

Genetic diversity of the apple leaf blotch fungus *Marssonina coronaria* in Europe

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Abstract

Apple blotch caused by Marssonina coronaria (Mc) is reported in Europe since 2001. It has become a serious problem in organic low input orchards and in cider production areas in Northern Italy, Switzerland, Austria and Germany. It is assumed that it has been introduced from East Asia where it is known since 1907. However, its invasion route to Europe and the genetic diversity of established populations in Europe is unknown. To better understand its epidemiology and to reveal its population genetic structure, we developed Mc specific SSR markers (SSR: short sequence repeats) and studied European samples. They showed the typical genetic signatures of a recently introduced species such as lower number of alleles per SSR markers, and higher clonality compared to samples from Asia. The observed low genetic diversity and high clonality suggests that management strategies (such as resistance breeding) might be successful, always supposing that further introduction of novel M. coronaria genotypes into Europe is prevented.

Keywords: Marssonina apple blotch, *Diplocarpon mali*, SSR markers, genetic diversity

Introduction

Marssonina coronaria (Mc) (Ellis & Davis) Davis; teleomorph *Diplocarpon mali*), causing chlorotic leaf blotch and premature leaf fall in apple, was first described in the USA (1903) and in Japan where it has been known since 1907. Further observations of this disease now and then indicated that the disease was globally present in apple orchards, however, mainly limited to sub-tropical regions (Leite et al. 1986). Only as of the 1990s it became a significant problem in the main apple production areas, such as in China, Korea, and India. Since 2010 it has also been established in Middle Europe mainly in cider-production areas or in low-input and organic orchards with scab-resistant cultivars (Bohr et al. 2018, Wöhner and Emeriewen 2019). It is hypothesized, that climate change with warmer conditions in early summer is one of the main driving forces for the epidemic spread of the disease in our regions.

So far nothing is known, however, on the genetic variability and the pathogenicity of different isolates of the pathogen and a large part of the life cycle, such as overwintering and frequency of sexual reproduction, are still unknown (Wöhner and Emeriewen 2019). The aim of this study was to develop molecular tools to investigate the population genetics and the evolutionary potential of Mc in Europe as a base for the development of new prevention strategies such as resistance breeding (Wöhner et al. 2019), or targeted and sustainable spray programs.

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Material and Methods

Twelve Mc specific SSR markers were developed, which were polymorphic between DNA extracts of a Swiss and Chinese Mc isolates, and showed no cross-reaction with healthy plant tissue. A total of 313 samples were collected from the main European apple production sites around Lake of Constance and those in South Tyrol as well as some samples from Central and Western Switzerland, Northern Germany, and Easter part of Italy/Slovenia. Ten outgroup samples were obtained from Korea, China, Japan, and the USA. These samples were amplified in multiplex PCR reaction grouping 3 markers each, and then the fragment lengths determined in capillary electrophoresis. Data were analysed with the R package *poppr* and Bayesian cluster analysis software *Instruct*.

Results and Discussion

A single and dominant genotype was found in 66% of all European samples, regardless of whether they were collected in Italy or Northern Germany. This finding and other genetic diversity parameters indicate a strong clonality and a geographically mostly unhindered gene flow within the European populations. A certain variability was found in the populations of the Lake Constance and South Tyrolean regions, both of which are among the most intensive apple-growing areas in Europe. However, these variabilities were small compared to the outgroup genotypes of Asian and American samples, which differed significantly in their genetic distance to the European genotypes.

The conclusions are that the actual epidemic Mc-populations in Europe, which vary in severity from year to year, (i) originate from a recently introduced founder population and spread rapidly in all major European apple production areas. This means that (ii) breeding programmes and other disease control strategies will only be successful and of durability, as long as no new and potentially more aggressive Mc genotypes are introduced in Europe.

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