

Tracking host infection and reproduction of *Peronospora salviae-officinalis* using an improved method for confocal laser scanning microscopy

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Abstract

Peronospora salviae-officinalis, the causal agent of downy mildew on common sage, is an obligate biotrophic pathogen. It grows in the intercellular spaces of the leaf tissue of sage and forms intracellular haustoria to interface with host cells. Although *P. salviae-officinalis* was described as a species of its own 10 years ago, the infection process remains obscure. To address this, a histological study of various infection events, from the adhesion of conidia on the leaf surface to de novo sporulation is presented here. As histological studies of oomycetes are challenging due to the lack of chitin in their cell wall, we also present an improved method for staining downy mildews for confocal laser scanning microscopy as well as evaluating the potential of autofluorescence of fixed nonstained samples. For staining, a 1:1 mixture of aniline blue and trypan blue was found most suitable and was used for staining of oomycete and plant structures, allowing discrimination between them as well as the visualization of plant immune responses. The method was also used to examine samples of *Peronospora lamii* on *Lamium purpureum* and *Peronospora belbahrii* on *Ocimum basilicum*, demonstrating the potential of the presented histological method for studying the infection processes of downy mildews in general.

KEYWORDS

aniline blue, confocal laser scanning microscopy, *Peronospora belbahrii*, *Peronospora lamii*, *Peronospora salviae-officinalis*, trypan blue

1 | INTRODUCTION

The downy mildew *Peronospora salviae-officinalis* causes a severe disease on the medicinal plant *Salvia officinalis* (common sage, Lamiaceae). Since its first report as a *Peronospora* sp. on *S. officinalis* in 1993 (McMillan, 1993), the disease has been reported from all over the world (Gamliel and Yarden, 1998; Hill *et al.*, 2004; Belbahri *et al.*, 2005; Liberato *et al.*, 2006; Humphreys-Jones *et al.*, 2008). It is of increasing economical concern and can be regarded as a newly

emerging disease. Although *P. salviae-officinalis* was described 10 years ago (Choi *et al.*, 2009) the infection process has never been studied. Knowledge about the epidemiology and biology of this pathogen is, however, crucial for the development of forecast models, and appropriate combat strategies against it in sage production.

Downy mildews of the genus *Peronospora* are obligate biotrophs, which means that they are dependent on living host cells for nutrition (Thines and Choi, 2016). They belong to the Peronosporaceae within the phylum Oomycota of the kingdom Straminipila (Beakes and

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