



**Improved pathogen management
in crops using rapid in-field
diagnostics**

Catherine Harrison



Septoria the disease

- *Mycosphaerella graminicola* (*Septoria tritici*, leaf blotch) is the most common foliar disease of wheat in Europe.
- Most fungicides used on wheat are targeted against its control.



Economic losses due to leaf blotch

- Up to 16% of the global harvest is lost to plant diseases.
- In the UK, foliar diseases of wheat account for losses of up to 12%.
- *Septoria tritici* residual yield losses £43-53 million p.a. UK
- Estimated than £82million p.a. UK is targeted against its control.

Azole resistance

- Azoles are the primary control method in the UK.
- CYP51 gene is important in the biosynthesis of a fungal cell membrane component.
- 33 known mutations on the CYP51 gene.



Aim of project

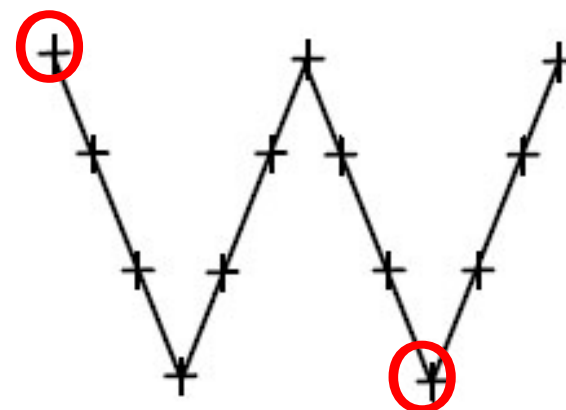
- To develop a rapid, in-field test to detect fungicide resistant genotypes.
- To enhance decision making, achieving effective disease control and more responsible use of chemicals in crops.
- The tests will be deployed on a hand held platform with automated result calling.

Septoria population evaluation

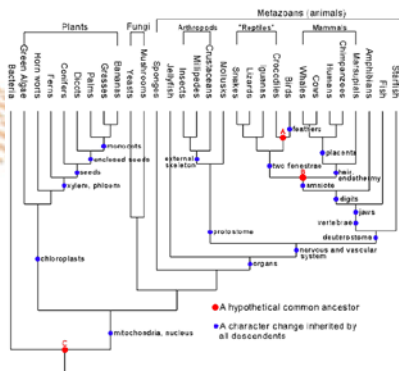
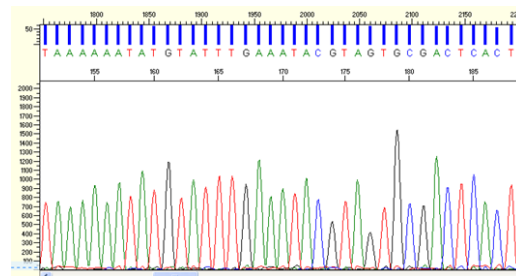
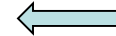
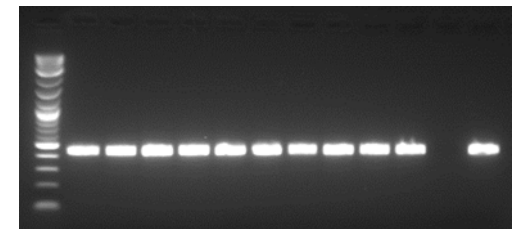
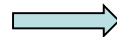
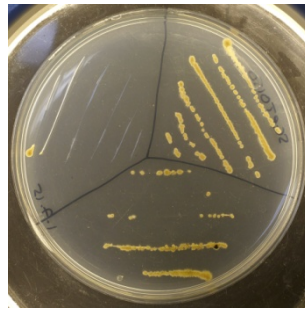
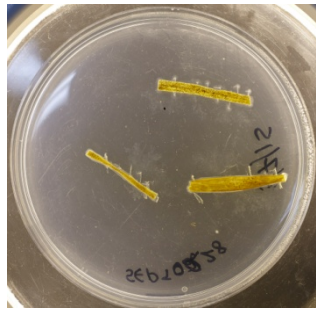
- Evaluate the population structure of pathogens to enable effective sampling in the field.
- How common are the known mutations.
- How variable are populations:
 - From farm to farm
 - From field to field
 - Within a field

Septoria sampling plan

- To establish a sampling strategy
- 4 Farms
- 3 fields per farm
- 20 samples from each field using a W grid
- 2 intensive sampling points
- Total 2160 samples



Septoria sequencing process



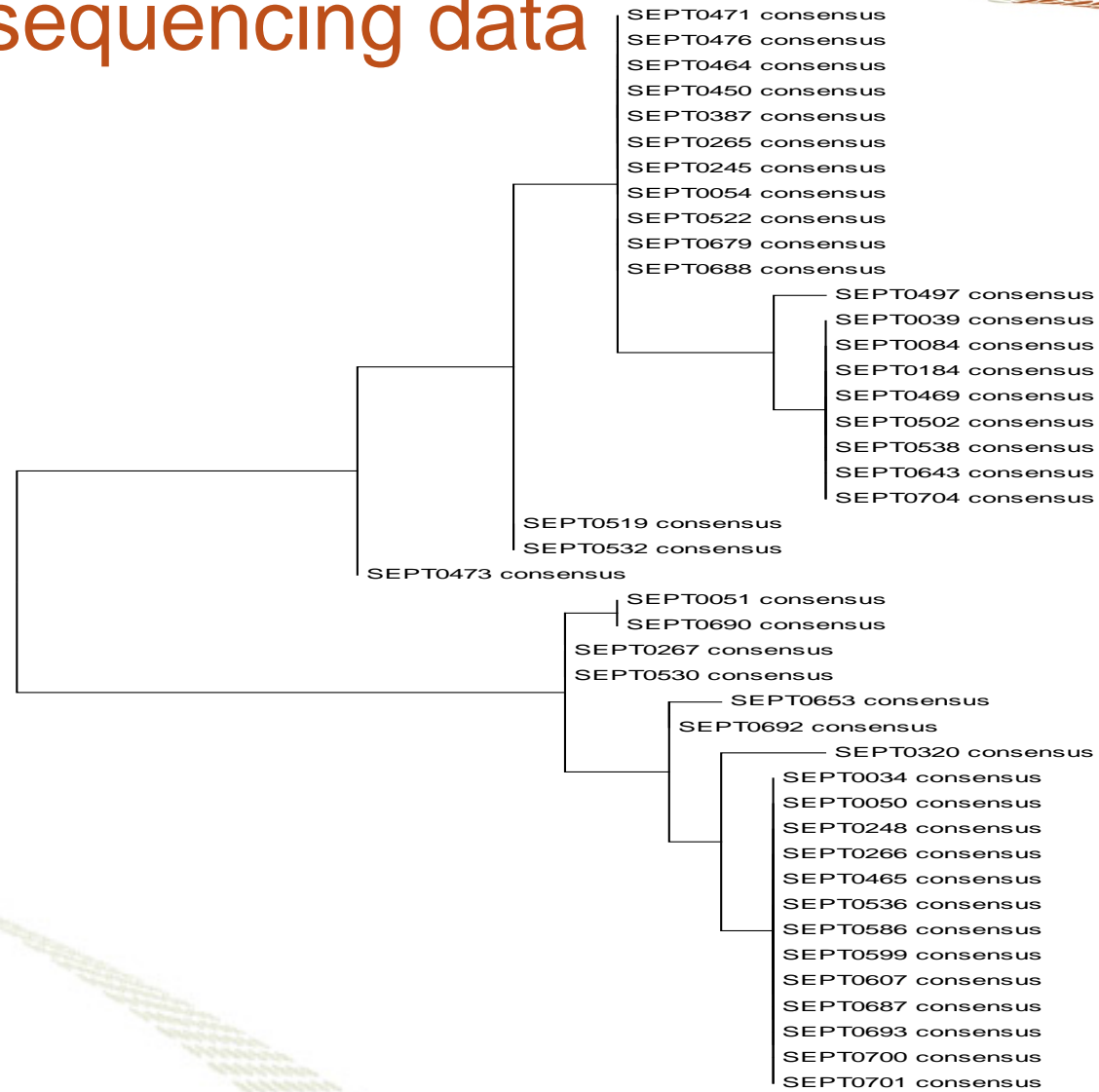
Sequence variation



DNA Sequences		Translated Protein Sequences	
Species/Abbrv	Gr	*****	*****
1. SEPT0320 consensus		CTCCTGTC	TTTGGCAAGGATGTGCTTTATGATTGTCCCAATTCGAAGCTCATGGAGCAGAAGAAGG
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14. SEPT0445 consensus		CTCCTGTC	TTTGGCAAGGATGTGCTTTATGATTGTCCCAATTCGAAGCTCATGGAGCAGAAGAAGG
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Septoria sequencing data




Importance of mutations found

- **D134G** – Causes reduced Azole sensitivity in combination with changes at 459-461
- **V136A** – Causes reduced Azole sensitivity in combination with changes at 459-461
- **A379G** – Found in resistant strains in combination with 381
- **I381V** – Causes reduced Azole sensitivity – in combination with changes at 459-461

Importance of mutations found

- **Y461H** – Found in resistant strains in combination with other changes
- **459-461** - deletion causes azole sensitivity
- **50 and 188** – No effect on azole sensitivity

The assay requirements

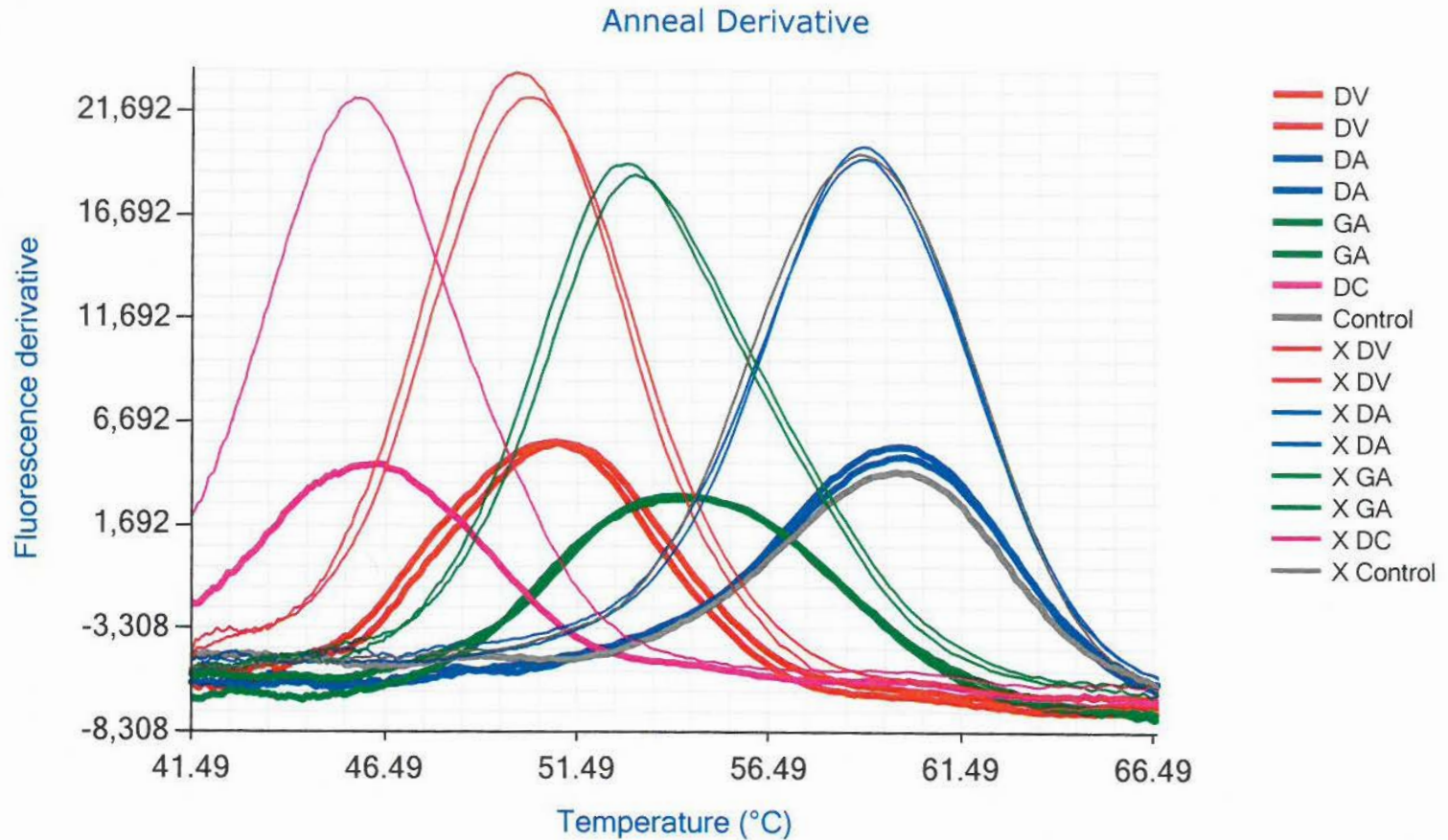
- The assay is a DNA based LAMP assay
 - Must be able to detect SNPs (single nucleotide polymorphisms).
 - Ideally be a multiplex reaction.
 - Must be rapid (less than 20 minutes)
 - Must be able to be performed in the field.
- 
- A decorative graphic in the bottom left corner consisting of a series of parallel lines that fan out from the left edge towards the center, transitioning from orange to light green.



New developments required

- To develop new probe methods to enable SNP detection and multiplexing. (Fera and GeneSys Biotech)
- Improved LAMP chemistry/reagents (GeneSys Biotech).
- To develop improved software for LAMP probe assays (OptiSense).

Initial method comparison



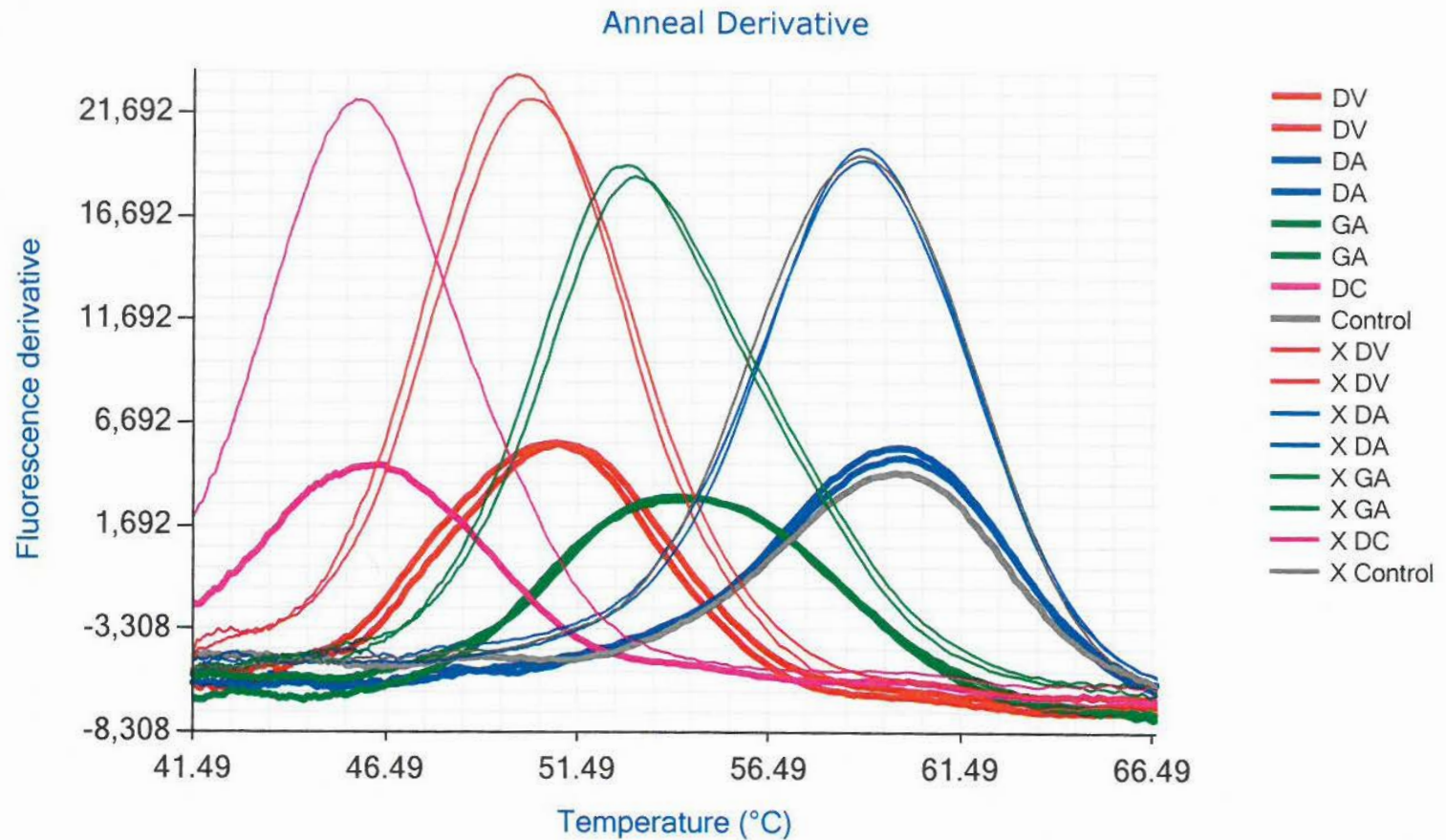
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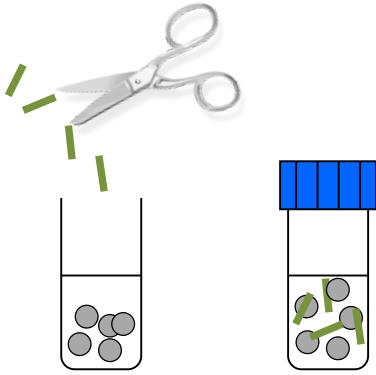
Initial method comparison



LAMP for detection of *Septoria tritici*



1. Preparation of samples



Shake for 1 minute



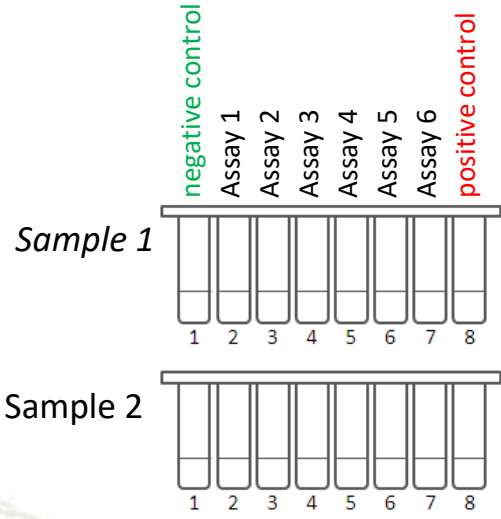
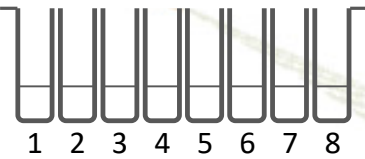
Dilute 1 in 10



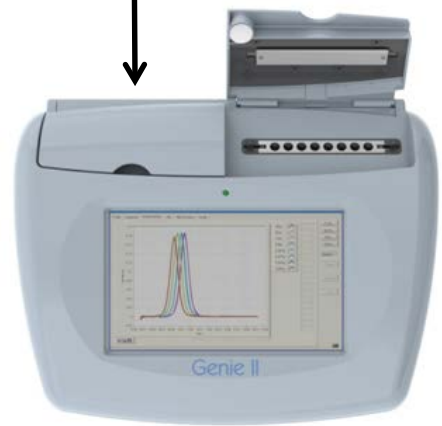
2. Preparation of test strips



Transfer sample into LAMP reactions



Block A: Sample 1 Block B: Sample 2



Acknowledgments

- Project Funded by Innovate UK
- Project partners
 - Agrii
 - Optisense
 - GeneSys Biotech
- Neil Boonham - Fera
- Emily Roberts – PhD student Fera