The Biology of *Ramularia collo-cygni*[1]

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INTRODUCTION

Ramularia collo-cygni Sutton & Waller was discovered by Cavara (1893) in northern Italy and described as *Ophiocladium hordei* Cav. Jørstad (1930) recorded this fungus on barley in Norway, Sutton and Waller (1988) on Triticale in Mexico. *Ramularia collo-cygni* is also known as a pathogen of barley in Germany, Switzerland, the Czech Republic, New Zealand, Uruguay and Argentina (Sachs, 2002). The first report of significant yield losses (-10%) caused by massive infection of barley was based on observations carried out in Austria (Huss *et al.,* 1987). Subsequently, *Ramularia collo-cygni* has become more and more established as a pathogen of barley, causing yield losses of 16 - 18% (Huss, 2000). A similar development was observed in southern Germany, especially in Bavaria. Heavy infections were also reported from Scotland and Ireland in 1997 (Millar, 1998; Burke *et al.,* 2000), and from Norway (Salamati, 2001).

Without any doubt, we are just at the beginning of in-depth investigations into the biology of *Ramularia collo-cygni*. Nevertheless, this attempt to document the knowledge available is important to summarize the current understanding of the disease process, especially from a practical point of view. The German term currently used for the disease caused by *Ramularia collo-cygni,* 'Sprenkelkrankheit', was proposed by Huss and Neuhold (1995) and could be translated as 'speckle disease'.

MATERIALS AND METHODS

Environmental conditions

The observations and experiments communicated here were carried out between 1986 and 2002 at the Lambach Research Station at Stadl-Paura located in the pre-alpine region of Upper Austria at 336 m altitude, in the transitional zone between Atlantic and Continental climate annual (precipitation 981 mm, average temperature 8.1°C, snow cover 53 days per year1). The dominant crops of the surrounding fields are winter-barley (30%) and maize (30%), followed by winter wheat (15%), rape seed and peas (together 15%) and 10% of fields under fallow.

[1] Key Note Address: Biotechnology and barley diseases

Method of spore counts

The spore counts were carried out with two spore traps. Slides with a thin layer of vaseline and paraffin were fixed at an angle of 45° in a mounting support at the height of the flag leaves of barley. All together, the conidia on 4800 grid units (0.196 mm^2 each) have been counted at a magnification of 400.

RESULTS AND DISCUSSION

Morphology of conidiophores and conidia, dispersal of conidia

The hyaline conidiophores grow out of the stomata in fascicles. Unlike the conidiophores of similar graminicolous *Ramularia* species (e.g., *R. pusilla or R. holci-lanati*), those of *R. collo-cygni* are spreading very conspicuously, becoming almost pressed to the surface, as if they wanted to avoid too much exposition to air currents. The terminal part of the conidiophore is strongly curved, very much like a swan's neck. The apical region may successively produce up to 5 conidia (Fig 1). Only rarely, can two still attached conidia be found on the same conidiophore. The surface ornamentation of the conidium wall is spiny-verruculose. The average conidium size is 8.0 x 4.4 µm, ranging from 6.2-10.6 µm in length and 3.2-6.0 µm in width.*i*

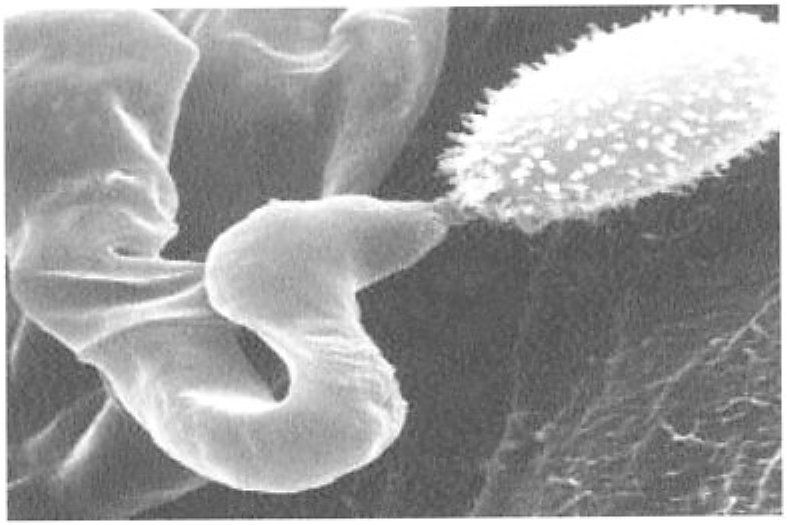


Fig 1. Conidiophore of *Ramularia collo-cygni* on wheat, with one conidium still attached; note the slightly widened sub-apical region of the conidiophore, with three scars below the conidium.

Normally, the caespituli develop only after the infected leaf tissue shows distinct necroses. On barley, the most important and most susceptible host of *Ramularia collo-cygni,* the culms, the lemma (especially the awn), and the palea may also be infected (Neuhold, 1995). The fungus grows preferably, but not exclusively, on the somewhat more protected lower (ab-axial) surface of the leaf blade. A massively infected leaf blade produces up to 50,000 conidia.

In 2001, spore traps were installed at our field research station, in order to estimate the number of wind dispersed conidia. Between May 9 (GS 49 of barley) and May 29 (GS 73 of barley; i.e., the time when whole leaf blades of barley start to die from the Ramularia disease), 3716 conidia were counted on 1 cm^2, amounting to an average deposit of 186 conidia per day. This pathogen is apparently able to produce enormous quantities of conidia, which are presumably dispersed over long distances by air currents. This would explain its rapid spread in recent years.

Germination of conidia and infection of the host tissue

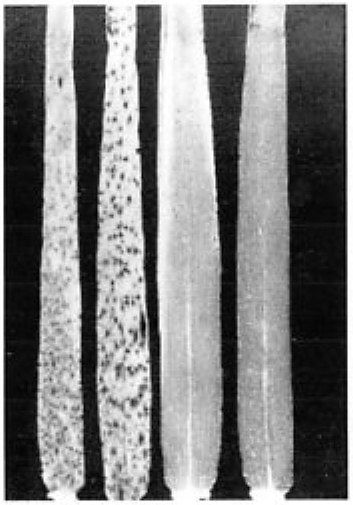
In most cases, spores of parasitic fungi require a host surface moistened by fog, dew or rain for germination and successful infection (e.g. Schrödter, 1987). Last year the influence of dew on the development of speckle disease of barley was tested by a simple exposure experiment.

Potted plants of the winter barley cv. Virgo were kept in an open polyethylene tunnel between May 12 (GS 39) and June 11 (GS 77) which was orientated parallel to the dominant wind direction. The plants were watered without wetting the leaves, in order to limit the access of conidia to dew as the only potential water source for germination and infection. Every evening at 21.00 h, a number of marked reference plants from this set were transferred into a dry room free of dew overnight, and returned into the polyethylene tunnel at 7.00 hours in the morning.

The result of this experiment is shown in Fig 2a. The leaves of dew-free barley plants were still green on 11 June, whereas the leaves of the plants exposed to night dew were all discoloured and necrotic, with richly sporulating caespituli of the Ramularia developed in the leaf spots.

This experiment demonstrates that night dew is a sufficient source of moisture to enable a massive *Ramularia* infection. It may also be concluded that direct sunlight does not influence the development of the disease symptoms, i.e., the leaf spots, contrary to the assumptions of Obst and Baumer (1998) and Habermeier *et al.,* (2002). It must be noted that the Ramularia leaf spots develop preferably on the exposed, pendent parts of the barley leaf blades, which is exactly where the highest concentration of dew droplets can be observed.

Germination and mycelial growth proceed rather rapidly in *Ramularia collo-cygni.* Sutton and Waller (1988) inoculated Triticale leaves and observed that *"both spores and mycelium inoculum grew rapidly over the leaf surface and some entered the leaf through open stomata within 24 h. Within the leaf, hyphae were intercellular and affected the staining reaction of cell cytoplasm some distance from their position indicating the probable production of a toxin"*. According to inoculation experiments with barley leaves carried out by E. Sachs, it takes 7 days from applying a suspension of conidia until the first disease symptoms become apparent (Huss and Sachs, 1998).



a)

b)

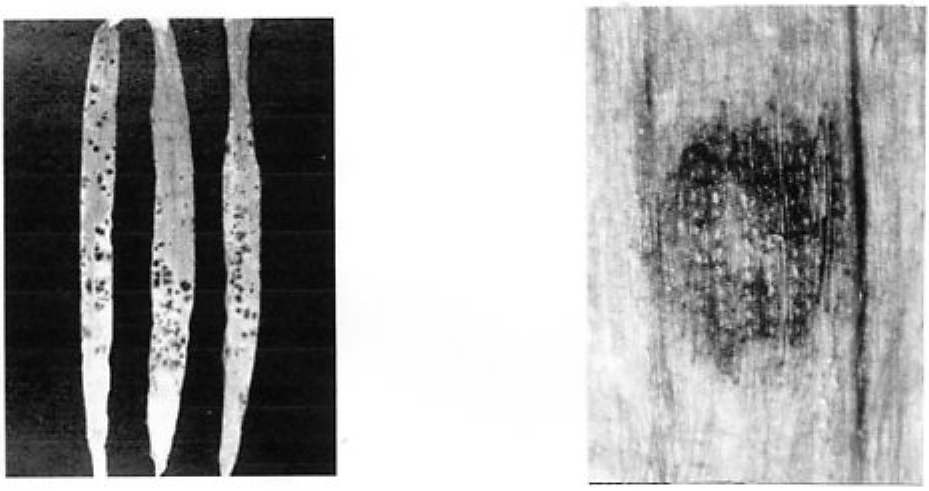
Fig 2. *Ramularia collo-cygni* on the winter barley Virgo, dew exposure experiments (see text). (a) The two barley leaves on the left have been exposed to dew and show a strong infection. The two leaves on the right were taken from the 'dew-free' reference plants where the Ramularia conidia did not germinate. (b) Close-up of a leaf spot showing the white, punctiform fascicles of conidiophores; taken from a leaf moistened exclusively by dew throughout the experiment.

Symptoms

Characteristic Ramularia leaf spots on winter and summer barley can be observed after heading. They are brown to blackish brown, 1-2 mm long, and usually sharply delimited by the leaf veins (Fig 2b). In addition to these, small punctiform leaf spots can be observed as well. Where neighbouring leaf spots meet, they may become confluent to form larger dark areas. Soon after the appearance of the leaf spots, the green leaf blade starts to fade to yellow and becomes necrotic, usually starting from the tip and the margins.

The symptoms observed on the leaves of winter barley in March differ considerably from those described above (Fig 3). In March they are usually circular and about 1 mm in diameter. The most conspicuous difference, however, is a ring of necrotic tissue all around the leaf spot which is probably due to the presence of a toxin (see above, Sutton and Waller, 1988).

While the disease caused by *Ramularia collo-cygni* may lead to considerable yield losses in barley, infections of wheat are of no economic significance. They have been observed at the research station at Lambach-Stadl-Paura since 1990, but only very weak symptoms could be noted. It seems that *Ramularia collo-cygni* behaved like a saprophyte or a very weak parasite until 1999, when a massive infection led to well-developed disease symptoms (Fig 4). The leaf spots are mostly 1 mm long, oval (to circular) in outline, white in the centre and surrounded by a very distinct brownish-black margin. The fascicles of conidiophores grow in the centre of the spot (Fig 4b).

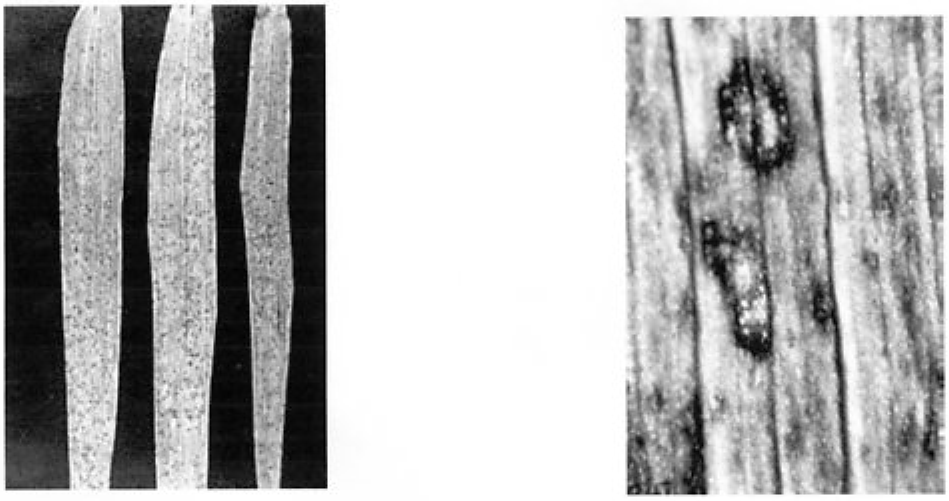


a) b)

Fig 3. *Ramularia collo-cygni* symptoms on the leaves of winter barley from March 2001: (a) Infected leaves, (b) circular leaf spot with white punctiform fascicles of conidiophores.

Parasitic and saprophytic lifestyle

During the time of stem extension of winter barley, *Ramularia collo-cygni* lives primarily as a saprophyte on the basal dead leaves. But it is also able to persist as a saprophyte on dead barley spikes, wheat and maize leaves, and rapeseed stems. On the other hand, it lives also as a parasite on barley, wheat, maize, and other grasses. This 'double' life strategy has great advantages for the fungus which can continue to grow oi the fields outside the normal growing season, and thus produce conidia throughout the year, ready to infect its favourite host barley at any suitable time.



a) b)

Fig 4. *Ramularia collo-cygni* symptoms on wheat: (a) infected leaves. (b) leaf spot with white fascicles of conidiophores.

Phenology and general development of the disease recorded at the Field Research Station

Soon after the snow has melted away, *Ramularia collo-cygni* can already be found on the leaves of winter barley. The disease process relevant to yield losses starts from the beginning of heading of winter barley. Subsequently, summer barley, rye, wheat and oat are infected, i.e. all the traditional grain crops. Summer barley is massively infected, in some years also oat. On rye the symptoms appear quite regularly, on wheat they are only found under favourable conditions. Maize is colonized in autumn, but mainly saprophytically. However, just like on wheat, certain breeds may develop characteristic disease symptoms.

Common couch (*Elymus repens*) is an important host for *Ramularia collo-cygni.* It is a perennial native grass, which serves as a host throughout the year. The annual wild barley *Hordeum murinum* is massively infected. The annual panicoid grass *Echinochloa crus-galli,* a common weed in many fields, is not infected in a significant manner. It may be concluded that the establishment of *Ramularia collo-cygni* as a regularly occurring parasite of barley has led to a considerable infection pressure on other grain crops and grasses. Probably one of the best examples for this tendency is false oat (*Arrhenatherum elatius*), a species which only shows *Ramularia* symptoms when barley is heavily infected.

The most curious example is certainly the record of *Ramularia collo-cygni* as a leaf spot parasite of *Cannabis sativa* by Scheuer and Mayrhofer (in Scheuer 1999). The Cannabis plants were grown in the Botanical Garden of the Institute of Botany in Graz, next to a small bed with various grain crops, including at least two different barley varieties.

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