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Differences in the gene transcription state of *Botrytis cinerea* between necrotic and symptomless infections of lettuce and *Arabidopsis thaliana*

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Botrytis cinerea can establish long-lived, symptomless, systemic infections in plant species. It is unclear how the fungus colonizes plant tissues without causing tissue damage and necrosis. Three hypotheses are: (i) the fungus state is similar in the two forms of infection, but the plant defences are more effective, leading to multiple small quiescent centres; (ii) excreted molecules that would trigger plant defences are suppressed; (iii) signal exchanges occur avoiding both extensive host cell death and complete spatial restriction of the pathogen. These hypotheses were tested by comparing transcript levels of a set of *B. cinerea* genes between symptomless and necrotizing infections. Four genes were analysed that participate in signalling pathways required for virulence, as well as five genes that directly participate in causing host cell death or degrading plant cell wall polysaccharides. In lettuce, necrotic infections on detached leaves (12–48 h after inoculation) had similar gene expression patterns to necrotic infections on leaves 44 days after inoculation of the seedlings. Symptomless infections on leaves that expanded after inoculation of young seedlings had similar fungal gene expression patterns at 14, 24 and 34 days after inoculation, which clearly differed from those in necrotizing infections. In *Arabidopsis thaliana*, there were differences in gene expression patterns between droplet inoculations on leaves, resulting in necrotic lesions, and symptomless infections in stems and leaves. The fungal gene expression patterns differed in detail between lettuce and *A. thaliana*. The observations suggest that the physiological state of *B. cinerea* during symptomless infection is distinct from necrotizing infections.

Keywords: botrydial, endophyte, *Lactuca sativa*, latent, systemic, transcription

Introduction

Botrytis cinerea is a plant pathogenic fungus causing grey mould disease and post-harvest losses in more than 1000 crops, ranging from ornamentals to vegetables and field crops (Elad *et al.*, 2016). Symptoms produced by *B. cinerea* range from restricted lesions to dry or spreading soft rots that often produce conspicuous sporulating colonies (Williamson *et al.*, 2007). In general, *B. cinerea* is considered to be a necrotroph, which draws nourishment from dead host tissue and produces initially local ('primary') necrotic lesions, which subsequently expand to actively cause plant tissue decomposition (Horst, 1983; Jarvis, 1994; Coertze & Holz, 2002; Elad *et al.*, 2004). In contrast, recent studies have revealed that *B. cinerea* can also cause symptomless systemic infection in several host plants including *Primula* spp., lettuce (*Lactuca sativa*), *Arabidopsis thaliana* and *Taraxacum vulgare* (Barnes & Shaw, 2003; Rajaguru & Shaw, 2010; Sowley *et al.*, 2010; Shaw *et al.*, 2016). In this type of infection the fungus grows along with the plant and enters newly expanding organs, without producing symptoms, until the plant becomes physiologically susceptible, typically at flowering. At this point extensive areas of host tissue death develop simultaneously, followed by sporulation of the fungus. Several species in the genus *Botrytis* are able to infect in this way (Shaw *et al.*, 2016). The physiological relationship between host and pathogen during symptomless systemic growth is unresolved. It is unclear how a fungus that can produce such a large arsenal of phytotoxic metabolites and proteins (van Kan, 2006) is able to grow inside plant tissue without causing extensive tissue damage and visual disease symptoms. The aim of the present study was to obtain preliminary insight into this question by comparing the expression of a set of fungal genes that participate in regulating virulence or in causing host cell death, between symptomless and necrotizing infections, in two host species.

A previous study illustrated that in some species a high proportion of symptomless plants can be infected with *B. cinerea*. The distribution of the fungus appeared to be discontinuous and scattered over distinct tissues

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