

# Septoria Epidemics on Wheat: Combined Use of Visual Assessment and PCR-Based Diagnostics to Identify Mechanisms of Disease Escape

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## Abstract

The effect of crop height on the epidemics of *Septoria tritici* and *Stagonospora nodorum* was investigated using visual assessment and PCR-based assays. Based on the results of our study, the primary mechanism of disease escape in tall crops is through a reduction of spore arrival. Real-time PCR is an important tool to quantify spore arrival and can, in combination with visual assessment, identify factors involved in the onset and extent of disease development.

**Keywords:** Septoria diseases; spore arrival; diseases escape and real-time PCR

## INTRODUCTION

Leaf blotch caused by *Septoria tritici* is the most serious foliar fungal disease in NW Europe. Control measures are primarily focused on fungicide sprays and deployment of resistant germplasm. Previous studies identified a relationship between crop architecture and disease levels in field crops (LOVELL *et al.* 1997). The aim of this study was to establish how upper canopy leaves of tall crops escape disease. For this purpose Septoria epidemics in a non-dwarf (*rht*) and a dwarf (*Rht10*) near isogenic line of cultivar Mercia (Figure 1) were followed by both visual assessment and PCR-based methods.

## MATERIAL AND METHODS

**Field trial and visual assessment.** During 1999–2000, *rht* and *Rht10* isogenic lines of cultivar Mercia were grown in replicated field plots (12 × 8 m) at Long Ashton, SW England. After flag leaf emergence, disease severity was recorded weekly on ten main tillers per plot using a leaf grid aid as described by PARKER *et al.* (1995).



Figure 1. Dwarf (*Rht10*) and non-dwarf (*rht*) near isogenic lines of cultivar Mercia

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Table 1. Primer sequences

Primers	Sequence (5'-3')	Target	GenBank Accession number
TAATF2	tgctcgtattatagctcttggtga	$\alpha$ -subunit ATP synthase of <i>T. aestivum</i>	M16842
TAATR1	actcacgggtatctgagggtattct	chloroplast ATP synthase of <i>T. aestivum</i>	
SNF3	acaccaggaacaactgtaacagc	$\beta$ -tubulin of <i>S. nodorum</i>	S56922
SNR	tatgcgcgctgctgcaaattcga	$\beta$ -tubulin of <i>S. nodorum</i>	
STCYTF2	ctgatgatggcaaccgattctta	cytochrome <i>b</i> of <i>S. tritici</i>	AY247413
STCYTR4	gctgtatcgtgtaaagctattaga	cytochrome <i>b</i> of <i>S. tritici</i>	

**DNA extraction.** Total DNA was extracted from ten leaves per plot, sampled twice weekly, and 100 ng of DNA tested in PCR. DNA extraction and quantification were done according to FRAAIJE *et al.* (1999).

**Multiplex PCR.** Using multiplex PCR, samples were checked for PCR inhibition (+/- amplification of *Triticum aestivum* DNA) and tested for presence of *S. tritici* and *Stagonospora nodorum*. Using a PCR protocol based on Red Hot DNA polymerase (ABgene, Epsom, UK) as described by FRAAIJE *et al.* (1999), PCR products of 187 bp (*T. aestivum*), 248 bp (*S. tritici*) and 276 bp (*S. nodorum*) were generated with species-specific primers (Table 1). For testing wheat leaf samples, 100 ng of template DNA and 0.3  $\mu$ M of each primer per reaction of 30  $\mu$ l were used. The

PCR conditions were 94°C for 2.5 min, followed by 50 cycles at 94°C for 30 s, 58°C for 45 s and 72°C for 1.0 min. The PCR was terminated with a DNA extension at 72°C for 9.0 min. PCR products were separated in ethidium bromide-stained 1.3% (w/v) agarose gel run in 1X Tris-Borate-EDTA buffer and exposed to UV light to visualise DNA fragments.

**Quantitative real-time PCR.** To quantify infection levels of *S. tritici*, a real-time PCR assay based on cleavage of fluorogenic TaqMan probes (LEE *et al.* 1993) was developed. The probe and primers (Table 2) were designed with Primer Express software (PE Applied Biosystems). An ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems) was used for amplification and fluorescence measurement. Cy-

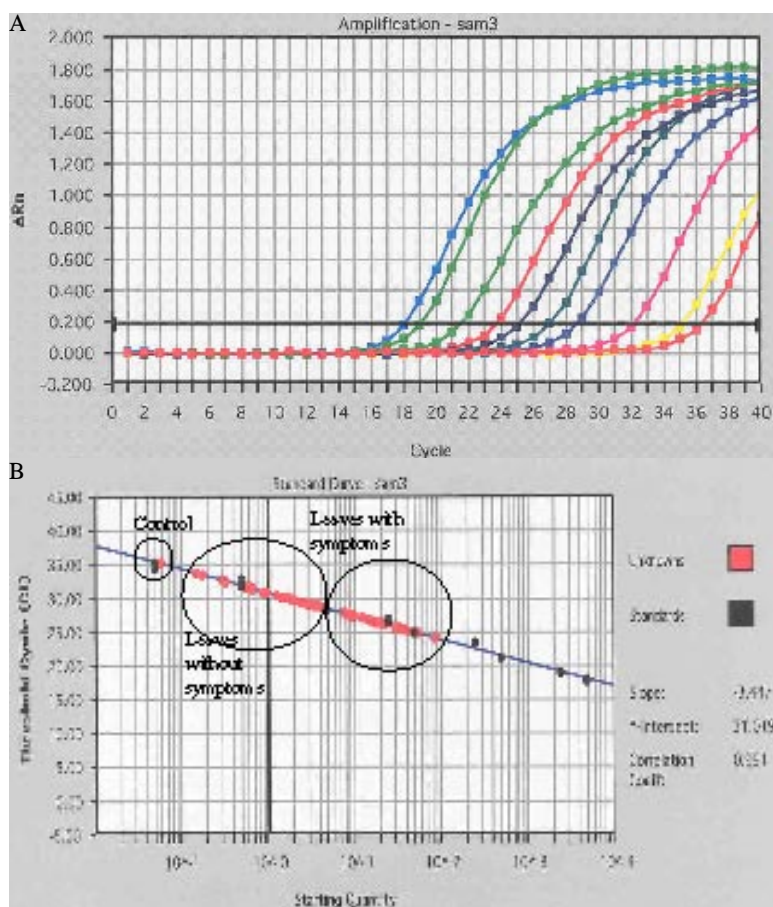


Figure 2. Real-time PCR assays. A) Fluorescence signals of spiked wheat samples. From right to left increasing amounts of *S. tritici* DNA. B) Relationship between *S. tritici* infection levels and PCR threshold cycle number (Ct). *S. tritici* infection levels expressed in pg pathogen DNA per 100 ng of wheat leaf DNA tested

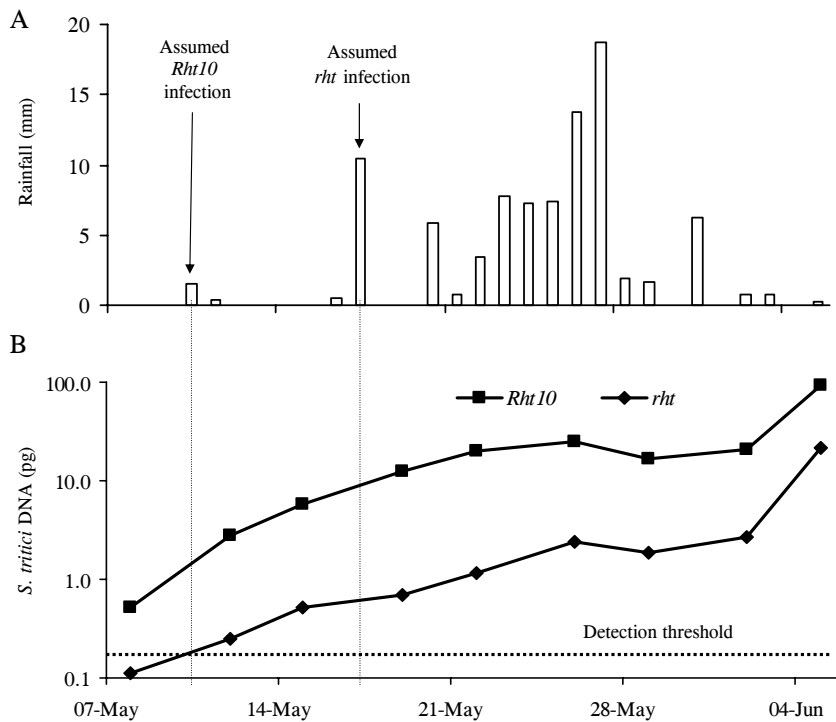


Figure 3. The relationship between rainfall (A) events and spore arrival determined by quantitative real-time PCR (B) measurements. Results are expressed as the amount of *S. tritici* DNA (in pg) per 100 ng wheat leaf DNA (mean value of three plots)

Table 2. Oligonucleotides used in real-time PCR assay

Oligonucleotide designation	Sequence (5'-3')
Primer STTAQF	acattaacatgaacaatcggtactataatactag
Primer STTAQR	tgttgctctcataaagacatttga
Probe STTAQDET	FAM-acataccctaagaatgcggttgccatca-TAMRA*

\*FAM: 6-carboxy- uorescein ( uorescent reporter dye); TAMRA: 6-carboxy-N, N, N', N'-tetramethyl-rhodamine (quencher)

clinging conditions were 94°C for 10 min, followed by 40 two-step cycles of 10 s at 95°C and 1 min at 60°C. Assays were performed with TaqMan PCR Master Mix (Applied Biosystems) supplemented with 0.9 μM forward primer, 0.3 μM reverse primer and 0.1 μM TaqMan probe. Using fluorescence measurements, PCR products (105 bp of cytochrome *b* gene) could be quantified individually, by reference to a standard curve generated by adding known amounts of target DNA using Sequence Detection System Software 1.6 (Figure 2). After extensive evaluation of the TaqMan assay, it appeared that *Puccinia* spp. could produce a weak false positive signal. However, no yellow or brown rust was detected during this study with visual assessment or PCR using specific primers described by FRAAIJE *et al.* (2001).

## RESULTS

Predictions of assumed infection dates were made through analysis of rainfall data and splash intensity.

Based on vertical distance measures between sporulating lesions and emerging flag leaves, primary infections for the *rht* and *Rht10* lines were estimated as 10<sup>th</sup> and 17<sup>th</sup> May, respectively (Figure 3A). In both lines, visual symptoms were detected three weeks after the assumed infection events (Figure 4), corresponding to the latency period of *S. tritici*. Real-time PCR (Figure 3B) showed that both lines became infected

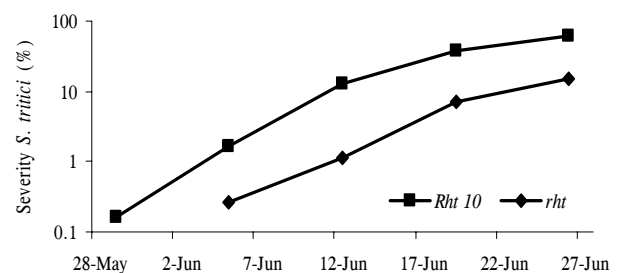


Figure 4. Flag leaf disease severity scores for *rht* and *Rht10* lines of cultivar Mercia. Values represent the mean of three plots

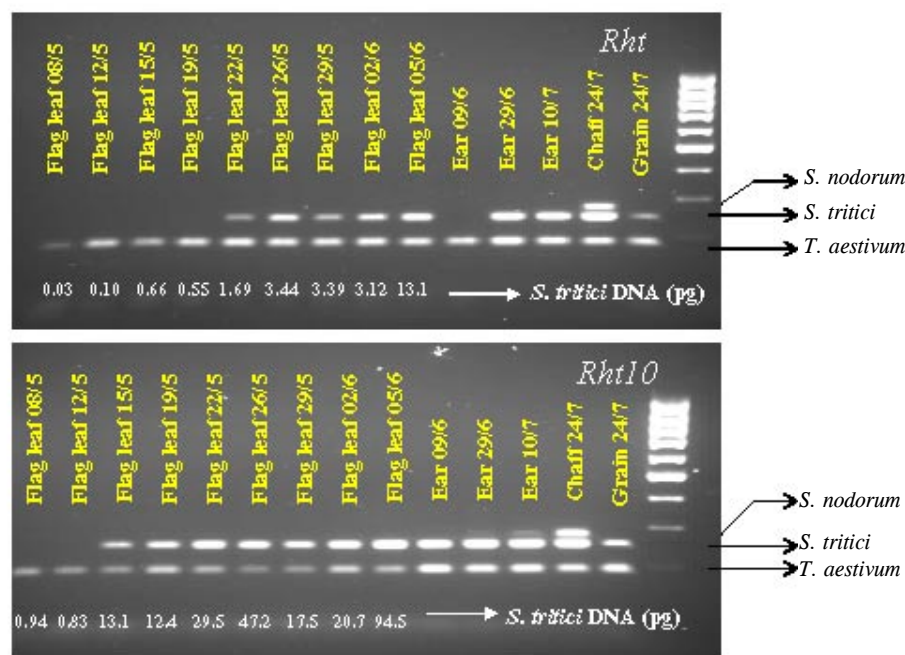


Figure 5. Multiplex PCR detection of *S. tritici* and *S. nodorum* in wheat leaves and ears of *rht* and *Rht10* lines. DNA levels of *S. tritici* were quantified by real-time PCR. No pathogens were detected in sterilised grains (results not shown)

with low levels of spores, unable to cause significant disease, before the assumed primary infection events. For *Rht10*, spores were probably transferred through contact with infected overlapping lower leaves, while for *rht* some spores arrived after rainfall on 10<sup>th</sup> May. The trend of increased fungal DNA detected over time and differences between the lines correspond well to the disease progress recorded by visual assessment. Differences in crop height profoundly affected spore arrival, but had no effect on relative colonisation and subsequent disease severity. The high level of spore arrival in the *Rht10* line after the first rainfall event was equivalent to leaves showing few lesions (see Figure 2), indicating levels > 1000 spores per leaf. Real-time PCR results were confirmed by multiplex PCR (Figure 5). No inhibition was detected. In comparison with the *Rht10* line, epidemics of both *S. tritici* and *S. nodorum* were delayed in the ears of the *rht* line. High infection levels of both pathogens were detected in the chaff.

## DISCUSSION

Crop height encoded by *rht* and *Rht10* genes is a major trait conferring disease escape and, depending on environmental interactions, can play a major role in expression of field resistance towards leaf blotch caused by *S. tritici* and *S. nodorum*. Based on the results of our study, the primary mechanism of disease escape in tall crops is through a reduction of spore arrival. Real-time PCR is an important tool to quantify spore arrival and can, in combination with visual assessment, identify factors involved in

the onset and extent of disease development. Pre-symptomatic PCR detection can further be used as an assessment of likely future disease and therefore rationalise fungicide inputs.

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