Ramularia collo-cygni

A New Disease and Challenge in Barley Production

First European Ramularia Workshop

12-14th of March 2006

Book of Abstracts

Georg-August University Göttingen, Germany

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17:00-19:00 Welcome reception (*Reception Hall, Hotel am Papenberg*)
19:30 Welcome dinner (*Restaurant 'Mazzoni'*, *Hotel am Papenberg*)

Monday 13 March *(Lecture Hall MN06, North Campus, Grisebachstraße 6)*

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Edelgard Sachs
Federal Biological Research Centre, Kleinmanchow, Germany

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Short movie

Session 1 Epidemiology

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Kvithamar Research Centre, Stjørdal , Norway

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Edith Stabentheiner, Tobis Minihofer, Herbert Huss
Institute of Plant Sciences, University of Graz, Austria

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11:30-12:00 Investigating the life cycle of *Ramularia collo-cygni* using a PCR based diagnostic
Neil Havis and Simon Oxley
Scottish Agricultural College, Edinburgh, Scotland

12:00-12:30 Ramularia leaf spot in barley. A new disease in Sweden?
Annika Djurle and Morten Rasmussen
Department of Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden

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16:00-16:30	Does the mlo resistance gene increase the susceptibility of spring barley to spotting diseases? *Joanne Claire Makepeace, James K.M. Brown, Simon Oxley and James I. Burke* John Innes Center, Norwich Research Park, United Kingdom
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P4 Breeding for resistance against non-parasitic leaf spots in barley

Markus Herz, Anita Behn, Lorenz Hartl, Günther Schweizer, Max Baumer

Bayerische Landesanstalt f. Landwirtschaftnstitut für Pfanzenbau und Planzenzüchtung, Freising, Germany

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Friedrich-Schiller-Universität Jena, Germany

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Syngenta Agro, Saint-Cyr-l'Ecole, France

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Scottish Crop Research Institute, Invergowrie, Dundee, United Kingdom

Evening Programme

18:00 Guided sight seeing tour through the old city of Göttingen

19:30 Workshop dinner at the historical Rathskeller in the old Town Hall

Tuesday 14 March *(Lecture Hall MN06, North Campus, Grisebachstraße 6)*

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09:30-10:00	Control of *Ramularia* in winter barley and spring barley using different fungicides-experiences from Denmark *Lise Nistrup Jörgensen and Anne Christiansen* Danish Institute of Agricultural Sciences, Department of Crop Protection, Research Center Flakkebjerg, Slagelse, Denmark
10:00-10:30	Strategies of chemical control of *Ramularia collo-cygni* *Torsten Balz, Günter Prigge, Ulrich Krieg, Ulf Sattler and Andreas von Tiedemann* Department of Crop Sciences, Division of Plant Pathology and Crop Protection, University of Göttingen, Germany
10:30-11:00	***Coffee Break***
11:00-13:00	Round table meeting on a concerted action regarding *Ramularia collo-cygni* research in Europe
13:00	End of the workshop

Welcome and Opening words

Introduction

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

Edelgard Sachs

Federal Biological Research Centre for Agriculture and Forestry, Kleinmachnow Branch, Germany

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 Zeeland, Mexico and the Argentine 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.

Introduction to the epidemiology of *Ramularia collo-cygni*
Short movie

APS- Video DVD
The Biology of Fungal Pathogens,
Vol. 3 Fungal Pathogens and Diseases of Cereals.
3.1 Barley Leaf Spots – Fungal Disease or Stress Response? (14:00 min), APS-Press

Editors: Prof. Dr. Joseph-Alexander Verreet and Dr. Holger Klink, Christian-Albrechts University, Kiel
Scientific Advisor: Prof. Andreas von Tiedemann, Georg-August University of Göttingen

DVD back cover information:
Necrotic leaf spots on barley caused by abiotic or biotic factors have gained considerable importance in some regions where the crop is grown. This disease complex may significantly affect yield and represents a challenge both to proper diagnosis and disease management.
A new biotic barley disease caused by the fungal pathogen *Ramularia collo-cygni* causes necrotic leaf spots. The video illustrates the life cycle of the pathogen and differentiates the disease symptoms and etiology from abiotic leaf spots, the so-called 'physiological leaf spots' (PLS). PLS are induced by a complex of environmental stress factors including, excess irradiation, drought, air pollutants, or extreme temperatures, which cause elevated levels of free unscavenged oxygen radicals in the leaf tissue. The video represents the sequence of events leading from generation of toxic oxygen radicals to induction of cell death and necrosis. Finally the impact of modern fungicides on this leaf spot complex is represented, with special emphasis on physiological fungicide effects on the plant.

Session 1: Epidemiology

|| *Refer also to poster P2 and P3* ||

Ramularia collo-cygni on spring barley, an overview of its biology and epidemiology

Saideh Salamati[1] and Lars Reitan[2]

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[2)] *Graminor AS, branch Holthe, N-7650 Verdal, Norway.*

Abstract: *Ramularia collo-cygni* (Rcc) is now gaining more attention as a serious pathogen of barley. Even though several scientists have considered it the main cause of the typical necrotic spotting of barley, still as late as 2004 obvious doubts are registered on it being a real pathogen (Heiser *et al*, 2004). A great deal of uncertainties is related to the fact that hitherto the attempts for artificial inoculation in controlled environments have not given the exact disease symptoms. Central Norway has cold and humid summers. The typical leaf spotting which later received the name Ramularia Leaf Spot of Barley (RLSB) was observed in early 1980s in this area. Since 1998 we have registered the disease every year. After about eight years working with the disease we have acquired some understanding about its causal agent, Rcc. In April 2005 a detailed experiment was carried out in the green house and 3 barley varieties were artificially inoculated with Rcc. Typical disease symptom was registered on the most susceptible variety (Lavrans) four weeks after inoculation. Later the pathogen was isolated from all three varieties with and without obvious disease symptom. In central Norway RLSB was the main disease of spring barley in 2005. Rcc developed similar type of spotting on oat and couch-grass (Elytrigia repens). Over 230 Rcc isolates were collected. We have looked at their sporulation abilities, growth habits on different cultural media and colour differences.
In connection with the decision support system VIPS (NCRI) we looked at the historical weather data in central Norway. Humid weather conditions at the beginning of barley growth which in central Norway occurs around the first 10 days of June is highly correlated with the final RLSBs severity, suggesting an early infection. We recognize the disease much later often at or late flowering. Based on an overview of all the results we will hypothesise a relationship between the pathogen and the barley plant, this to understand some of the disease epidemiology.

Key words: *Hordeum vulgare* L., *Ramularia collo-cygni* Sutton & Waller, biology, epidemiology.

Reference

Heiser, I., Heß M., Schmidtke K.-U., Volger U., Miethbauer S. & Libermann B., 2004. Physiol. & Molecul. Plant Pathol. 64: 135-143.

Scanning electron microscopic investigations on leaves of barley and maize infected with *Ramularia collo-cygni*

Edith Stabentheiner[1)], Tobis Minihofer[1)] and Herbert Huss[2)]

[1)] *Institute of Plant Sciences, University of Graz, Schubertstraße 51, 8010 Graz, Austria;*
[2)] *Institute of Organic Farming, HBLFA Raumberg-Gumpenstein, Field Trial Station Lambach-Stadt-Paura, Gmundnerstraße 9, 4651 Stadl-Paura, Austria*

Abstract: *Ramularia collo-cygni* B. Sutton & Waller was investigated using scanning electron microscopy on leaf samples of barley and maize sampled in the summer and the winter season. The host is invaded via the stomata. The conidiophores emerge through the stomata but are also capable of breaking through the epidermis of heavily infected leaves. Caespituli are concentrated in patches, but cover the surface completely on heavily infected chlorotic and necrotic leaves, with the highest density always on the lower surface. *R. collo-cygni* is able to infect and sporulate on necrotic, chlorotic and green leaves in summer and winter conditions with low temperatures seeming to have no effect on either its morphology or life cycle.

Investigating the life cycle of *Ramularia collo-cygni* using a PCR based diagnostic

Neil Havis and Simon Oxley

Scottish Agricultural College, West Mains Road, Edinburgh, Scotland, EH9 3JG, UK.

Abstract :The life cycle of the barley disease, *Ramularia collo-cygni* was studied using a recently developed PCR based diagnostic test. The appearance of the pathogen was monitored in field trials over a number of seasons. Controlled environment experiments focused on a potential seed-borne stage for the fungus. Results indicated that the fungus can be detected before the appearance of visible sypmtoms but the earliest detection date varies between season and variety. In addition the pathogen is widespread at the end of the growing season in harvested grain samples and can be transmitted to developing plants from infected seed stock. Examination of infected seedlings did not reveal the presence of spores but fungal structures were found within the leaf. The implications of potential seed-borne stage in the pathogen life cycle are discussed.

Ramularia leaf spot in barley. A new disease in Sweden?

Annika Djurle [1] and Morten Rasmussen [2]

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[2] *Swalöf Weibull AB, BA Cereals, Cereal Breeding Department Landskrona, Disease Resistance Lab, SE-268 81 Svalöv, Sweden*

Abstract: The barley pathogen *Ramularia collo-cygni* has recieved increasing attention in Europe during the last years. It was found in Sweden on two occations in 2002 (M. Rasmussen and S. Salamati, oral comm.). During 2005 spring barley fields in Sweden were surveyed for *R. collo-cygni* and samples with possible symptoms were sent in for diagnosis. The fungus was present in 13 out of 42 fields and in 3 out of 7 samples. The presence of the pathogen in different geographical areas was thereby confirmed. It is assumed that occurrence of Ramularia leaf spot often has been hidden behind other diseases and the diagnosis physiological leaf spots (Twengström *et al.*, 2004). For 2006 field experiments addressing questions about cultivar susceptibility, yield loss and control are planned in a field where last year's barley crop was diseased by *R. collo-cygni.*

Disease scorings from these trials will be compared to similar datasets from locations in Scotland, Norway and Germany. A preliminary study on Nordic spring barley material from NGB indicates differences in resistance reactions between locations.Knowledge on pathogen population structure and dynamics as well as on pathogen virulence and plant genetic resistance interactions is still too limited for any substantial interpretation of these differences.

This work is in part financed by the Swedish Farmer's Foundation for Agricultural Research.

Key words: *R. collo-cygni,* survey

References

Twengström, E., Rasmussen, M. & Waern, P. 2004. Fysiologiska fläckar i korn. SLU, Faktablad om växtskydd - Jordbruk 117.

Session 2: Pathogenicity

|| *Refer also to poster P5 and P6* ||

Phytotoxins from *Ramularia collo-cygni* – mode of action and contribution to pathogenicity

Ingrid Heiser[1)], Sebastian Miethbauer[2)], Kai-Uwe Schmidtke[2)], Michael Heß[1)] and Bernd Liebermann[2)]

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[2)] *Institute of Pharmacy, University of Jena, Neugasse 23, D-07743 Jena*

Abstract: Fungal and bacterial phytotoxins play an important role as virulence factors or even as determinants of pathogenicity in many plant-pathogen interactions. For *Ramularia collo-cygni* we were able to isolate several phytotoxic metabolites from the culture medium which could be identified as rubellins A-E. These red- or yellow-coloured anthraquinones showed toxicity on barley, the host plant of *R. collo-cygni*, as well as on tobacco, a non-host plant, and therefore can be addressed as non-specific phytotoxins. Symptoms induced by the rubellins appear as chlorotic and necrotic lesions which only develop in the light. The strong light-dependency of rubellin action can be explained by the photodynamic activity of these molecules. Analogous to cercosporin, a perylenequinone phytotoxin from *Cercospora* sp., the rubellins are activated by light and induce the formation of reactive oxygen species (ROS) after illumination. ROS like singlet oxygen, the superoxide radical anion, hydrogen peroxide and the hydroxyl radical are strong oxidants and induce the oxidative breakdown of membranes and pigments when the antioxidative capacity of the plant cell is exhausted. Therefore the rubellins may be responsible for the symptoms induced in barley leaves after infection with *R. collo-cygni*. The presence of rubellins in infected leaves further points to an important role for these toxins in the infection process.

Session 3: Resistance

|| *Refer also to poster P4 and P8* ||

Field screening in Norway for resistance to *Ramularia collo-cygni* in old and new barley material

Lars Reitan[1] and Saideh Salamati[2]

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[2)] Midt-Norsk Plantevern AS and the Norwegian Crop Research Institute (NCRI), Kvithamar Research Centre, N-7500 Stjørdal, Norway.

Abstract: In the Trøndelag region of Norway barley is important, and counts for about 20% of the total Norwegian barley production. However, the climate is quite different from the main cereal producing area (south-east Norway) through lower temperature, higher rainfall and higher leaf humidity (marine climate).
Typical leaf spotting symptoms caused by *Ramularia collo-cygni* (Rcc) was observed in this region in the 1980ies, although incorrectly connected to other fungi. Field experiments treated with and without fungicides showed effects on the symptoms, and it was clear that the most effective time of treatment was late in plant development (after heading).
In 1999 the connection between the symptoms and the *Ramularia collo-cygni* fungus was stated. Barley breeding material was scored for Rcc-symptoms, and in some years a yield loss of at least 1 ton/ha was documented for the susceptible check variety. Since 2000 there have been established field nurseries for Rcc-observations on breeding material, potential resistance sources and material from other breeders and collections. In 2004 and 2005 a Nordic Gene Bank (NGB) collection of Nordic old and younger varieties were tested in two environments in Trøndelag. (Similar nurseries in other Scandinavian countries failed to give Rcc symptoms). Although the environmental effects are very big, still the genetic effect is significant. Several thousand genotypes have been tested since the start, and so far, no varieties without Rcc-symptoms are found. There is big variation between cultivars, but difficulties with the scoring due to other diseases are present. Especially mildew (*Blumeria graminis)* and net blotch *(Pyrenophora teres*) can cause problems. There are indications that early mildew attacks may cause a partial induced resistance to Rcc. The higher Rcc-attacs in mildew resistant varieties were present both for the mildew resistance gene ml-o and for other sources.

Key words: *Hordeum vulgare* L., *Ramularia collo-cygni* Sutton & Waller, field testing

Screening for leaf spot resistance - results and impact on practical breeding

Herbert Bistrich[1)], Josef Breun[2)], Georg Emmert[3)], Andreas Fleck[1)], Heidi Jaiser[3)], Harald Kempe[2)] and Marc Lemmens[3)]

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Abstract: Within the scope of a EC funded project (CRAFT 1999-70866), spring barley genotypes were evaluated for resistance to a leaf spot complex caused by *Ramularia collo-cygni*, non parasitic leaf browning (=NBV), or other. A core set of 55 genotypes was tested in replicated field trials at four locations in 2002 and at three locations in 2003. In addition 850 advanced breeding lines (screening set) and more than 1200 genebank accessions (pre-screening set) were tested in two and one environments respectively. Evaluation was done using a percentage damaged leaf area for the core set and a 1-9 scale for the other genotypes. To combine the core set evaluation data from time series (3 evaluation dates) into one value a linear disease progress was assumed. The area under disease progress curve (AUDPC) was calculated for each plot and expressed in % of the worst possible value. Overall variance analysis revealed significant genotype, environment and genotype x environment effects. Across all environments a quantitative variation of means was observed. *IPZ 24727* siblings showed the best resistance in both seasons whereas cv. *Barke* was found to be highly susceptible. A common feature of the worst cultivars (cvs. *Extract, Aspen, Avilla*) was possession of the mlo gene. These results corroborate the findings of Behn-Günther (2003) for NBV-QTL mapping. No released cultivar in the core set was surpassed the resistant check cv. *Prolog*. Cvs. *Jacinta* and *Millena* might be valuable crossing parents and several new additional resistance sources were identified. According to multiple comparisons of AUDPC means *SZD 159, SZD 160* and *Br 6680d36* were not significantly different from *LBPB 24727B*. In comparison with cv. *Prolog* lines with good resistance were frequently showing up in the screening set (e.g. *SZD 042-02, Br 104-02, Br 134-02, SZD 020-03, SZD 069-03, SZD 086-03, PF 016-03, PF 036-03*, PF 037-03, ...) but promising lines were rarely found among the genebank accessions. The evaluation of those exotic lines was generally hampered by the high susceptibility to powdery mildew and leaf rust. Due to the severe drought in 2003, resulting in a lack of disease symptoms at all, a large part of the genebank material (trials at Herzogenaurach & Morgenrot) could not be evaluated. Nevertheless, cvs. *Chevron, Vairoga Priekuli, Emel Dschemal, Oberthal 7, Alpina, Irba Moda, Karez* and *Clermoni*, selected from the pre-screening set 2002, were showing good resistance at Lambach, Lipprichshausen and Großaitingen. For the core part, the correlations (spearmans ρ) between leaf spot susceptibility (AUDPC) and plant height or heading date were only weakly negative or not significant. The discovery of additional resistance sources combined with high operative heritabilities (0.8-0.9) and the given variation within ripening and plant height classes, should enable practical breeders to make a substantial progress in leaf spot resistance without a shift to late and tall genotypes. Resistance tests in controlled environments or techniques to ensure an even disease pressure (e.g. some kind of artificial inoculation) are not yet available. Hence the selection of suitable testing sites, providing reliable disease development without the interference of other factors, will remain an important challenge. Last but not least, the breeder has to be on the site at the right time (to do the scoring).

Expression of resistance of barley varieties to Ramularia leaf spot and the status of the disease in Denmark

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[2)] *Danish Institut of Agricultural Sciences, Department of Variety Testing, Teglværksvej 10, Tystofte, DK-4230 Skælskør. Hans.Pinnschmidt@agrsci.dk*

Abstract: Widespread and heavy infection of *Ramularia* leaf spot was for the first time observed in Denmark in 2002 on spring barley (Pinnschmidt & Hovmøller 2003). Survey activities and disease management-related research efforts such as investigations on the resistance properties of Danish barley cultivars have been initiated since then. Severe Ramularia attack at several sites was again observed on spring barley in 2004 and 2005 and on winter barley in 2005. Multi-environment disease assessment data obtained on spring- and winter barley varieties were analysed to characterise the disease reactions and resistance properties of the varieties as well as the disease situation in the various environments. Both spring- and winter varieties differed substantially in their expression of resistance. The winter barley varieties Vanessa, Mombasa, Hamu and Himalaya and the spring barleys Braemar, Cabaret, Alliot, Prestige and Stratego were amongst the most susceptible ones while the spring barleys Proctor, Modena and some other cultivars were quite resistant across the environments. The varieties Carola, Nobilia, Lomerit and some new breeding lines were amongst the most resistant winter barleys but they displayed some instability in their expression of resistance across the environments as did many other varieties, both winter and spring types. About 60% of the total variation of disease severity was explained by genotype and environment main effects. A substantial part of the remaining variation could be explained by genotype x environment interactions. The results indicate that effective varietal resistance against Ramularia leaf spot exists. They also indicate that local pathogen populations may vary with respect to their virulence profiles which may interplay with specific resistance profiles of barley genotypes and challenge the stability and durability of varietal resistance against *Ramularia* leaf spot. However, the *Ramularia*-reaction of some cultivars may have been affected by the presence of other diseases and/or physiological leaf spots in some cases. Developing practicable methodology for examining the variation in virulence and aggressiveness of the fungus and for assessing varietal resistance in artificially inoculated field trials is therefore a basis for progress in resistance testing and breeding.

Key words: *Ramularia collo*-cygni, resistance level, resistance stability, genotype x environment interactions, barley, Denmark

References

Pinnschmidt, H.O. & Hovmøller, M.S. 2003: Ramularia, a new disease of barley – a review of present knowledge. DJF rapport nr. 89 (2003), pp. 313-321.

Does the *mlo* resistance gene increase the susceptibility of spring barley to spotting diseases?

Joanne Claire Makepeace[1)], James K.M. Brown[1)], Simon Oxley[2)] and James I. Burke[3)]

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Abstract: The mutant form of the *mlo* gene has been used in plant breeding as it confers durable resistance to *Blumeria graminis* (powdery mildew). However, the mutant gene carries undesirable pleiotrophic effects including spontaneous necrotic flecking of leaves which leads to reduced yield. Recent work has also shown that plants carrying a mutant *mlo* gene are more susceptible to two necrotrophic pathogens, *Magnaporthe grisea* (Jarosch et al. 1999) and *Cochliobolus sativus* (Kumar et al. 2001).

The aim of this project is to test if plants carrying mutant *mlo* alleles are more susceptible to three faculatative pathogens which are important in barley producing areas of northern Europe. These are *Rhynchosporium secalis*, *Pyrenophora teres* and *Ramularia collo-cygni*.

In field trials, near-isogenic lines of cvs. Ingrid and Pallas with mutant *mlo* alleles were more resistant than their recurrent parents to *R. secalis and R. collo-cygni*. This contrasts with the published results on *M. grisea* and *C. sativus*.

Growth room studies on seedlings showed that the environmental conditions affected the resistance of lines with mutant *mlo* alleles to *R. secalis*. The resistance of *mlo* mutant lines was associated with increased levels of host cell wall appositions (HCWA) produced by plant cells. These HCWA were associated with a reduction in penetration by the fungus. However, if the environmental conditions in which the plants are growing were altered prior to inoculation, a reduction or loss of the resistance was achieved. High light conditions prior to inoculation resulted in reduced resistance to *R. secalis* in *mlo* plants, associated with an increase in penetration and a reduction in HCWA produced by plant cells. A combination of high light and temperature prior to inoculation resulted in increased susceptibility of mutant *mlo* lines to *R. secalis*.

References:

Kumar, J., R. Huckelhoven, U., Beckhove, S., Nagarajan, K., H., Kogel. (2001). A compromised Mlo pathway affects the response of barley to the necrotrophic fungus *Bipolaris sorokiniana* (teleomorph; *Cochliobolus sativus*) and its toxins. Phytopathology 91(2): 127-133.

Jarosch, B., K. H. Kogel, U. Schaffrath (1999). The ambivalence of the barley Mlo locus: Mutations conferring resistance against powdery mildew (Blumeria graminis f. sp, hordei) enhance susceptibility to the rice blast fungus Magnaporthe grisea. Molecular Plant-Microbe Interactions 12(6): 508-514.

Session4 Poster

P1

Effect of fungicide application on *Ramularia collo-cygni* infection in barley as analysed by chlorophyll fluorescence imaging

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Abstract: At the Research Centre Flakkebjerg in Denmark, a field trial with barley was initiated in order to test the effect of three fungicides: Acanto Prima, Opera and Bell on the infection of *R. collo-cygni.* The use of chlorophyll fluorescence imaging was to quantify both infection rates and the effect of these fungicides. This high-resolution technique enables the study of plants displaying heterogeneities in photosynthetic activity, which may be due to metabolic regulation, tissue differentiation, virus and fungi infections as well as biotic or abiotic stress.

Infection was followed by scoring the disease on the second last leaf, F1, according to the following scale: 0%, 0-0,1%, 0,1-1%, 1-5%, 5-10%, 10-25%, 25-50%, 50-75%, 75-100% in the untreated plots. Chlorophyll fluorescence imaging was measured on leaves for each category during three weeks after the onset of infection.

Overall, the photosynthetic activity was lowered in necrotic spots but the results indicate that areas around spots are able to compensate to a certain degree by increasing photosynthetic activity. Infection lowered photosynthetic light-use as indicated by quantum yield parameters, electron transport rate (ETR) and the relative rate of CO_2 fixation. The transient effect of the fungicides could be followed. Treatment with fungicides delayed the onset and progress of infection. Of the fungicides tested, Bell had the largest effect, while Opera and Acanto Prima were less efficient.

P2

Monitoring the epidemics of *Ramularia collo-cygni*: Comparison of varieties, sites, years and methods

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Abstract: Although recent research established *Ramularia collo-cygni* as an important cause of leaf spotting on barley, epidemics remain mostly uncertain and difficult to investigate.
The main method for quantifying the pathogen is the diagnosis of sporulation with the swanneck shaped conidiophores on the leaf surface with a microscope. Typically for these observations none or hardly any sporulation is found on the host plant in spring until mid May. About 2 weeks after flowering small necrotic lesions appear on the upper leaves followed by rapid breakdown of leaf tissue and heavy sporulation of *Ramularia collo-cygni* on the necrotic leaves.
Sporulation on the different leaf levels is compared with the occurrence and progress of the leaf spots. The results from trials from the past 5 years show influence from years, sites, variety and growth stage of the host plant on the epidemics of *Ramularia collo-cygni*. The method remains laborious and not sensitive enough for the onset and rapidity of the epidemics, since sporulation follows necrosis.
Additional observations were made collecting spores. The number of spores is compared to assessed progress of necrotic leaf area and the area with sporulation of *Ramularia collo-cygni* found under the microscope.
Using molecular methods PCR testing showed the presence of *Ramularia collo-cygni* DNA about 1 week prior to first spotting.
The results obtained with different methods will be discussed and conclusions for the epidemics of *Ramularia collo-cygni* will be drawn.

P3

A new direct PCR test detect *Ramularia collo-cygni* in barley

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Abstract: For a better understanding of the epidemiology of *Ramularia collo-cygni* B. Sutton & J.M. Waller, internal specific primers to the ITS1/2 rDNA regions were designed (Rcc1 and Rcc2) which amplify a 348 bp fragment. Direct –PCR, without any DNA purification steps, were performed during the whole period of vegetation of untreated winter and spring barley (respectively cv. Plaisant and cv. Celinka) to follow the epidemiology of the fungus. The plants were collected in two regions of Switzerland. Results show that, between growth stage DC 24 (4 tillers detectable) and DC 85 (grain content solid), *R. collo-cygni* is present in leaves, ears and awns after PCR amplification on homogenized plant juices. *R. collo-cygni* is therefore present during a long period of time in the plant which signifies that infection of barley takes place in autumn for winter varieties, where the fungus overpass the winter time and can infect spring barley very early in the season. The control of symptomless volunteers two months after harvest also showed the presence of the fungus in this plants and proves the “green bridge” between barley cultures.
As controls, other fungal pathogens and saprophytes isolated from barley, and also barley itself, were amplified with primers Rcc1 and Rcc2. No amplification products could be observed, this showing the high specificity of the primers.

P4

Breeding for resistance against non-parasitic leaf spots in barley

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Abstract: Non parasitic leaf spots (NPLS) are a phenomenon which causes severe losses in yield and decrease in quality of barley. Especially in regions which are affected by intensive global radiation the symptoms can be observed frequently every year. Intensive radiation induces a stress response of the barley leaves which results first in brown spots and leads in later stages of the disease to a too early ripening of the plant. Often the lesions caused by the stress reaction are the entrance for a secondary fungal infection, which include in particular *R. collo cygni*.

On a particular location in Bavaria the symptoms of the leaf spots can be very well studied.

Genetic diversity of the response to global radiation was identified within the German barley germplasm. One breeding line showed extreme good resistance. The genomic localisation of the factors responsible for resistance could be identified by a QTL mapping approach. The knowledge about chromosomal assignment and linked markers was used to develop nearly isogenic lines carrying QTL intervals for resistance against NPLS.

The developed differential plant material will be the basis for further studies assessing directly the expression of the involved genes. Also the interaction of RCC with NPLS can be illuminated on the basis of the genetic background.

For the introgression of the resistance into adapted breeding material, efficient use of molecular markers will be an essential tool. The presented results describe the path to develop useful molecular markers for an important agronomic character of barley.

P5

Biosynthesis of the rubellins, produced by the phytopathogenic fungus *Ramularia collo-cygni*

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Abstract: The phytopathogenic fungus *Ramularia collo-cygni* (Sutton and Waller) produces anthraquinone derivatives (rubellins) with photodynamic activities. Incorporation experiments with [1-^{13}C]-acetate and [2-^{13}C]-acetate, respectively, reveal that these rubellins were biosynthesised *via* the polyketid pathway. The labelling pattern in the anthraquinone derivatives after feeding with [U-$^{13}C_6$]-glucose proved the fungal folding mode of the poly-β-keto chain.

P6

The phytopathogenic fungus *Ramularia collo-cygni* produces different anthraquinone derivatives (rubellins) with photodynamic activity

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Abstract: The anamorph fungus *Ramularia collo-cygni* (Sutton and Waller) causes a leaf spot disease on barley and other *Poaceae*. Rubellins as non-host-specific toxins contribute to symptom development in the infected plant. In addition to the rubellins B, C and D, we isolated the yellow coloured anthrachinon derivate rubellin A from mycelium and culture filtrate. Furthermore, two novel compounds named rubellin E and dehydrorubellin D were isolated and elucidated in their structure. In comparison to the others, rubellin A shows increased photodynamic oxygen activation. The ability to produce rubellins is not limited in the anamorph genus *Ramularia* to *R. collo-cygni*.

P7

Benefits of triazole + chlorothalonil for the control of *Ramularia collo-cygni*, a new problematic in Barley in France

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Abstract: In 2002, *Ramularia collo-cygni* (R c-c) generated severe yield losses in Center France. Since this year, the presence of this fungus has been also frequently notified in others area of France. The symptoms of this disease are often considered as those of *Dreshlera teres*.
Since 2004, a monitoring was made in various areas of France ; isolations were made in Petri dishes and revealed the widespread presence of *R c-c*.
Some trials with exclusive detection of *R c-c* showed a very high level of efficacy of triazole + chlorothalonil based products (Bravo Premium or Citadelle). The results of these specialities are better than straight triazole applications.

Key words : *Ramularia collo-cygni*, chlorothalonil, Bravo Premium, Citadelle.

P8

Genetics of spots and blotches in spring barley

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Abstract: Random inbred lines from two contrasting spring barley crosses were used to study the genetics of Physiological Spotting (PS) and Ramularia Like Spots and blotches (RLS). The genetic control of PS was much higher than that of RLS and there appeared to be little association between the development of the two. Molecular marker maps of the two crosses were then used to search the genome for Quantitative Trait Loci affecting the expression of the two characters. Results showed that the *mlo* mildew resistance had a major effect upon the increased expression of PS. Apart from the *sdw1* dwarfing gene, no large genetic effects were detected in either cross for RLS, suggesting that deployable resistance was due to the cumulative action of minor genes in the crosses studied.

Session 5: Chemical Control

|| *Refer also to poster P1 and P7* ||

Impact of fungicides and varietal resistance on *Ramularia collo-cygni* in spring barley

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Abstract: *Ramularia collo-cygni* is now a major disease of barley in Scotland and Eire. Growers currently rely on fungicides to achieve effective control, and this has led to an increase in production costs at a time when grain prices are low. The efficacy and yield benefits of fungicides to control *R. collo-cygni* have been tested in Scotland and Eire in a three-year programme of 'Appropriate Fungicide Dose' trials. Breeding varieties resistant to the disease is a longer-term aim. The susceptibility of spring barley varieties to *R. collo-cygni*, losses in yield and green leaf associated with the disease has been recorded over four seasons from 2002-2005. Some varieties vary from one season to another in their susceptibility to the disease and associated yield loss. Others are more consistent showing either good or poor resistance to *R. collo-cygni*.

Control of Ramularia in winter barley and spring barley using different fungicides- Experiences from Denmark

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Abstract: For two seasons different fungicides have been tested for their efficacy for control of *Ramularia collo-cygni*. The trials have been carried out in the spring cultivar Alliot and the winter barley cultivar Vanessa. In both cultivars the disease did not appear before GS 55-65.

During 2004 and 2005 2 trials in spring barley were treated with fungicides applied at GS 45-51. As an average the fungicides Bell (boscalid+epoxiconazol), Opera (epoxiconazole + pyraclostrobin) and Proline (prothioconazole) 4 weeks after application gave between 87 and 96% control using 2/3 of normal rate. Slight dose responses were seen between 2/3 and 1/3 dosages. Yield responses from control of Ramularia in spring barley have, in trials dominated by Ramularia, varied between 5 and 11 hkg/ha. Best net yield was obtained following the use of a 1/3 rate of Opera and Bell or 2/3 rate of the mixture Amistar (Azoxystrobin) + Stereo (propiconazole + cyprodinil).

In 4 winter barley trials in 2005 fungicides were applied at GS 39-51. As an average of the trials comparing ½ rates best effect was obtained using Bell, Opus team (epoxiconazole + fenpropimorph), Opera and Proline. In winter barley the yield responses have been relative low (3-8 hkg/ha). The level of control was only slightly reflected in the yield responses partly due to the fact that also other diseases appeared in the trials.

In one winter barley trial in 2005 3 different timings for control of Ramularia were compared, using GS 37-39, 51-55 and 61-65. The results showed best control from application at either GS 37-39 or 51-55. The later timing (GS 61-65) gave generally inferior levels of control. In this trial Bell was better than Opera, which again was better than Acanto Prima (Picoxystrobin + cyprodinil).

One spring barley trial with the susceptible cultivar Alliot was sown in 2005 following a spring barley crop with severe attack of Ramularia in 2004. The field was either ploughed, harrowed a few times before sowing or direct sown. The trial developed severe attack of net blotch and Rhynchosporium in particular following minimal tillage and direct drilling. Tillage methods did not have any impact on Ramularia, which hardly could be identified in the trial. This could indicates that the disease is not easily spread from stubble or is weak in competing with Rhynchosporium and net blotch.

Key words: fungicides, Ramularia, winter barley, spring barley

Chemical control and strategies against *Ramularia collo-cygni*

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Abstract: Over the last few years *Ramularia collo-cygni* (Rcc) has been observed very late in the barley growing season. In 2004 we saw the first infections of Rcc at the end of May on plants at growth stage EC 69. On May 24th the causative agent was found on leaves 3 and 4 and one week later, on May 31st, it was found on the flag and first leaves. One explanation for this late infestation by Rcc is that older plants are more susceptible. Infection of the plants is initiated by spores and can be seen by studying wind borne spores and Rcc proteins in barley leaves. Examination of spores on water agar showed no pronounced optimum temperature for spore germination within a range of 4°C to 24°C. Fewer spores were seen to germinate above 24°C and germination rate was even lower above 32°C.

At present none of the barley varieties available provide a useful tool for a strategy against Rcc. All varieties are susceptible and analysis showed no differences in the levels of Rcc protein found in the straw. The timing of initial infestation and the speed of disease development varies with variety.

In contrast to control through resistant varieties, fungicides have been found to provide a useful control strategy against Rcc. We applied selected fungicides at one application timing, growth stage EC 37-39, at the full recommended rate. The treatments were compared according to direct disease control and persistence of effect and yield. Opera (50 g/l epoxiconazole and 133 g/l pyraclostrobin at 1.5 l/ha) showed a moderate control of disease. Champion (233 g/l boscalid and 67 g/l epoxiconazole at 1.5 l/ha) was better than Opera. Input (160 g/l prothioconazole and 300 g/l spiroxamine at 1.25 l/ha) gave very good disease control but the persistence of effect was not so good with Rcc symptoms appearing 6 weeks after treatment and disease developing thereafter. Fandango (100 g/l prothioconazole and 100 g/l fluoxastrobin at 1,25 l/ha) seems like the same like Input, but the persistence effect stops earlier. The treatments with the best combination of direct disease control and persistence of effect were Bravo (500 g/l chlorothalonil at 2 l/ha) and Amistar Opti (400 g/l chlorothalonil and 80 g/l azoxystrobin at 2.5 l/ha). Yields of Opera, Champion, Fandango, Input, Amistar and Amistar Opti treatments are on a comparable level. The results will be presented and discussed in the lecture.

Round table meeting on a concerted action regarding *Ramularia collo-cygni* research in Europe

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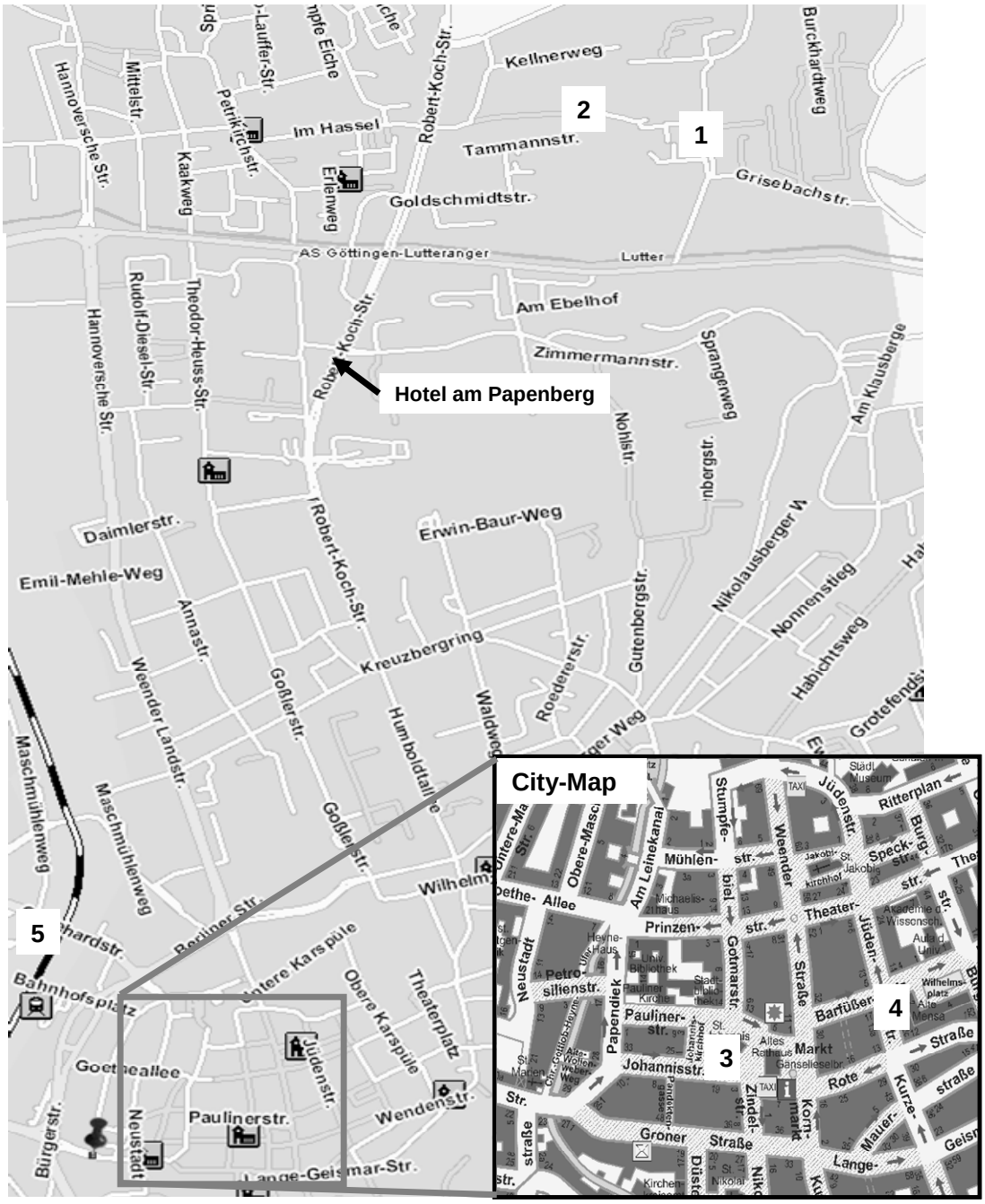
Map of the City of Göttingen

First Ramularia Workshop 2006 in Göttingen



Legend:

1 Workshop Venue: Plant Pathology and Protection Division, Grisebachstrasse 6, Lecture Hall MN 06
2 Bus-Stop Kellenerweg, Stop for bus 5 (closest bus-station to the workshop venue, ca 300 m)
3 Old Town Hall, Meeting for the guided City Tour. The Old Rathskeller is located in the basement.
4 Bus-Stop Jüdenstrasse, bus 5 and 8. Direct connection to the Workshop venue and Hotel Am Papenberg
5 Train Station