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Ramularia leaf spot (RLS) was in the Czech Republic first identified in 1998. Severe symptoms of RLS were detected on barley on whole area of the Czech Republic in years 2002 and 2009 and in the others years has this disease more likely local importance. Climatic conditions in the Czech Republic are more continental (less amount of precipitation during vegetation and colder winters) than in countries affected by oceanic climate (e.g. Scotland, Germany). There were tested 144 spring barley cultivars at 3 locations in the Czech Republic over 3 years (2009–2011). Only minor and statistically insignificant differences were observed among the individual cultivars in reaction to RLS. No cultivars were observed to have resistance to *Ramularia collo-cygni* (Rcc), but significant influence of location and year on the intensity of RLS infection in barley was observed. Isolates of Rcc mostly originated from the Czech Republic, but also from the Slovak Republic, Germany and Swiss were tested using amplified fragment length polymorphism (AFLP) analysis. The level of genotypic diversity was higher within populations than among them. No significant population differentiation was observed thus extensive gene flow is assumed among populations. The inferred clusters did not represent geographical populations. Czech Rcc isolates were screened for strobilurin resistance. Frequency of the mutant allele responsible for strobilurin resistance of Rcc was significantly affected by the application frequency of strobilurin fungicides. In locations with more frequent application of strobilurins, the frequency of the mutant allele in RCC isolates was higher (up to 100%). By contrast, in locations where strobilurin fungicides were not used the mutant allele occurred only in 0–5% of the isolates. A real time PCR assay was designed to quantify the pathogen in barley tissues. PCR primers and a TaqMan probe were designed to target Rcc-specific DNA sequence. The method was optimized using pure fungal DNA and plasmid standard dilutions. Barley kernels were dissected into lemma, pericarp, testa, endosperm and embryo which were individually tested by real time PCR for quantifying Rcc. *Ramularia* DNA was highest in the lemma, and occurred in lower amounts in the pericarp and embryo. Collection of Rcc isolates in Kromeriz contains more than 300 items and is fully available for everybody who is interested. We store isolates in freezer after drying for four weeks under vacuum pump.

Photos by Matusinsky