

High Resolution Imaging of
in situ Root Hair
Development to Assess
Oilseed Species Responses
to Water Stress

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Background

- **Root hairs** – extensions of trichoblasts, specific root epidermal cells, that enable plants to more effectively extract soil moisture and nutrients by increasing the effective surface area of roots.

Background

- Problems with current/previous techniques

- solution culture

(Ma et al., 2001a; Gahoonia and Nielsen, 2003)

- doesn't reflect soil conditions
- quantification difficult w/microscopy
- doesn't allow for repeated measure



http://roots.psu.edu/lynch_lab



Background

- Problems with current/previous techniques
 - excavation
 - damages root hairs
 - quantification difficult w/microscopy
 - doesn't allow for repeated measure

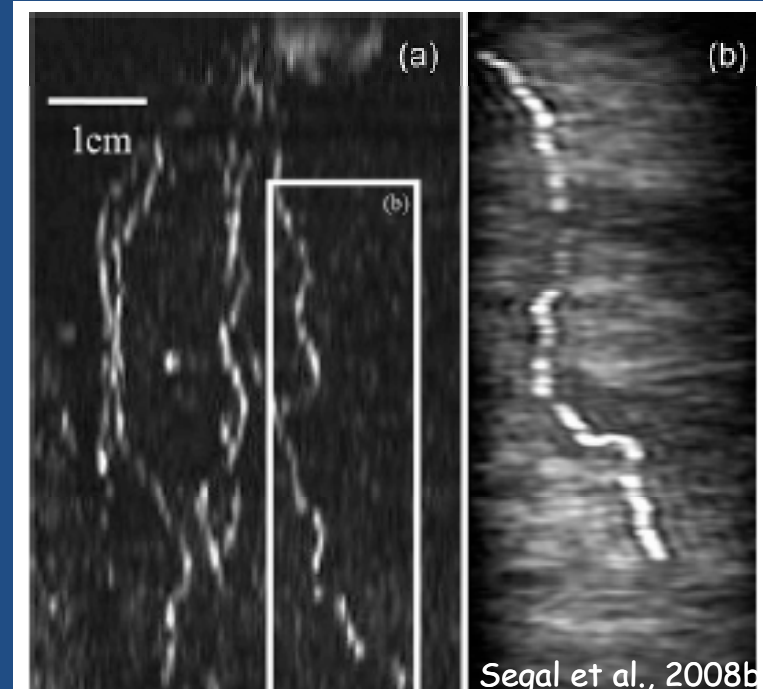


Background

- Problems with current/previous techniques
 - minirhizotrons/MRI
 - may be expensive
 - may not resolve root hairs

(Pan, et al. 1998; Segal et al., 2008b)

\$\$\$, £££ , €€€ ,
¥¥¥ , RpRpRp



Background

- Benefits of high resolution *in situ* root hair imaging

Detect Variation

- species
- environment
- nutrient

Improved
Surface Area
Estimation

Improved
Nutrient Uptake
Modeling

Background

- Benefits of high resolution *in situ* root hair imaging

Better decisions!!



History of Electronic Root Imaging

- 1980 analog video camera in situ variable res.
(Dyer and Brown, 1980)
- 1989 analog video camera ex situ variable res.
(Cunningham et al., 1989; Harris and Campbell, 1989)
- 1991 desktop digital scanner ex situ 30 - 236 pixels cm⁻¹
(Pan and Bolton, 1991)
- 1995 analog video camera in situ variable res.
(Majdi and Persson, 1995)
- 1998 portable digital scanner in situ 40 - 157 pixels cm⁻¹
(Pan et al., 1998)
- 2001 portable digital scanner in situ 79 pixels cm⁻¹
(Pan et al., 2001)
- 2009 portable digital scanner in situ 1890 pixels cm⁻¹
(Hamac et al., 2009)

Objectives

- evaluate the capability of high resolution scanners to distinguish crop species differences in root hair morphology
- determine if crop species differ in root hair responses to soil water availability

Materials and Methods

- Preparation
 - Canon® CanoScan LiDE 600 F (1890 pixels cm⁻¹)
 - DAP® silicone
 - Rubbermaid® plastic containers



Materials and Methods

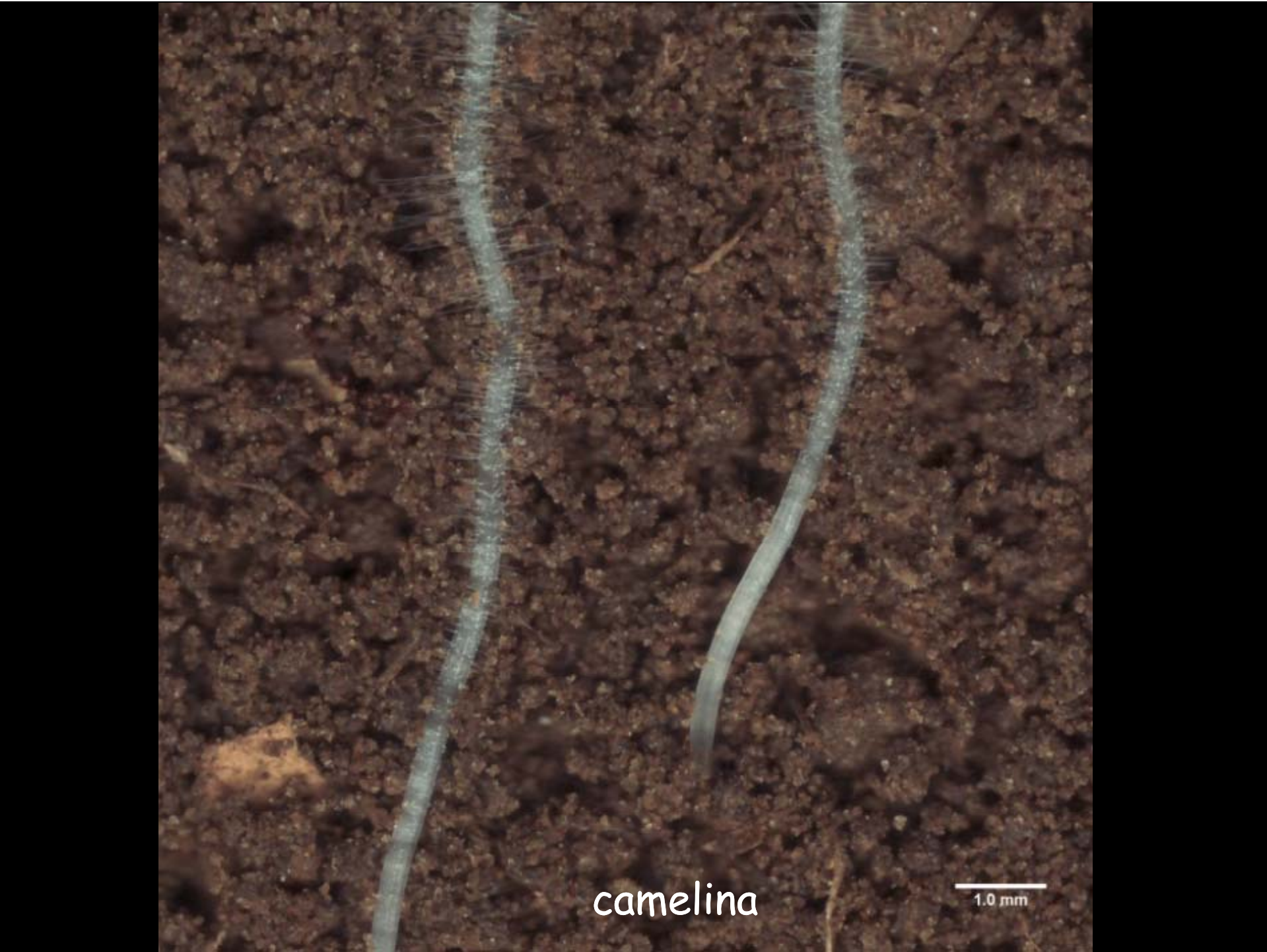
- Preparation
 - 2 scanners/container
 - Ritzville silt loam
(Coarse-silty, mixed, superactive, mesic Calcic Haploxerolls)
 - bulk density 1.1 g cm^{-3}
 - lighting
 - $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$
 - 12 hr day length



Experimental Design

- 4 species (camelina, flax, lentil, and canola)
- 2 levels of water availability
(-188 & -63 kPa)
- 4 replications
rep 1 & 2 --> run 1
rep 3 & 4 --> run 2





camelina

1.0 mm

A photograph showing a white, fibrous root system of a flax plant growing in dark brown soil. The root is the central focus, extending vertically from the top to the bottom of the frame. The soil is granular and contains some small, light-colored organic matter. In the bottom left corner, the word "flax" is written in white. In the bottom right corner, there is a white scale bar with the text "1.0 mm" below it.

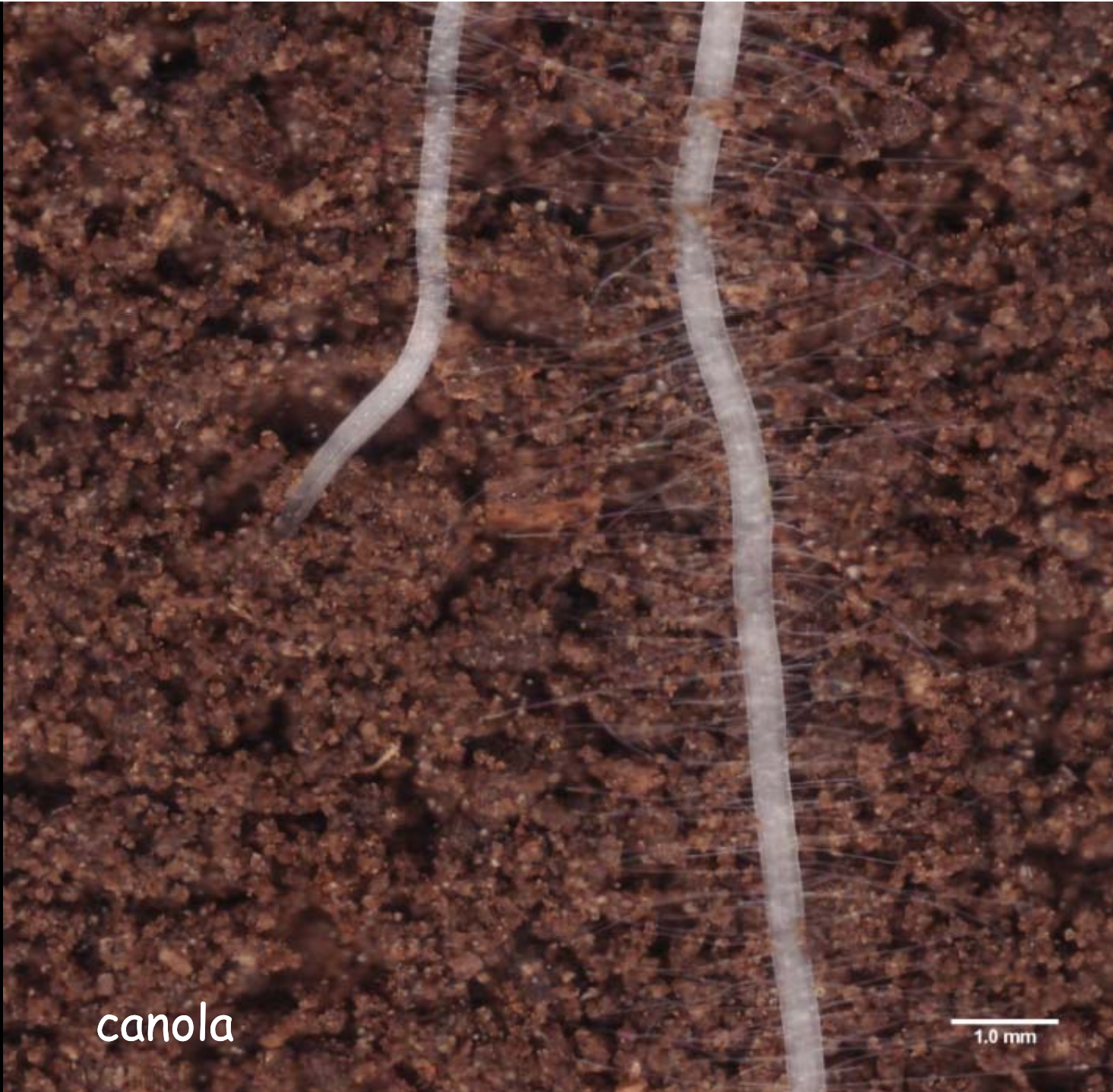
flax

1.0 mm



lentil

1.0 mm



canola

1.0 mm









Image Analysis

- 9 images per scanner
- Collected on laptop
- Transferred to PC
- Adobe Photoshop CS3 Extended
 - Root Hair Geometry
 - Max RHL
 - Root hair density
- Calculated Root surface area



Root Hair Geometry Data Analysis

Richards Growth Model

$$Y = A / (1 + B * e^{(-C * X_1)})^{1/D}$$

where:

Y - RHL

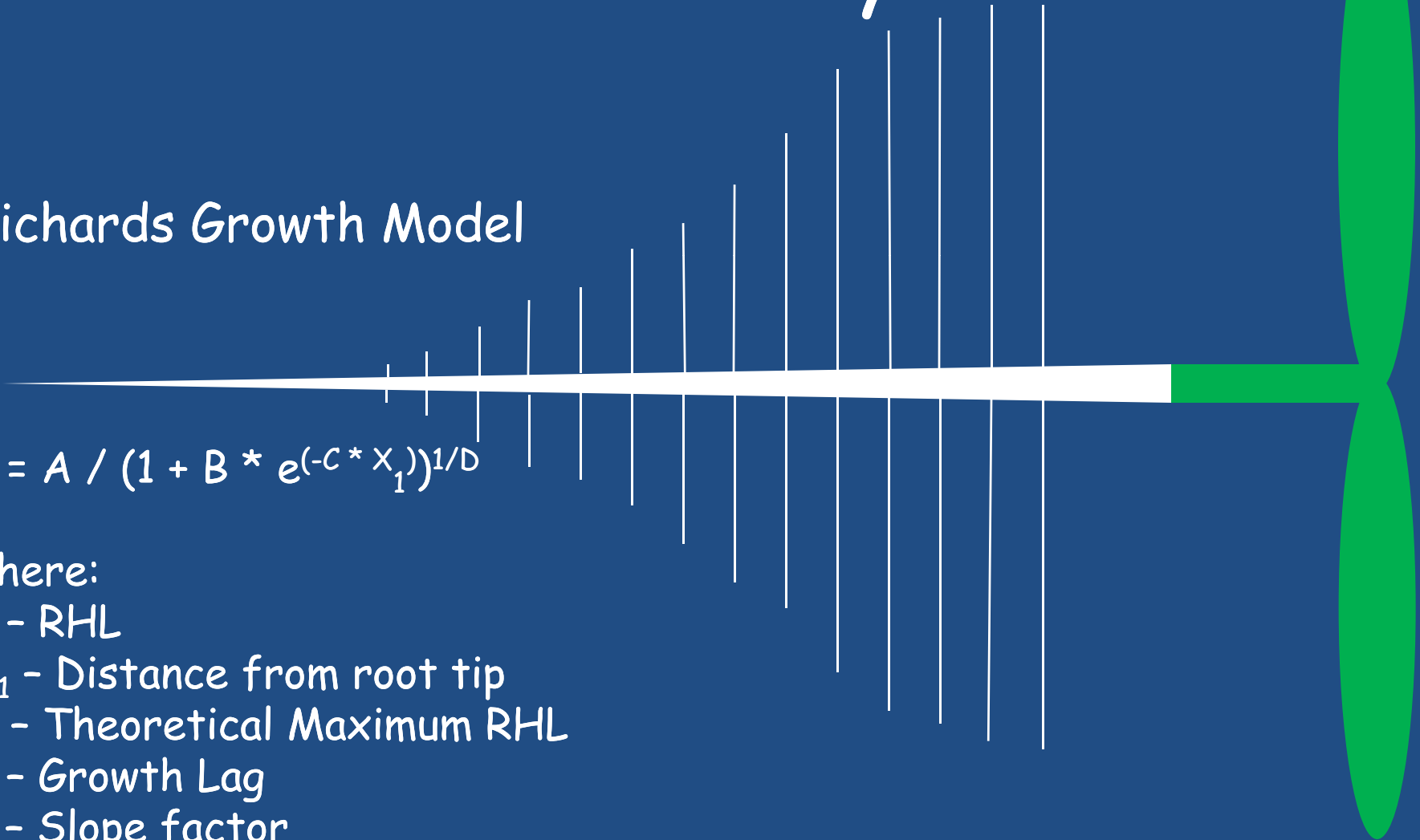
X₁ - Distance from root tip

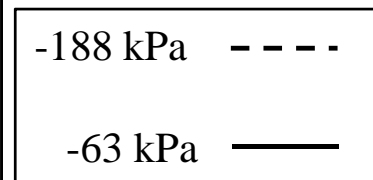
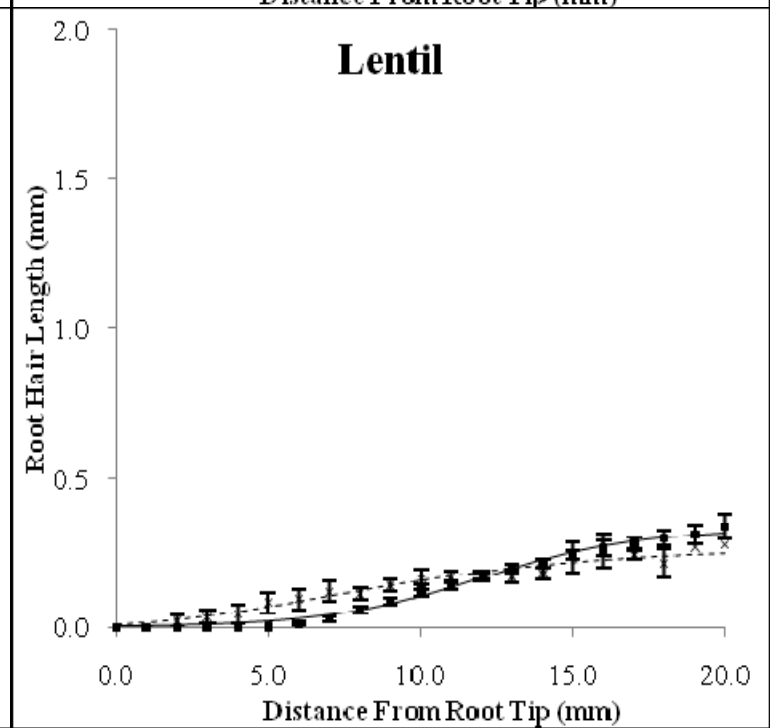
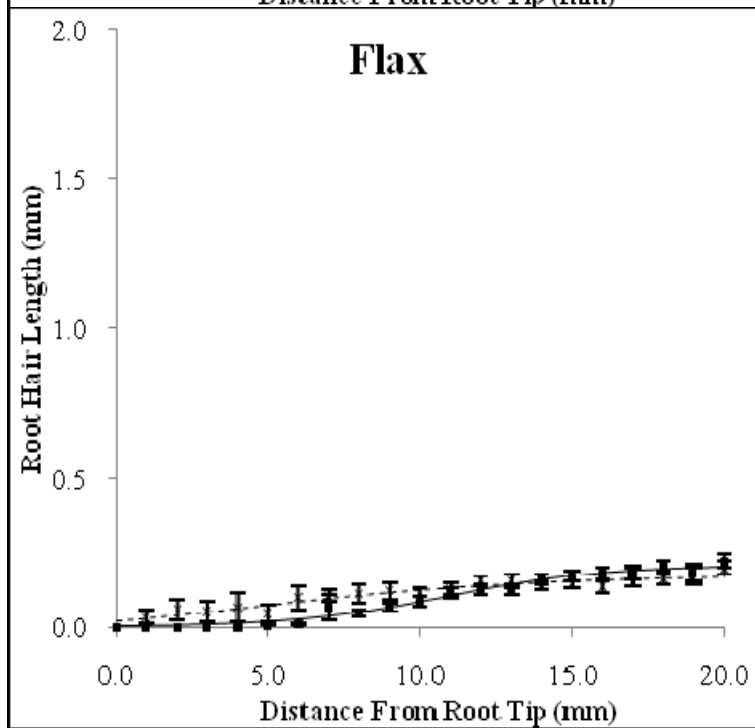
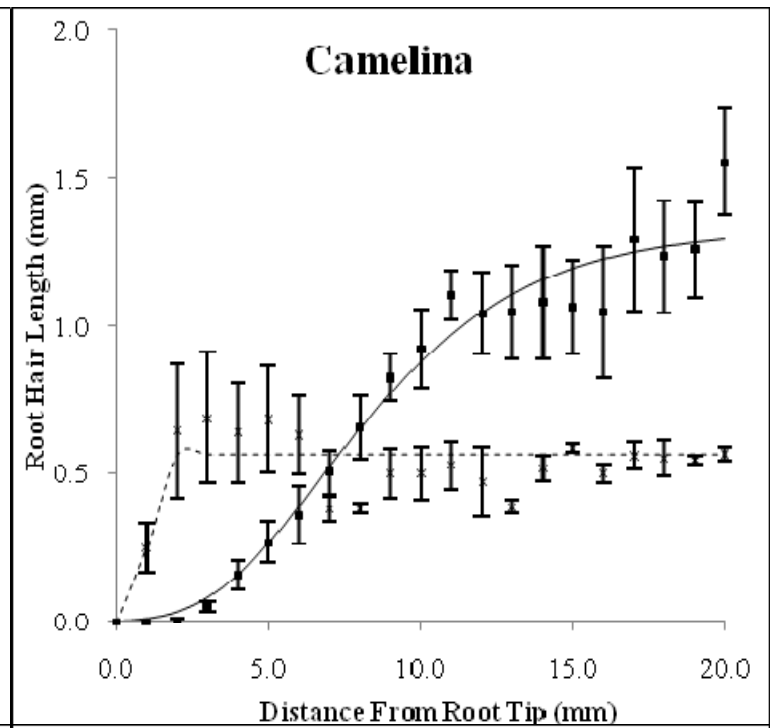
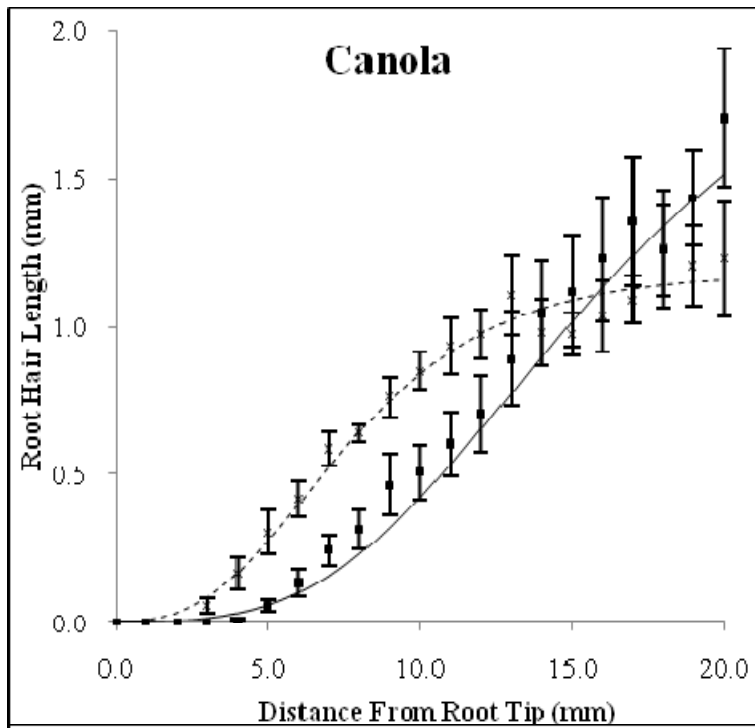
A - Theoretical Maximum RHL

B - Growth Lag

C - Slope factor

D - Growth Lag





Species	Root Hair Length (μm)			Root Hair Density (root hairs cm^{-1})			Root Surface Area ($\text{cm}^2 \text{cm}^{-1}$)		
	-188 kPa	-63 kPa	Mean	-188 kPa	-63 kPa	Mean	-188 kPa	-63 kPa	Mean
Canola	1007	1452	1230 a	132	157	144 a	1.33	2.59	1.76 bc
Camelina	607	1165	886 a	152	219	181 a	0.74	2.11	1.14 ab
Flax	191	224	207 b	328	328	328 b	0.79	0.93	0.86 a
Lentil	350	261	306 b	514	595	553 c	3.10	2.94	2.99 c
Mean	539 a	776 b		232 a	278 b		1.21 a	1.80 b	

Conclusions

- Useful and low cost technique for root measurement
- Species effect
 - inverse relationship w/RHL and RHD
 - extremes for RHL and RHD had highest RSA
- Water availability effect
 - ↑ water ↑ RHL, RHD, and RSA
 - ↑ water ↑ length from tip to initiation zone
- Has utility in other areas of soil and rhizosphere biology exploration

Questions

Thanks to the Washington State
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