

Following the epidemics of *Rhynchosporium secalis* in susceptible and resistant barley cultivars using quantitative real-time PCR assays



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Introduction

Leaf blotch caused by *Rhynchosporium secalis* is the most important foliar disease of barley in the UK. To control epidemics, resistant germplasm and fungicides are used. The aim of this study was to develop a greater understanding of the spatial and temporal distribution of *R. secalis* field populations in resistant and susceptible cultivars. To monitor epidemics, pathogen infection levels were determined by quantitative real-time PCR and results compared with visual assessment.

Methods

From ten barley cultivars with different resistance ratings, the top three leaf layers were sampled and visually assessed for symptoms in Cambridgeshire at GS 72-75 in 2002. Small plots of cultivars Vertige and Leonie were grown in Hertfordshire and every leaf layer sampled during the growing season in 2000/2001. For each leaf layer sample, DNA was extracted from ten leaves. To check simultaneously for inhibition and for presence of *R. secalis*, samples were tested in a multiplex PCR. The PCR product sizes were 420 bp (chloroplast ATPase subunit A of *Hordeum vulgare*) and 350 bp (cytochrome *b* gene of *R. secalis*). To quantify infection levels, a real-time PCR assay was developed using a FAM-labelled TaqMan probe. PCR-amplified products (77 bp fragment of a β -tubulin intron) could be quantified individually, by reference to a standard curve generated by using known amounts of target DNA (Figure 1).

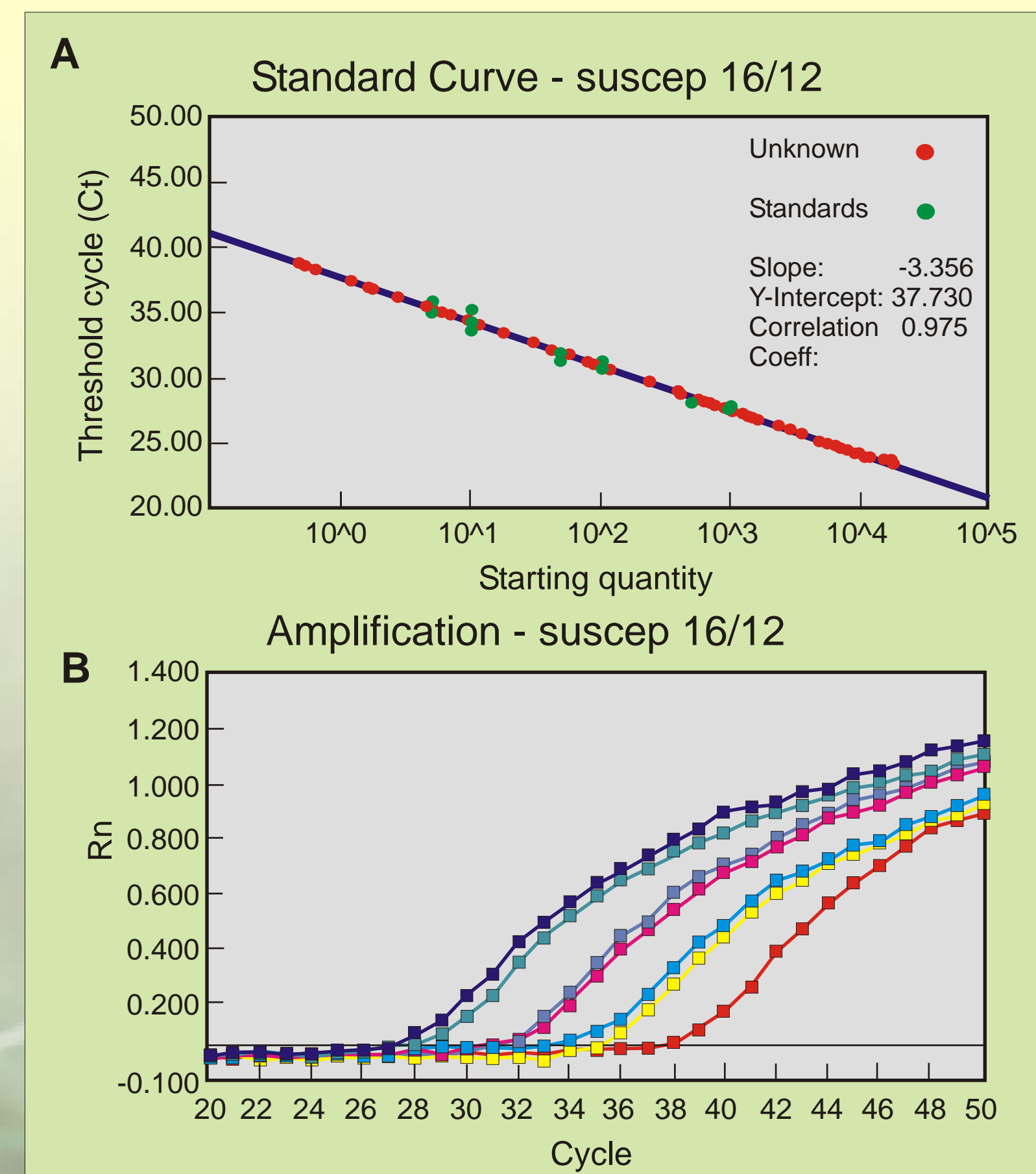


Figure 1. (A) Real-time PCR calibration curve for quantification of infection levels of *R. secalis* in barley leaves. Calibration samples were obtained by spiking barley DNA (100 ng) with different amounts (in pg) of pathogen DNA. (B), (FAM) fluorescence signals from calibration samples, (amount of *R. secalis* DNA decreasing from left to right).

Results & Discussion

The detection threshold of the real-time PCR assay was 3 pg of *R. secalis* DNA in the presence of 100 ng barley DNA. For the ten barley cultivars tested, infection levels determined by real-time PCR correlated well with those of visual assessments (Table 1). However, based on the resistance ratings, some resistant cultivars (e.g. Jewel and Pearl) showed high disease levels, while low infection levels were detected in the susceptible cv. Heligan. This might be due to breakdown of race-specific resistance genes and the uneven geographic distribution of particular *R. secalis* pathotypes in the UK.

For the susceptible cv. Vertige (5) and resistant cv. Leonie (8), the temporal distribution of *R. secalis* in the top three leaves was monitored. Although, detection of *R. secalis* using multiplex PCR showed similar results for both cultivars (Figure 2), infection levels determined by real-time PCR were on average ten times lower for the resistant cv. Leonie (Figure 3). Since the plants were asymptomatic, the presence of relative high levels of *R. secalis* in subsequent leaf layers of cv. Leonie indicated that extensive spore arrival, colonization and/or subsequent dispersal had occurred without (visual) infection.

Cultivar/sample	resistance rating 1	Replicate 1		Replicate 2	
		Visual assessments 2	Real-time PCR 3	Visual assessments	Real-time PCR
Sumo flag	5	0.2	41	0.5	62
Sumo leaf 2		5.8	1325	2.2	212
Sumo leaf 3		2.3	609	2.4	1072
Heligan flag	6	0.5	14	0.1	< 3
Heligan leaf 2		0.0	8	0.0	8
Heligan leaf 3		Trace	29	0.1	29
Regina flag	7	0.0	8	0.3	176
Regina leaf 2		1.0	52	1.0	75
Regina leaf 3		1.0	290	1.3	112
Siberia flag	7	0.5	< 3	0.2	8
Siberia leaf 2		0.0	5	0.1	< 3
Siberia leaf 3		0.0	< 3	0.1	< 3
Pearl flag	8	0.50	48	0.2	56
Pearl leaf 2		0.8	114	0.6	186
Pearl leaf 3		2.2	453	0.7	717
Vanessa flag	8	0.0	4	0.0	< 3
Vanessa leaf 2		0.0	< 3	0.0	< 3
Vanessa leaf 3		0.0	245	0.2	46
Angela flag	8	0.2	64	0.3	107
Angela leaf 2		0.2	11	0.2	14
Angela leaf 3		0.1	4	0.1	8
Jewel flag	8	6.5	1243	0.2	123
Jewel leaf 2		3.5	978	0.8	202
Jewel leaf 3		1.5	413	0.4	723
Antonia flag	8	0.1	33	0.1	< 3
Antonia leaf 2		0.4	163	0.3	107
Antonia leaf 3		0.2	58	0.3	124
Leonie flag	9	0.2	< 3	0.0	< 3
Leonie leaf 2		0.0	< 3	0.0	< 3
Leonie leaf 3		0.2	< 3	0.1	< 3

1, Resistance ratings: 5, susceptible; 9, resistant.
2, Scores are expressed as percentage infected leaf area.
3, Real-time PCR results are shown as amount of pathogen DNA (pg) per 100 ng of barley DNA.

Table 1. Real-time PCR and visual assessment scores for ten different cultivars of barley.

Vertige

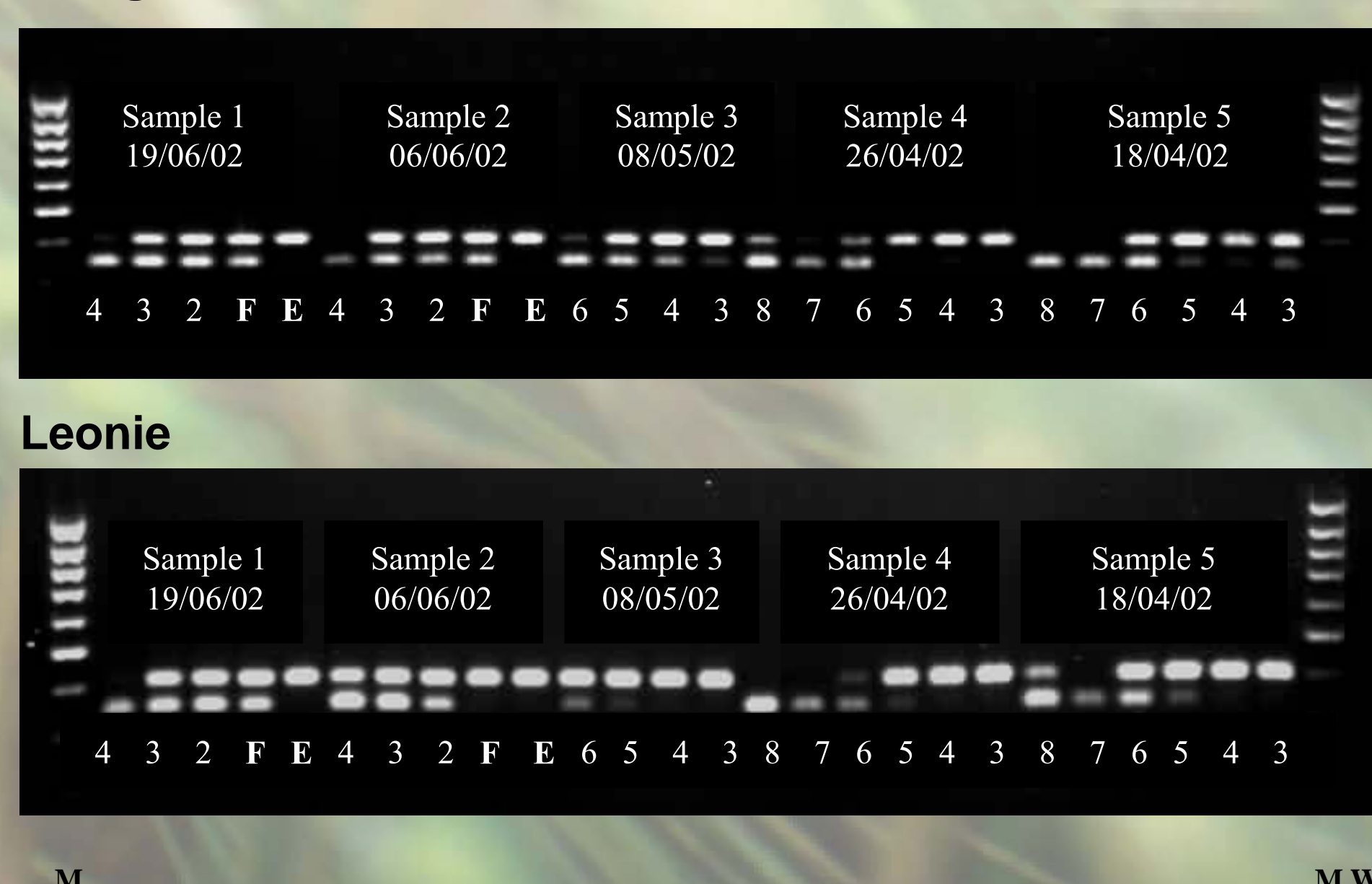


Figure 2. PCR detection of barley and *Rhynchosporium secalis* in leaves and ears of cultivars Vertige and Leonie

The DNA samples are shown as (E) ears, (F) flag and subsequent leaf layers (2,3 etc.); M, 100bp marker, W, water blank; higher band barley (420bp), lower *R. secalis* (350 bp)

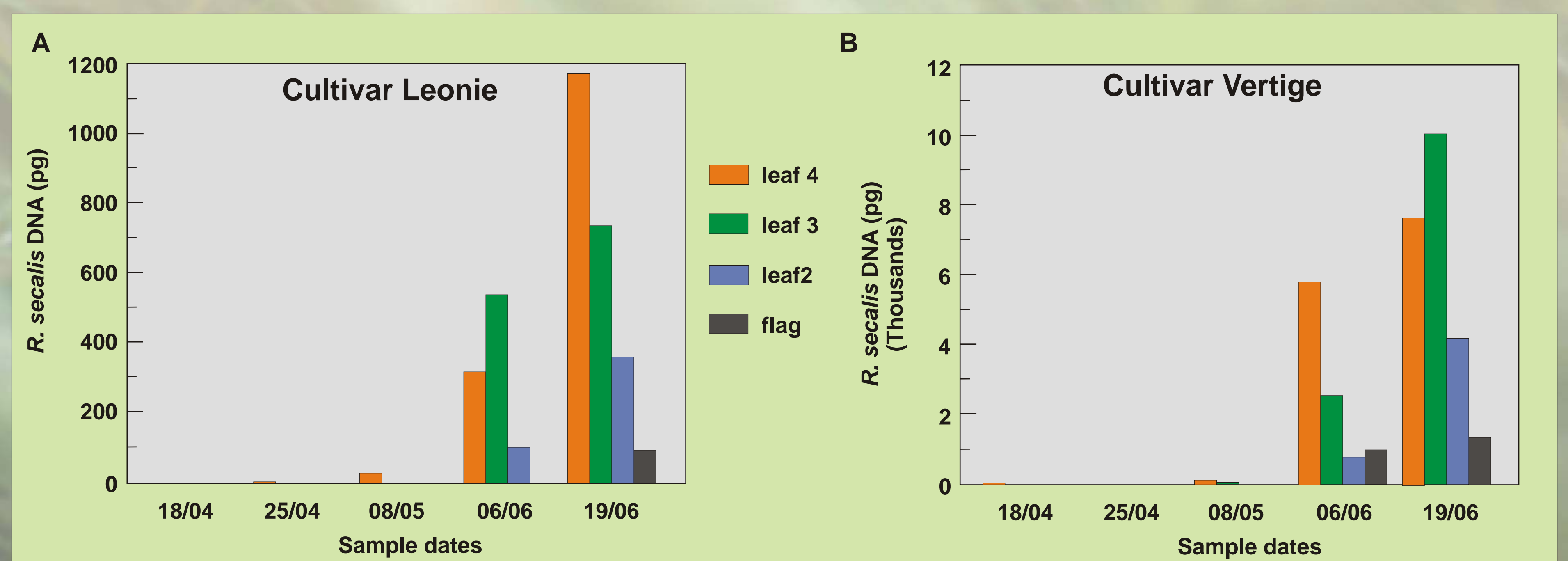


Figure 3. *Rhynchosporium secalis* DNA infection levels for barley cv. Vertige (A) and Leonie (B) measured using real-time PCR. The results are expressed as the amount of *R. secalis* DNA (in pg) per 100 ng of barley DNA.

Conclusions

Real-time PCR is an important tool to quantify infection levels, and can, in combination with visual assessment, increase our understanding of how epidemics evolve under different genetic and environmental conditions. More knowledge on the role of primary inoculum (e.g. seedborne infection and (a)sexual reproduction) and plant-pathogen interactions is needed in order to maximise the epidemic-intervening effects of resistance genes and/or fungicides.

Acknowledgements

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