



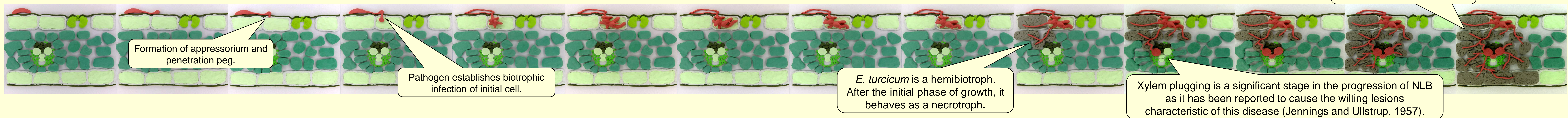
How do disease resistance QTLs affect the development of northern leaf blight in maize?

Ellie Walsh^{1*}, Joy Longfellow^{1*}, Chia-Lin Chung¹, Jesse Poland², and Rebecca J. Nelson^{1,2}

¹Dept. of Plant Pathology and Plant-Microbe Biology, and ²Dept. of Plant Breeding and Genetics, Cornell University, Ithaca NY 14853, USA; *Joint First Authors.

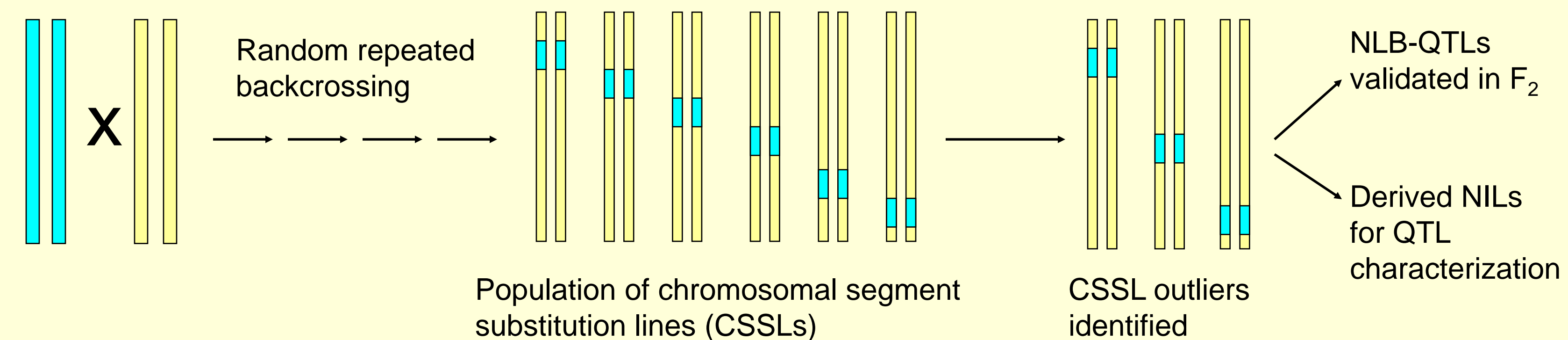
Introduction: With the ability to map disease resistance quantitative trait loci (QTLs) and transfer them to near-isogenic lines (NILs), it is possible to study specific QTLs and their roles in fungal pathogenesis in maize. Here we show our analysis of two QTLs for resistance to northern leaf blight (NLB) – B73 allele at bin 1.02 (*qEt1.02_{B73}*) and Tx303 allele at bin 1.06 (*qEt1.06_{Tx303}*). We applied microscopy to investigate the interaction between the fungal pathogen *Exserohilum turcicum* and the host plant as influenced by these two loci. We hypothesized that these individual QTLs affect distinct stages of disease development (Fig. 1). Pyramiding favorable alleles of QTLs for resistance against fungal penetration and colonization would provide a complex defense for pathogens to overcome.

Fig. 1: Claymation representation of *Exserohilum turcicum* infection of maize leaf in cross-section.



Background: Our general objective is to understand how disease QTLs affect fungal pathogenesis in maize. Studies on host-pathogen interactions in a range of pathosystems have revealed an array of mechanisms by which plants reduce the efficiency of pathogenesis. With the aim of relating specific loci with effects on specific stages of fungal development, we have mapped QTLs for resistance to NLB and transferred them into near-isogenic lines (NILs).

Fig. 2: The development of NILs for the purpose of characterizing disease resistance QTLs.



NLB-QTLs were identified using conventional “macroscopic” disease components, including incubation period (IP), lesion expansion (LE), diseased leaf area (DLA) and area under disease progress curve (AUDPC).

A set of 82 chromosomal segmental substitution lines (CSSLs) derived from a cross of B73 x Tx303 was used in this study. The full set of 82 CSSLs together contains ~ 89% of the Tx303 genome in the B73 background, and each line has ~ 5% of the donor plant (Szalma et al., 2007). Phenotypic screening was conducted in the field in NY and NC from 2006 – 2007. Lines that differed significantly in disease resistance from the B73 recurrent parent were identified, and two QTLs (*qEt1.02_{B73}* and *qEt1.06_{Tx303}*) were validated (Table 1 and Fig. 3; for details, see poster #197 by Chung et al.).

Fig. 3: B73 NILs inoculated with NLB in the field.



From our multiple field and greenhouse trials on diverse maize materials, marked phenotypic variation in terms of the number, size, and type of flecks (initial infection sites) and mature lesions caused by *E. turcicum* has been generally observed. Some NLB-QTLs were found to be more effective for certain disease components, suggesting the existence of QTLs affecting specific pathogenesis stages. To further investigate this question, we hypothesized that the two identified QTLs – *qEt1.02_{B73}* and *qEt1.06_{Tx303}* play distinct roles in pathogenesis of *E. turcicum* on maize.

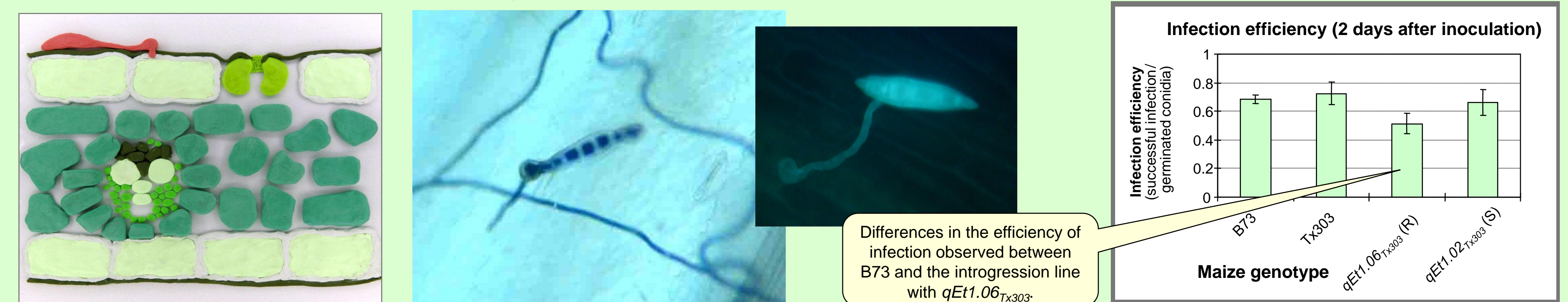
Table 1: Macroscopic parameters used for NIL development and QTL characterization; values represent allele effects (Mean_{Tx303} – Mean_{B73}).

	Identified NLB-QTLs				Resistant parental genotype	
	<i>qEt1.06_{Tx303}</i>		Loss of <i>qEt1.02_{B73}</i>		Tx303	
	GH	Field	GH	Field	GH	Field
IP (days after inoculation)	-0.3	2.1 **	-0.6 ***	-1.6 ***	-0.4	2.4 ***
LE (mm)	0.5	-1.9 *	2.4 *	3.5 ***	5.9 ***	-0.6
DLA (%)	-	-6.4 ***	-	18.9 ***	-	-4.8 ***
	-	-6.8 ***	-	19.6 ***	-	-8.1 ***
	-	-5.3 ***	-	20.1 ***	-	-10.6 ***
AUDPC (area unit)	-	-120.8 ***	-	391.3 ***	-	-157.5 ***
IP:AUDPC		0.16		.004		

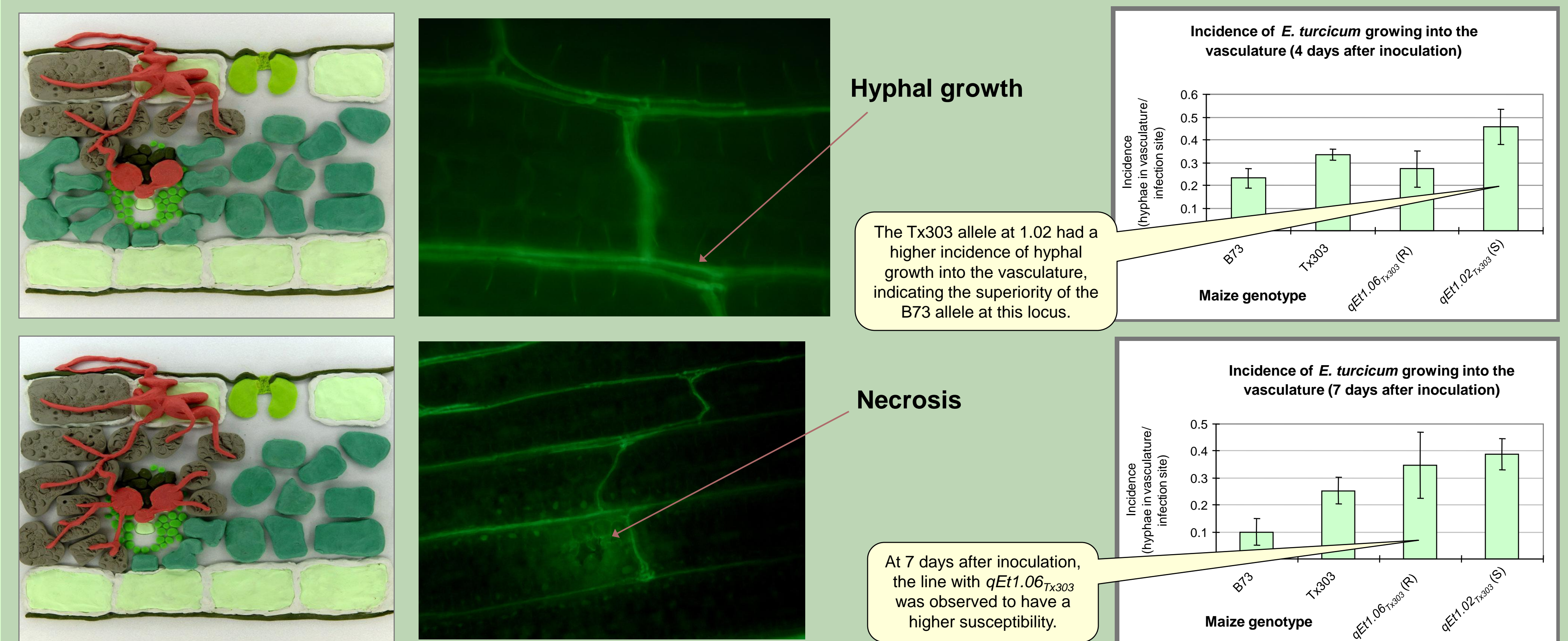
* P < 0.05, ** P < 0.01 and *** P < 0.001.

Fig. 4: Utilizing techniques for microscopic disease components; the two QTL were observed to have significant effects at distinct stages of pathogenesis.

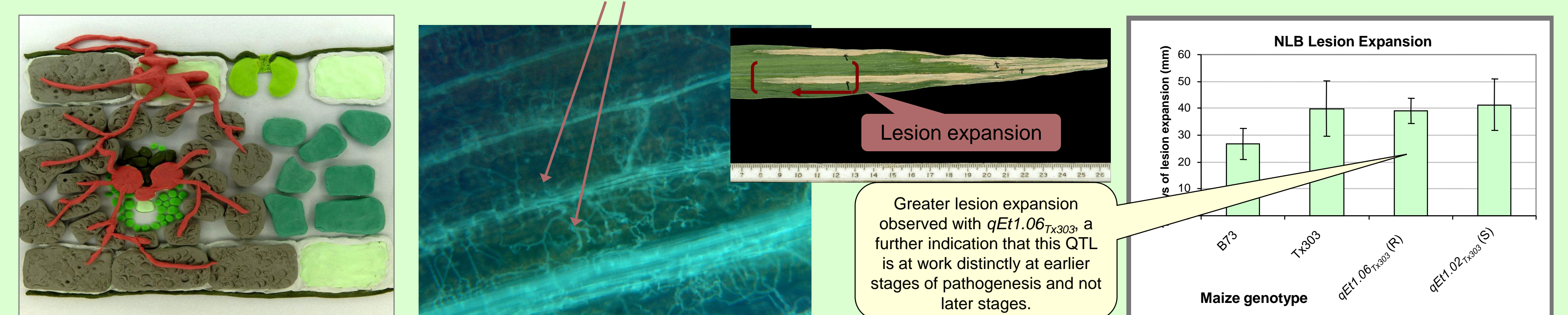
Penetration: Spores with or without germination tube, appressorium and infection peg were observed using trypan blue staining or aniline blue – KOH fluorescence microscopy.



Hyphal invasion into the vasculature: Intracellular hyphal growth from initially infected epidermal cell to surrounding mesophyll cells, and the subsequent invasion into the vascular bundle was investigated by aniline blue – KOH fluorescence microscopy.



Destructive hyphal growth: Intracellular hyphal growth continues, and regions of necrosis expand into lesions.



Conclusions:

- To test the hypothesis of QTL affecting distinct stages of *E. turcicum* pathogenesis, and to complement conventional macroscopic components of disease resistance, we developed a series of microscopic parameters.
- Preliminary evidence revealed the distinct features of two NLB-QTLs:
 - In greenhouse conditions, *qEt1.06_{Tx303}* is effective only against fungal penetration. It is unclear whether this QTL also confers resistance to later stages of pathogen development in the field.
 - The *qEt1.02_{B73}* is effective for inducing necrosis surrounding infection sites, as well as inhibiting hyphal growth into the vascular bundle, and the subsequent necrotrophic colonization in the leaves.
- Delaying the invasion and extension of *E. turcicum* in the vascular system is confirmed to be critical for quantitative resistance to NLB in maize. The complementary effects of these two QTLs suggest that they could be useful targets for marker assisted selection (MAS) in maize improvement.

References:

Jennings, P. R. and Ullstrup, A. J. 1957. A histological study of three Helminthosporium leaf blights of corn. *Phytopathology* 47:707-714.
Szalma et al. 2007. QTL mapping with near-isogenic lines in maize. *Theor. Appl. Genet.* 114(7): 1211-1228.

Acknowledgements:

•The Generation Challenge Program
•The McKnight Foundation
•The Rawlings Cornell Presidential Research Scholars Program