# Early season detection of *Marssonina coronaria* spore dispersal with selected spore traps and qPCR

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## Abstract

*Marssonina apple blotch, caused by the fungus Marssonina coronaria (teleomorph Diplocarpon mali) causes severe defoliation of apple trees in late summer, therefore leading to reduced yield and fruit quality as well as to a weakening of the trees over time. M. coronaria is one of the most important diseases in apples in Asia and has increasingly become a problem in European apple production in recent years, especially in low-input organic apple production. However, knowledge on the biology of M. coronaria in Europe is still scarce. The aim of our study was to gain a better understanding on spore dispersal in combination with disease progress in spring and early summer. To this end we developed a method that enables to capture and quantify M. coronaria spores in the field.*

**Keywords:** Marssonina apple blotch, rotating-arm spore trap, Mycotrap, disease forecast models, *Diplocarpon mali*

## Introduction

*Marssonina coronaria* (Ellis & Davis) Davis (teleomorph *Diplocarpon mali* (Harada & Sawamura)) is an ascomycete fungus causing Marssonina apple blotch (MAB) on apple trees (*Malus domestica*). The pathogen can lead to severe tree defoliation, weakening the trees and resulting in a decrease in yield and fruit quality. The disease has a major economic impact especially in South and East Asia. However, in the last years MAB started to also cause problems in Europe (Wöhner and Emeriewen 2019) and in the USA (Aćimović and Donahue 2018, Khodadadi and Aćimović 2019), especially in organic orchards with low input of pesticides and untreated orchards for juice production (Bohr et al. 2018). Still, knowledge about the biology, epidemiology and disease control of the pathogen mostly comes from Asia and data on how the primary infection develops, from which inoculum it starts and how the epidemic progresses is missing for Europe and the USA. The quantification of spores in the air allows to assess the potential for a primary infection and for subsequent outbreaks of epidemics. Moreover, knowledge on spore dispersal combined with the observation of disease progress is an important aspect for understanding the disease dynamics and for developing disease forecast models. However, to our best knowledge, no report on the detection of *M. coronaria* spores in the air exists for Europe and data on spore dispersal and its combination with disease development is missing.

## Material and Methods

MAB disease development was monitored in an extensively managed apple orchard in Switzerland in the years 2017, 2018 and 2019. In 2019, the spore dispersal of *M. coronaria* was investigated by different spore traps from May to July. Beforehand, the optimal spore trap to capture *M. coronaria* spores in the field was evaluated by comparing different spore

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traps under experimental conditions. Moreover, we developed a new quantitative real-time polymerase chain reaction (qPCR) method to quantify *M. coronaria* spores in spore trap samples. We designed new primers as well as a new hydrolysis probe and assessed them for optimal PCR conditions. We further evaluated different protocols for DNA extraction from spore trap samples. To quantify spore numbers in spore trap samples an in vivo standard curve was generated that allowed the conversion of $C_q$ values into spore numbers.

## Results and Discussion

Our assessment of different volumetric impaction spore traps under experimental conditions revealed the Mycotrap and the rotating-arm spore trap to be the most suitable traps to monitor temporal and spatial spore dispersal of *M. coronaria*, respectively. Furthermore, the newly developed qPCR method proved to be highly specific and also sensitive enough to detect even less than ten *M. coronaria* conidia per spore trap sample. The method can be used with a hydrolysis probe or a DNA intercalating dye like SYBR green. With these new tools, we monitored the spore dispersal as well as disease progress within an extensively managed apple orchard. Our results suggest that *M. coronaria* spore dispersal starts before May and that primary infections occur already in late April or at the very beginning of May. Secondary infections may then start at latest beginning of June. Higher numbers of spores were found within and close to susceptible cultivars compared to a not susceptible cultivar. Our data further suggest that weather conditions are more important for infection than the number of spores in the air. Knowledge on the spore dispersal and therefore the amount of inoculum present in the air combined with the observed disease progress is an important aspect for understanding the disease dynamics and can be used to improve disease forecast models. The here developed qPCR method and the optimization of spore traps further represent valuable tools for future more in-depth investigations of the *M. coronaria* spore dispersal and thus help to improve our understanding of this emerging disease.

## Acknowledgements

We thank the Interreg V program project number ABH003 for financing this study.

## References

Aćimović, S. G., and D. Donahue. (2018). Marssonina leaf blotch of apple. Pages 1-4 Tree Fruit News.

Bohr, A., S. Buchleither, M. Hechinger, and U. Mayr. (2018). Symptom occurence and disease management of Marssonina blotch. 18th International Conference on Organic Fruit-Growing 19-21 February 2018, Hohenheim, Germany.

Khodadadi, F., and S. G. Aćimović. (2019). Marssonina blotch of apple caused by *Marssonina coronaria* in New York. Plant Health 2019 - American Phytopathological Society Annual Meeting, Cleveland, OH, USA.

Wöhner, T., and O. F. Emeriewen. (2019). Apple blotch disease (*Marssonina coronaria* (Ellis & Davis) Davis) - Review and research prospects. *European Journal of Plant Pathology* **153**:657-669.

## Citation of the full publication

The citation of the full publication will be found on Ecofruit website as soon as available.