

Phenotype Screening Corporation

Ronald Michaels, PhD
Technical Director
Knoxville, Tennessee, USA

Represented by

Zealquest Scientific Technology Co.,
Ltd.

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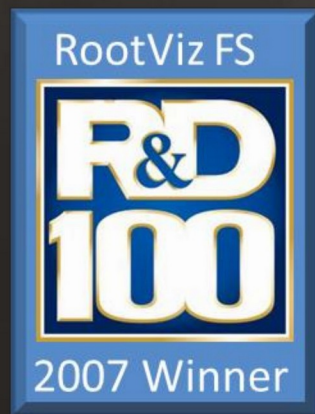
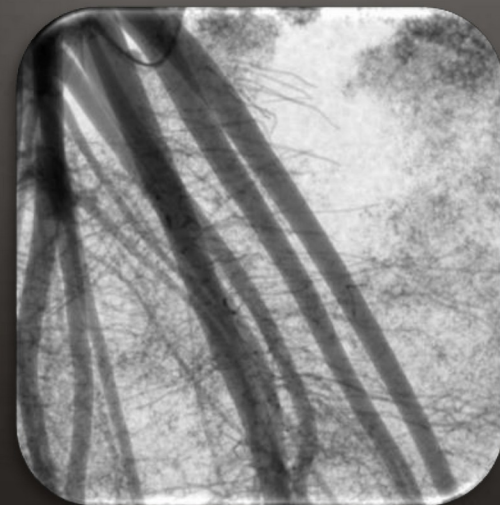
About Us



RootViz FS Imaging System



Root Lab



The Power of the Whole Picture

- ✓ Plant Developmental Stage
- ✓ Plant Height
- ✓ Stalk Diameter
- ✓ Leaf Area
- ✓ Chlorophyll Content

- ✓ Projected Root Area
- ✓ Total Root Length
- ✓ Total Root - Transect Crossings
- ✓ Root Distribution by Depth



- ✓ Total Dry Weight Biomass
- ✓ Shoot Dry Weight Biomass
- ✓ Root Dry Weight Biomass
- ✓ Root/Shoot Dry Weight Biomass Ratio

Our Method

- Very detailed evaluation of a relatively small number of plants
- Multiple measurements over time
- Seed and seedling evaluation
- Plant root imaging and analysis over time
- Above surface measurement of plant development
- Integrated analysis

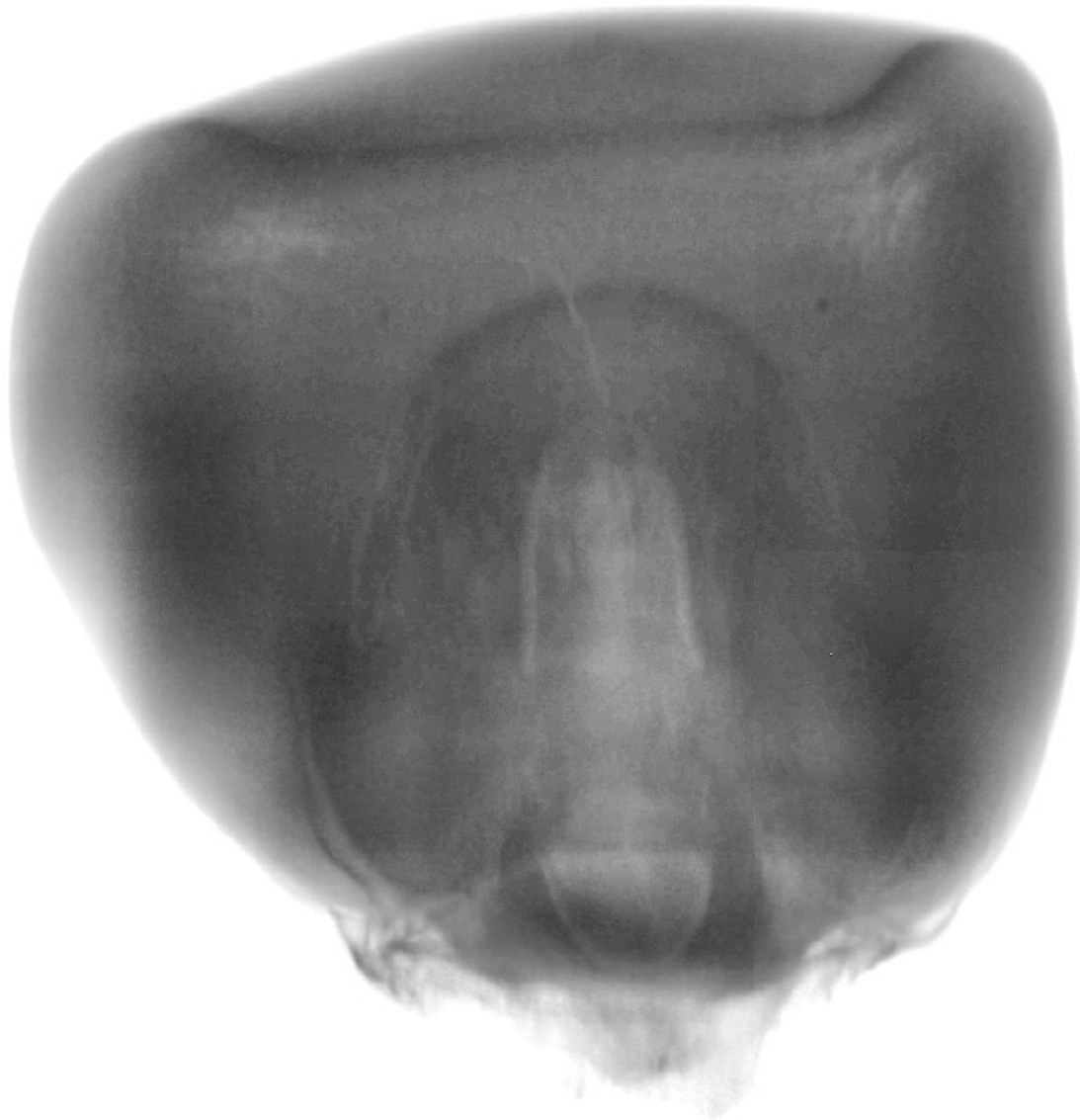
Applications

- Genetic Evaluation
 - Size Distribution of Maize Roots
 - Drought Tolerant Gene in Soybean
 - Rate of Development of Maize Roots
- Treatment Evaluation
 - Chemicals
 - Extracts from plants and bacteria
 - Living Bacteria and Fungi

Plant Growth

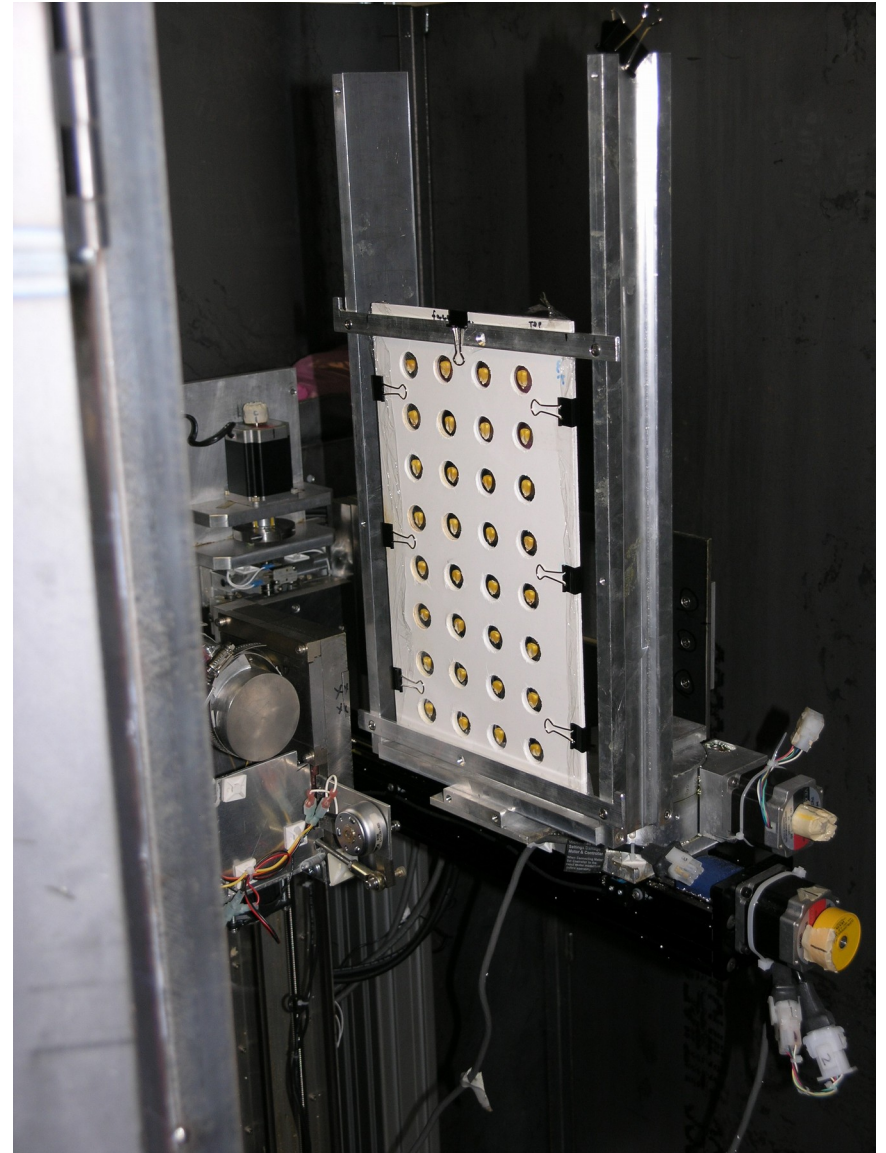
- Seed Imaging and Analysis
- Germination
- Seedling Imaging and Analysis
- Seedling Selection
- Transplanting to Growth Container
- Nutrient Solution and Application

X-Ray Image of Maize Seed



Seed X-Ray Image Acquisition

- 32 Maize seeds in fixture
- Computer controlled positioning
- Automated Image Capture



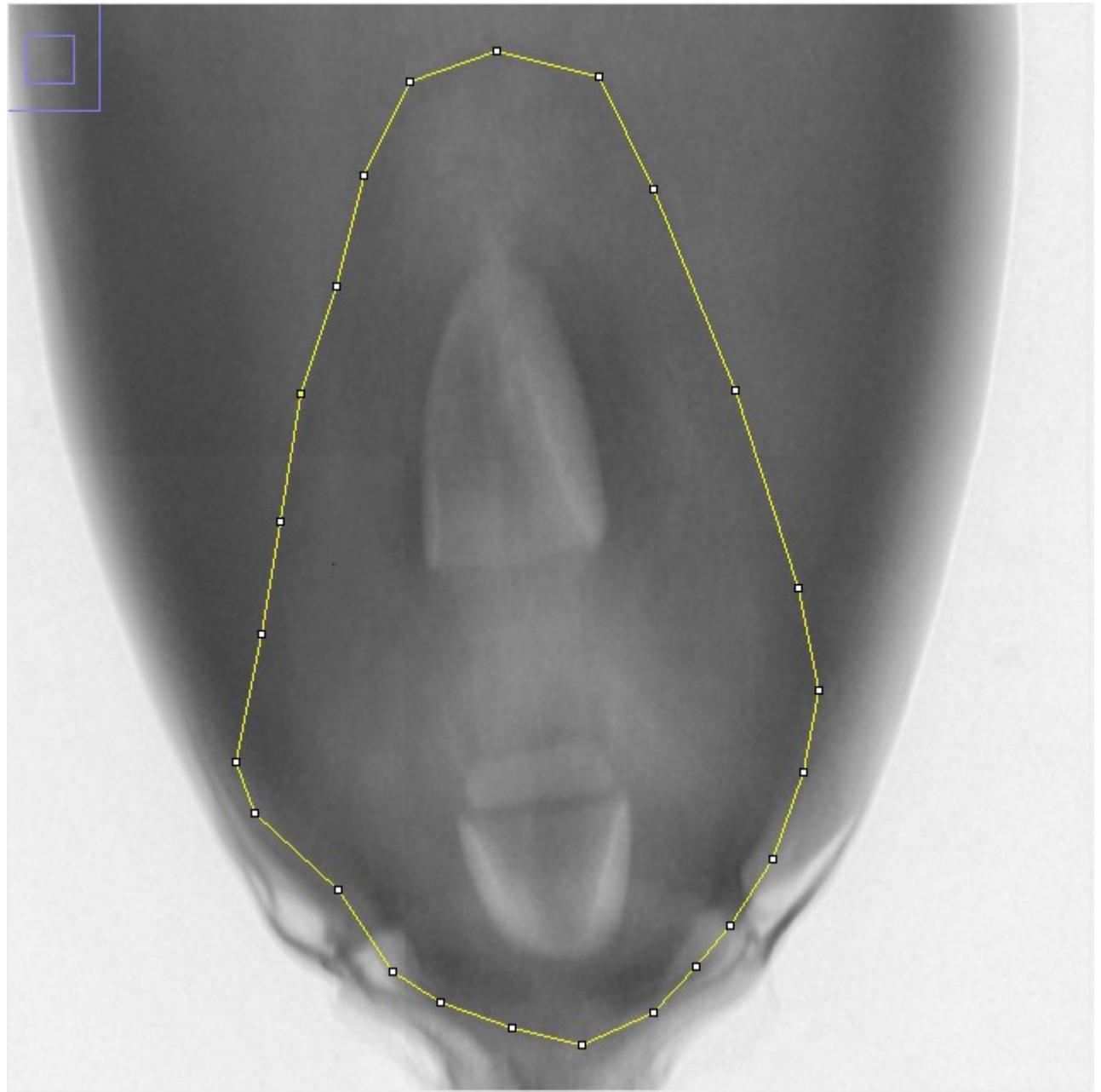
OCT Image of Maize Seed

- This is preliminary work
- Optical Coherent Tomography
- Uses near infra-red light
- Reflection occurs at a change of optical index of refraction
- Sensitive to “layers”



Seed Analysis

- Example:
- Scutellum Area
- Done manually using ImageJ



Germination

- Seeds placed in germination pouches
- Pouches on hangers
- Each pouch photographed 4 days after germination



Seedling Analysis



- Example: Primary Root Length
- Done manually using ImageJ

Seed vs. Plant

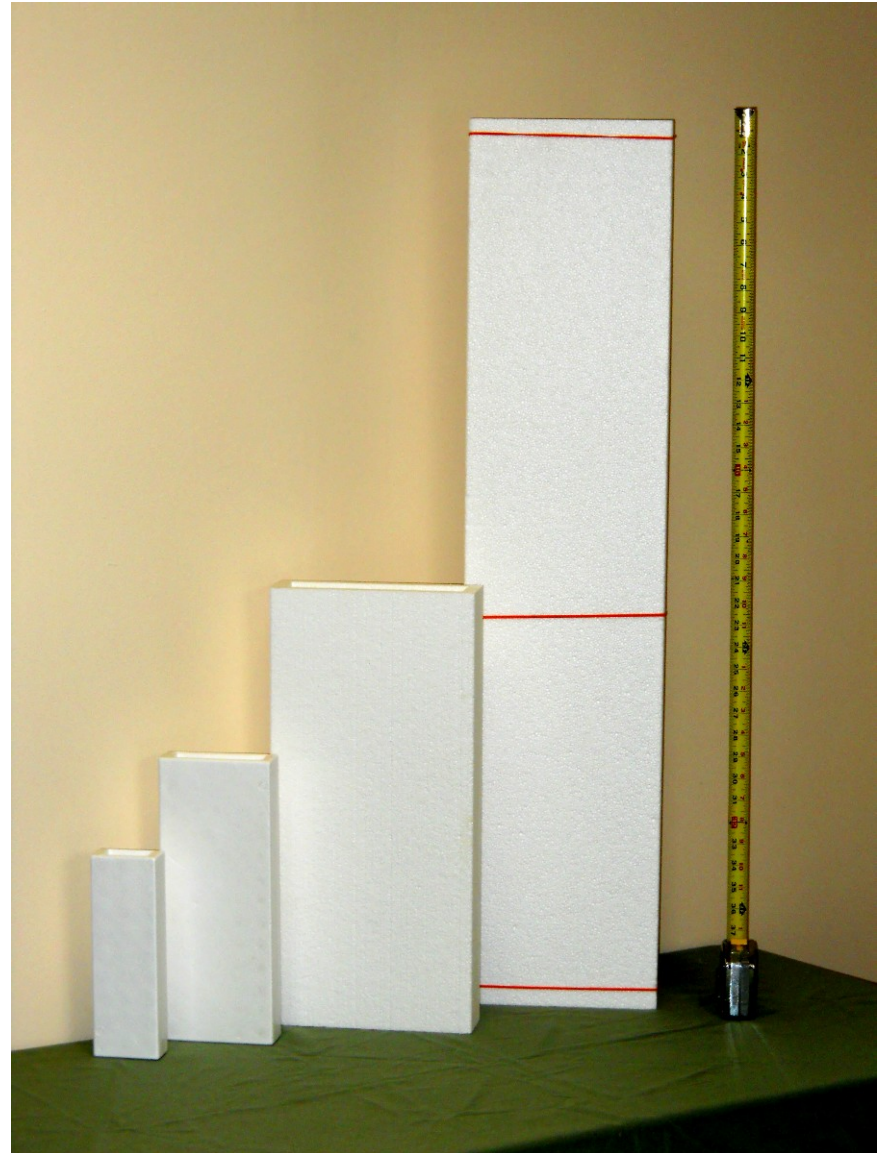
	Seed Internal Structure				
	<i>ScutellumAreaPx</i>	<i>InternodalArea</i>	<i>SeedMass/RadicleArea</i>	<i>ScuA/seedA</i>	<i>EmbryoAxisA/SeedA</i>
NumberOfSeminalRoots4DAI	0.305348372	0.390666137	-0.134750918	0.216031893	0.156178176
PrimaryRootLength4DAI	0.412293364	0.170812209	0.167621338	0.253794135	0.274469306
ShootLength4DAI	0.604765501	-0.004477519	-0.299471811	0.642179711	0.14983289
AxisSum4DAI	0.526426356	0.128586543	-0.043142298	0.457195579	0.243288934
SumLengthOfSeminalRoots	0.917195554	0.350312993	-0.339688862	0.864025158	0.51521736
VigorRating	0.923471607	0.331052187	-0.296644496	0.858368748	0.503077961

	Seed Internal Structure			
	<i>RadicleArea</i>	<i>ScuA/seedA</i>	<i>RadicleA/SeedA</i>	<i>PluA/InterA</i>
HeightatV6(mm)	-0.402993629	-0.529233373	-0.393430539	0.217730979
V6Date	0.125953443	0.10573364	0.144666434	0.728915869
DaysToV6	0.125953443	0.10573364	0.144666434	0.728915869
HeightAtV5(mm)	-0.697007613	-0.667458299	-0.708944656	0.196562404
V5Date	0.087595686	0.037891182	0.090944806	0.726376639
DaysToV5	0.087595686	0.037891182	0.090944806	0.726376639
HeightV4(mm)	-0.794815444	-0.849886148	-0.799598381	-0.094424125
V4Date	0.249859467	0.081561666	0.255539703	0.822131391
DaysToV4	0.249859467	0.081561666	0.255539703	0.822131391

- Green shows high correlation

Plant Growth

- Seedlings are transplanted from germination paper to our plant growth containers and substrate



Drip Irrigation

- Modified Hoagland Nutrient Solution
- Pressure Compensated Emitter Tips
- 30 seconds on 5 minutes off
- Pump driven recirculating nutrient solution



Our Grow Room



Above Surface Measurements

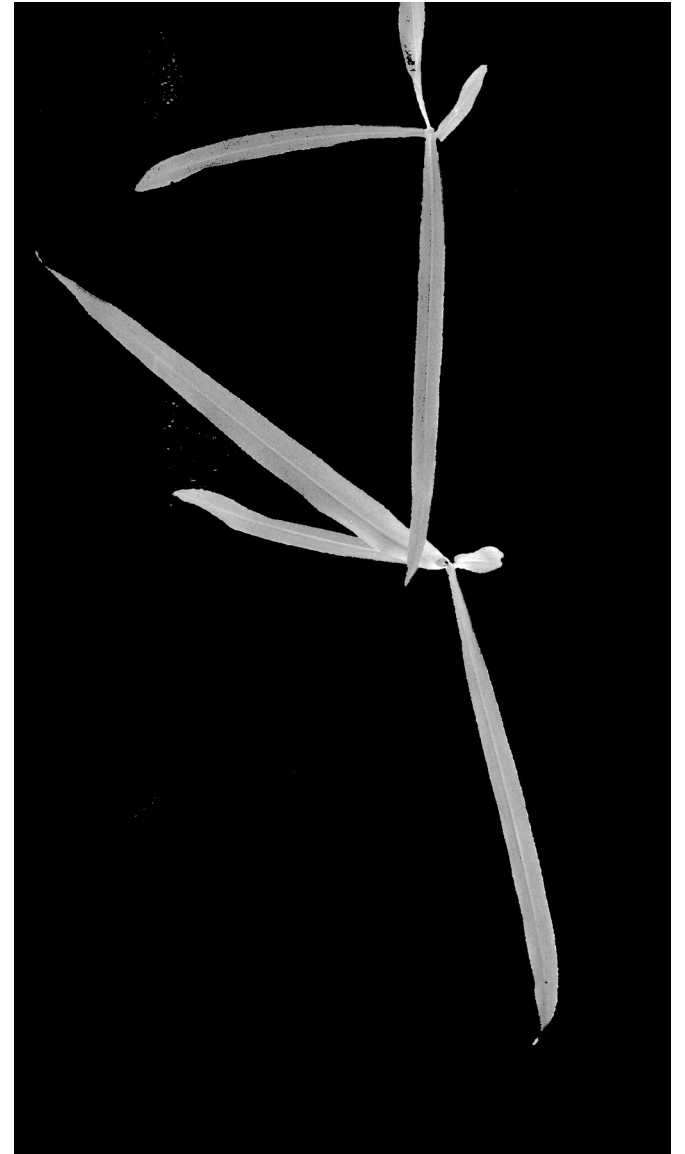
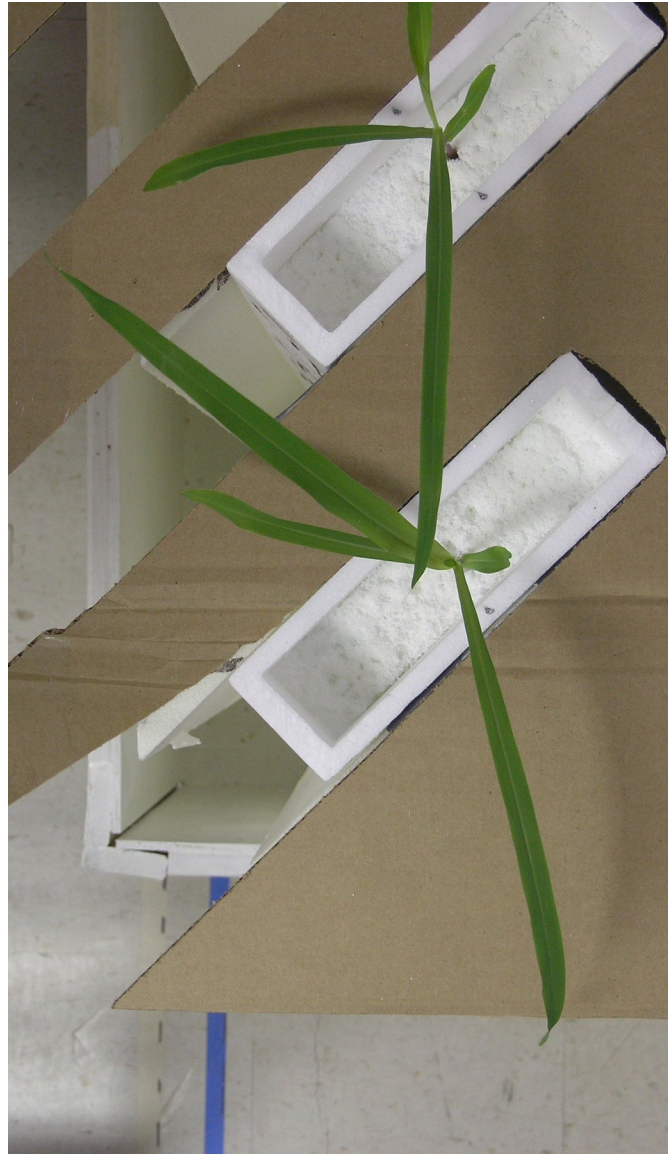
using maize as an example

done manually and entered into spreadsheets

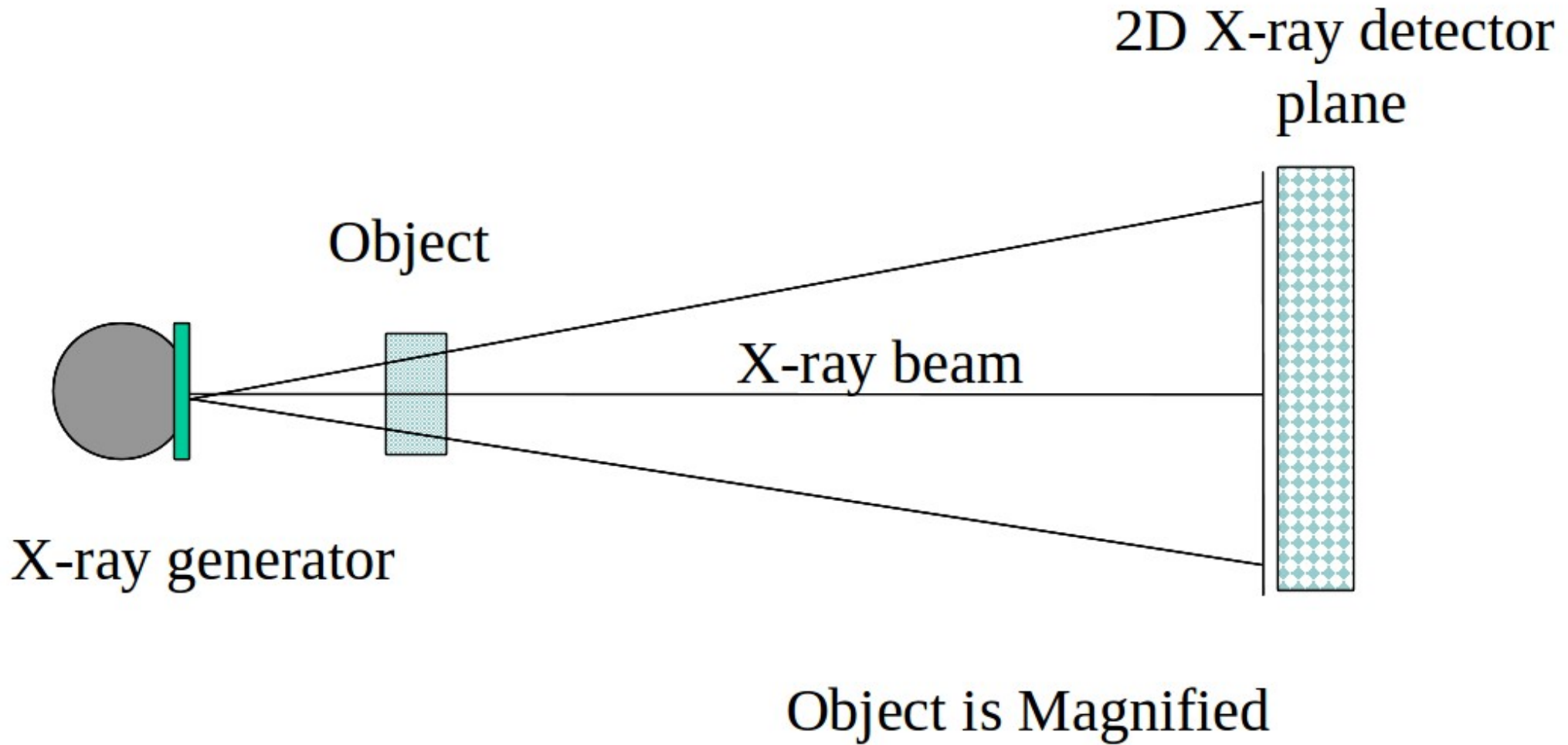
- Periodic Measurements
 - Vegetative State
 - Plant Height
 - Leaf Vertical Intercept Area
 - Leaf Chlorophyll Content
- End of Experiment Measurements
 - Stalk Diameter
 - Flat Leaf Area
 - Dry Root Mass
 - Dry Shoot Mass

Leaf Vertical Intercept Area

- Useful for determining foliar spray dose to plant
- Illustration of Color Segmentation Software
- Note that only a specified range of green is shown. All other colors are rejected.



X-Ray Imaging

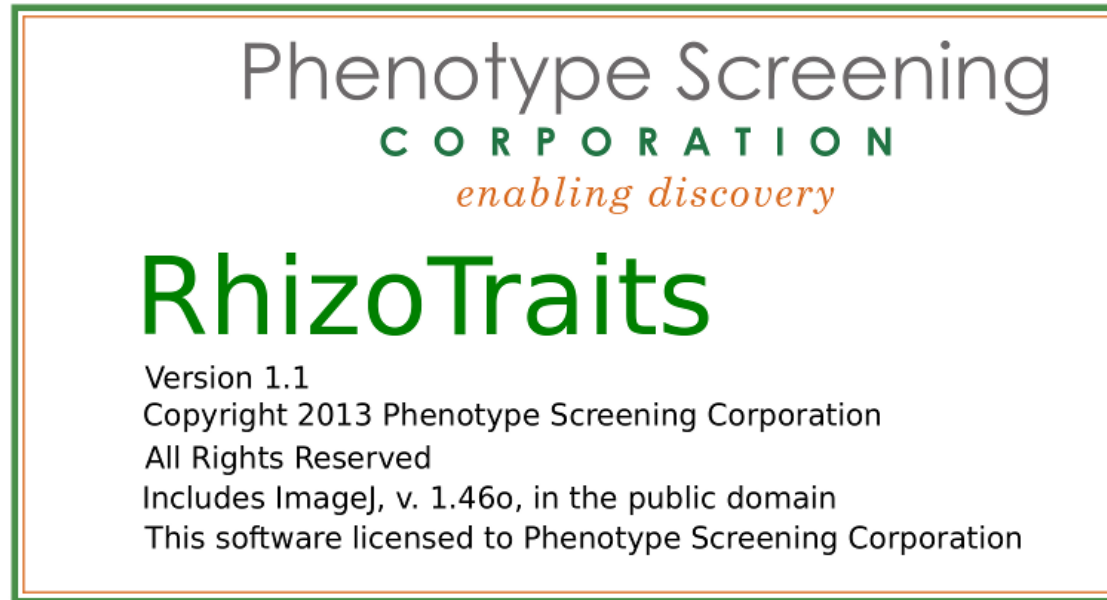


X-Ray Imaging

- X-Ray Generator on Left
- X-ray Camera on right
- Place plant into x-ray machine
- Plant rotates 90 degrees
- Plant moves up and down and back and forth
- 40 images are captured
- This example is 500cm plant container

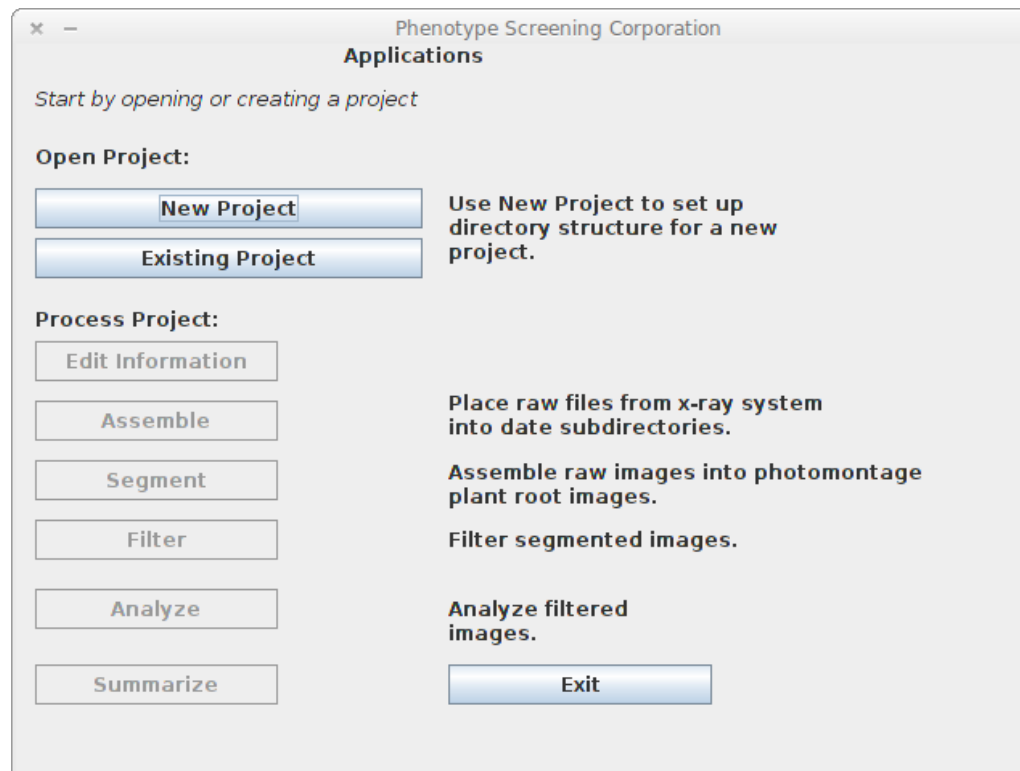


RhizoTraits Software



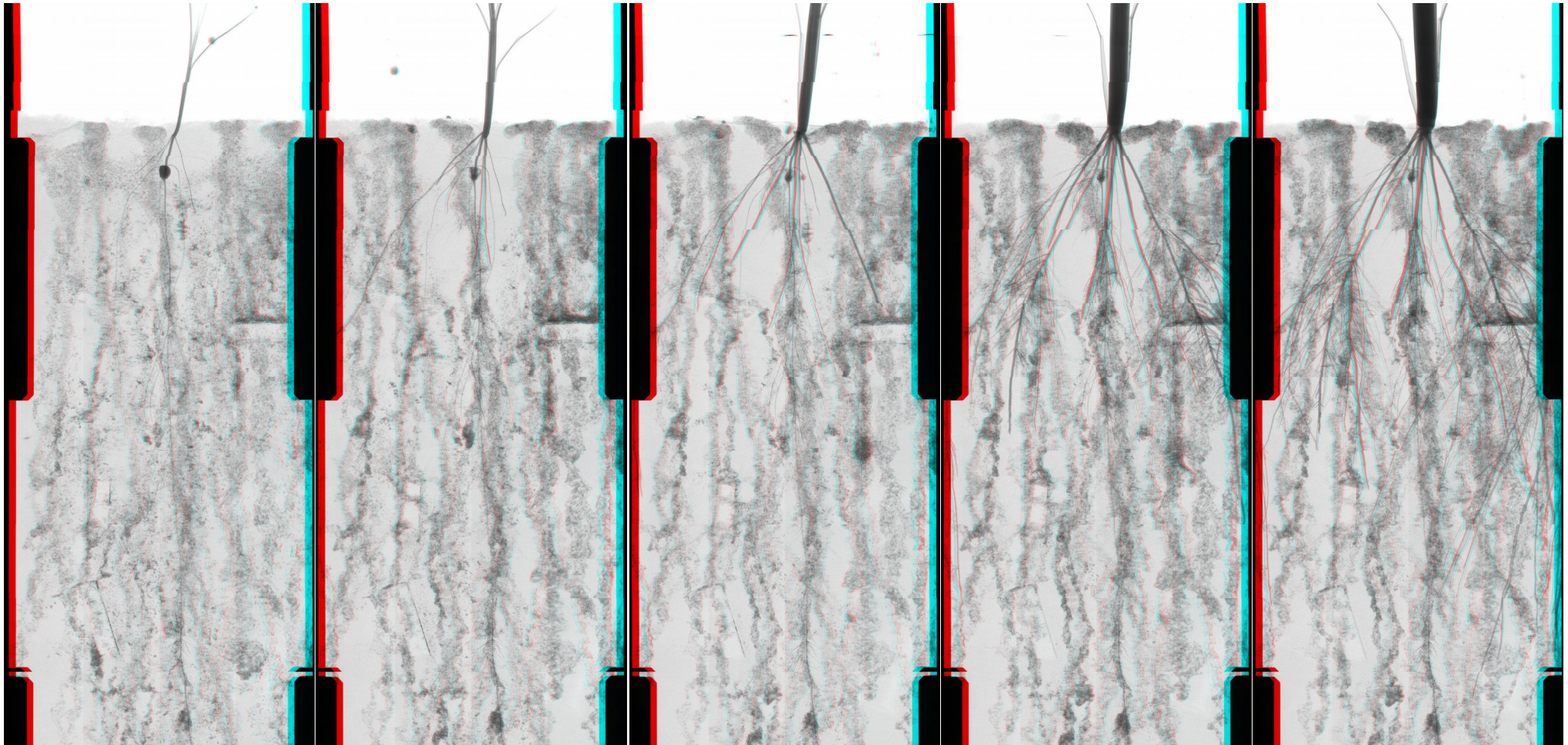
- Version 2.0 Release to Customers Fall 2016
- Automates
 - Image Assembly
 - Root Segmentation
 - Data Analysis to Spreadsheet Format

RhizoTraits Start Screen



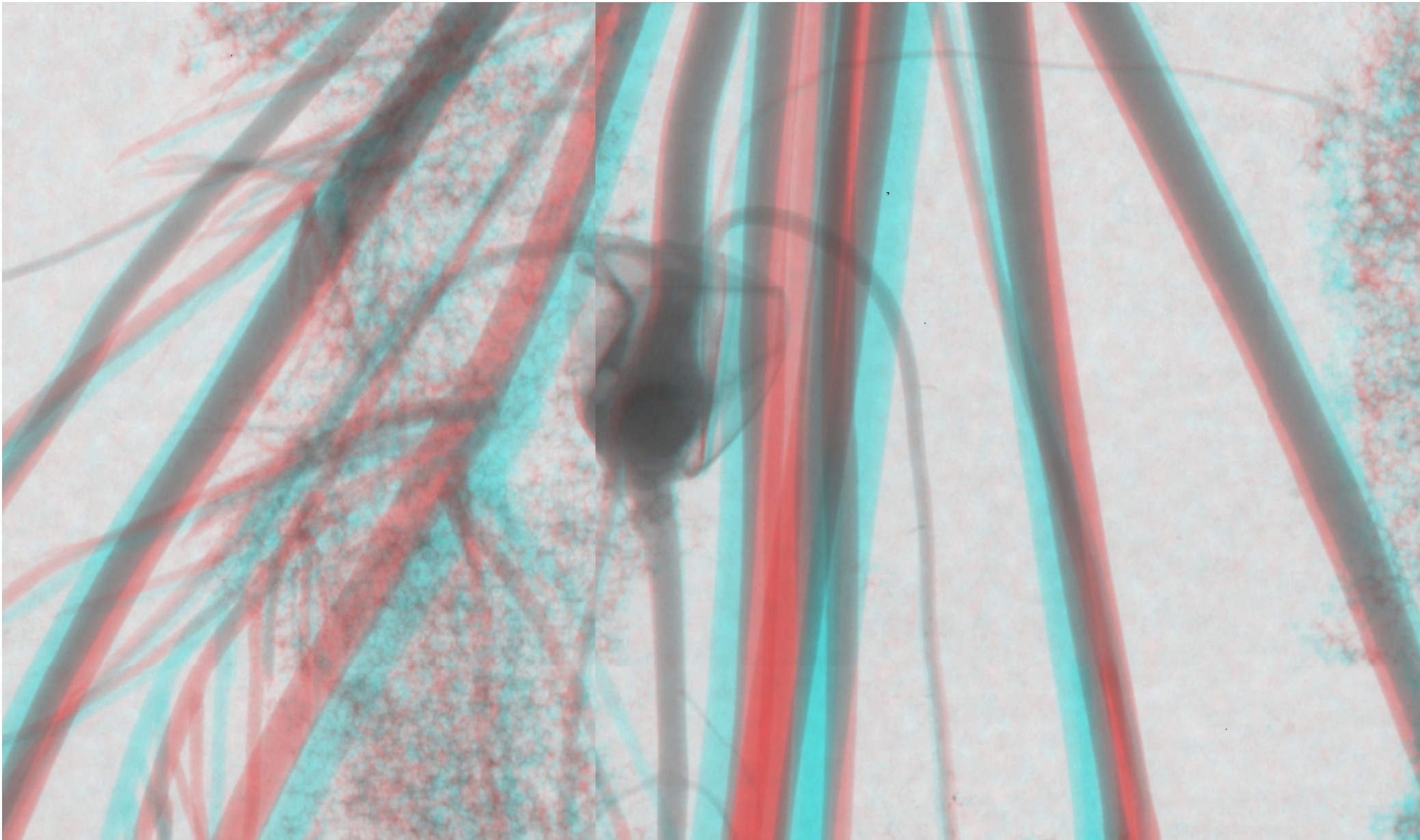
- A Java Front End calls scripts and executables.
- This is very flexible software, and works well with default settings.
- Many settings can be modified for specific cases.

Example Images of Maize Assembled by RhizoTraits

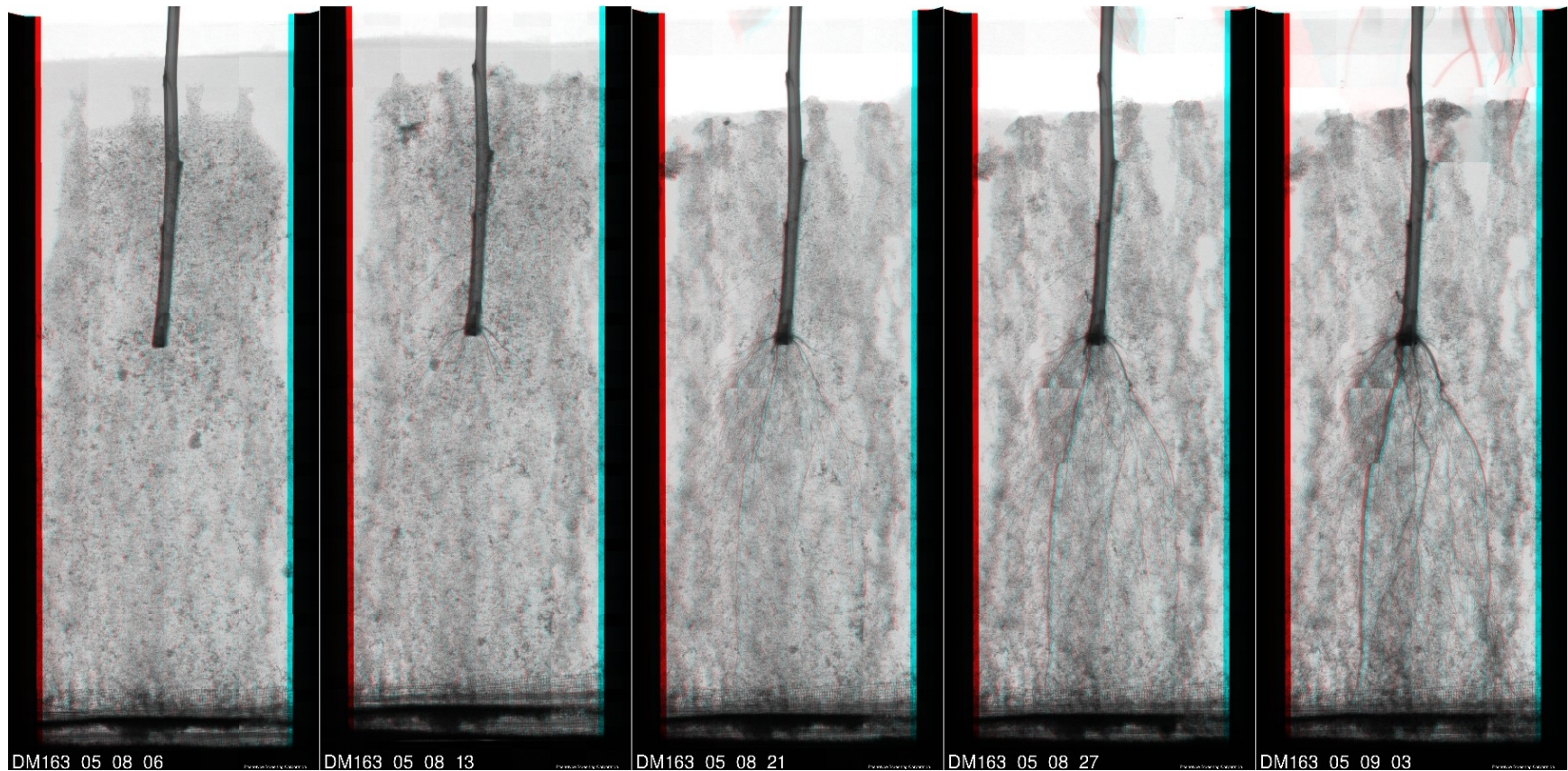


Weekly Images

Full Resolution – Maize Kernel



Weekly Images of Poplar Cuttings



Root Segmentation by Size Class



Original X-ray
Image

Large
Roots

Coarse
Roots

Medium
Roots

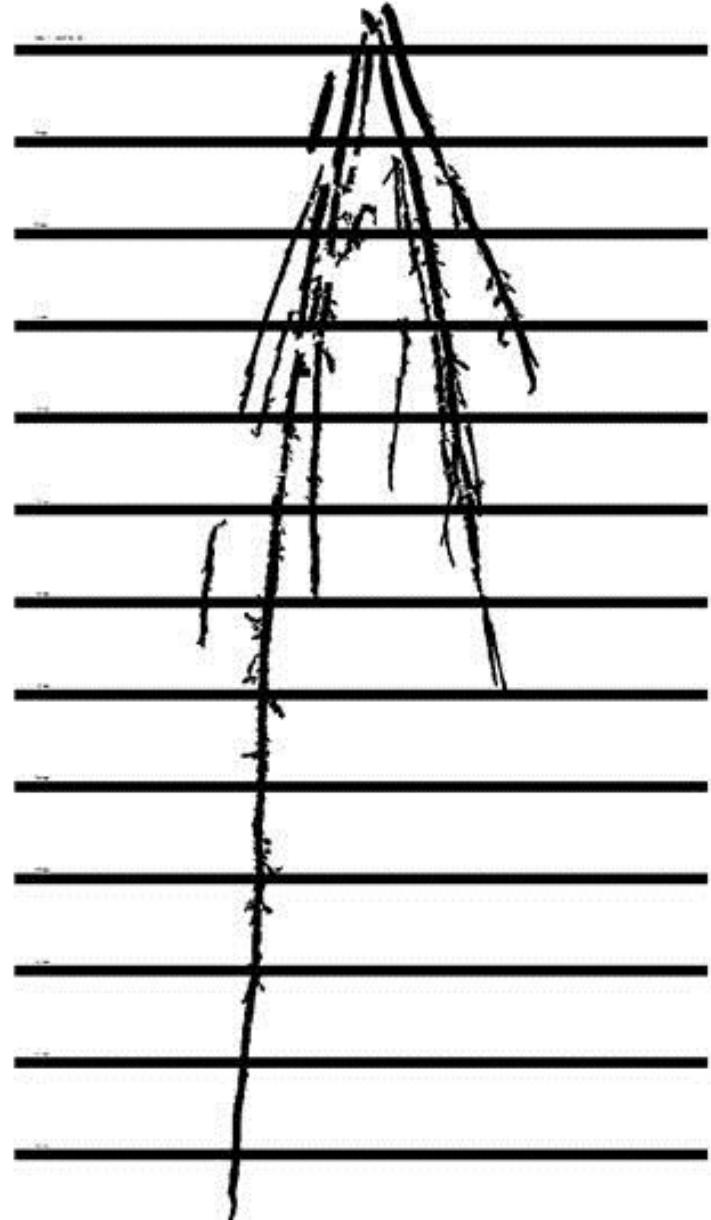
Fine
Roots

Ultra-fine
Roots

Reconstructed
Composite Image

Transect Analysis Example

- Horizontal Lines across segmented root image
- At each line, software finds position and root diameter at each crossing
- Data entered into spreadsheet automatically



Other Information

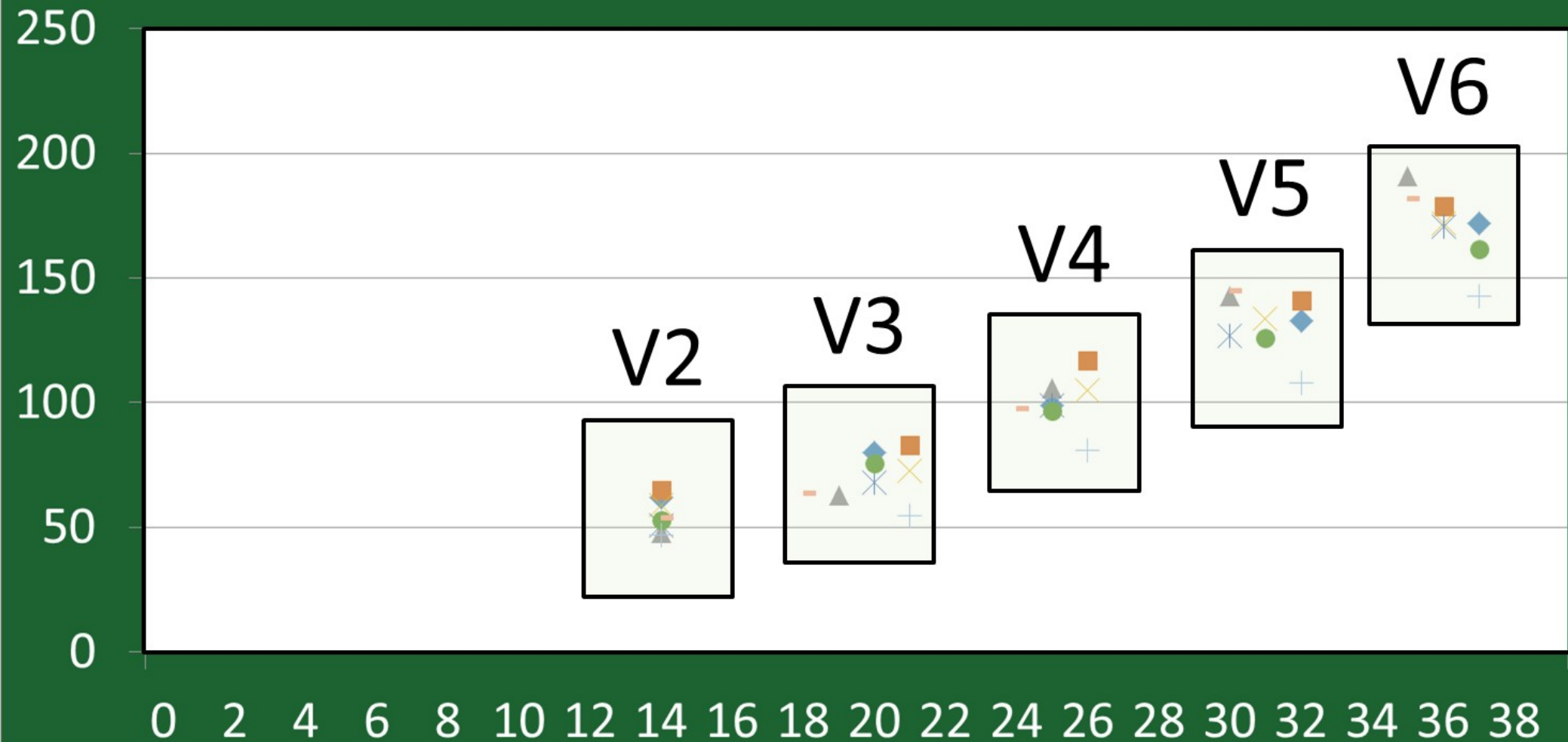
- Total root length
- Total projected area
- Root system width at each depth
- Root angle (not currently implemented)
- All root measurements automatically recorded in spreadsheet.

Analysis Result Spreadsheet

Plant_ID	Size_Class	Projected_Area	TRL_(m)	xval_(px)	yval_(px)	RC#	Rtdiam_(mm)	TDepth_(mm)	WidthAtDepth_(mm)	CountDensity_	DSFFlag
M1101	SizeClass3	6357270	5.576	3235.781	3312	1	1.477	25	30.769	0.1	DSF
M1101	SizeClass3	6357270	5.576	3775.241	3312	2	0.116	25	30.769	0.1	
M1101	SizeClass3	6357270	5.576	3880.75	3312	3	1.826	25	30.769	0.1	
M1101	SizeClass3	6357270	5.576	4174.241	3312	4	2.784	25	30.769	0.1	
M1101	SizeClass3	6357270	5.576	2829.812	4174	1	0.953	50	62.234	0.123	DSF
M1101	SizeClass3	6357270	5.576	3088.741	4174	2	1.595	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	3486.741	4174	3	2.871	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	3658.241	4174	4	1.334	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	4089.741	4174	5	3.045	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	4155.241	4174	6	0.116	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	4181.741	4174	7	0.319	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	4342.784	4174	8	0.839	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	4704.741	4174	9	3.045	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	4879.741	4174	10	3.045	50	62.234	0.123	
M1101	SizeClass3	6357270	5.576	2003.873	5036	1	1.877	75	89.639	0.12	DSF
M1101	SizeClass3	6357270	5.576	2136.741	5036	2	2.581	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	2256.741	5036	3	1.131	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	2409.741	5036	4	0.957	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	2703.741	5036	5	2.175	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	3277.241	5036	6	2.842	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	3473.741	5036	7	2.175	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	3553.241	5036	8	0.754	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	3628.751	5036	9	0.434	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	3737.741	5036	10	2.001	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	4065.741	5036	11	1.247	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	4491.741	5036	12	1.769	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	4596.803	5036	13	2.055	75	89.639	0.12	
M1101	SizeClass3	6357270	5.576	4997.241	5036	14	2.32	75	89.639	0.12	

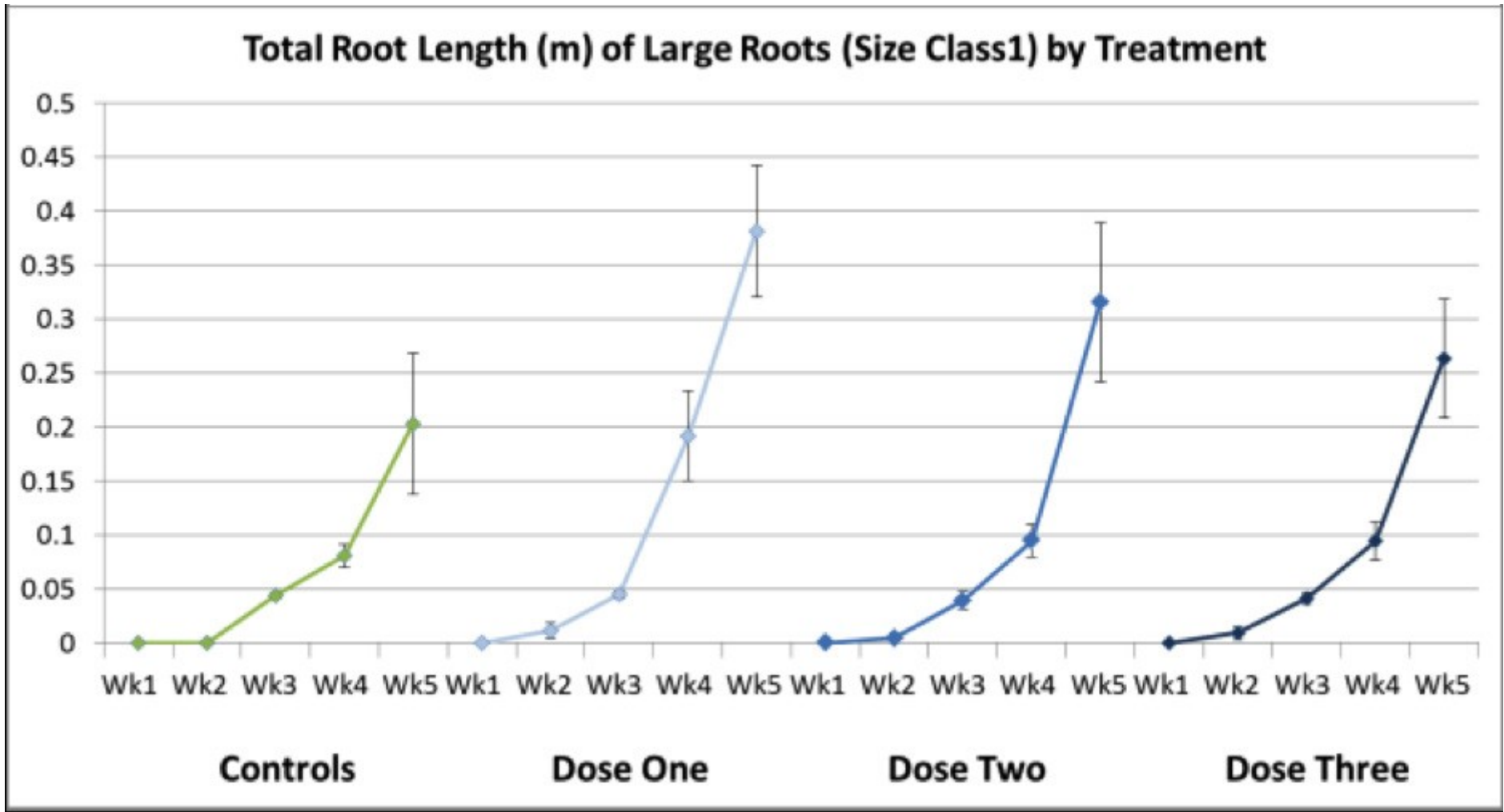
Replicate Growth through Time

Untreated Maize Height (mm) Through Time (days)



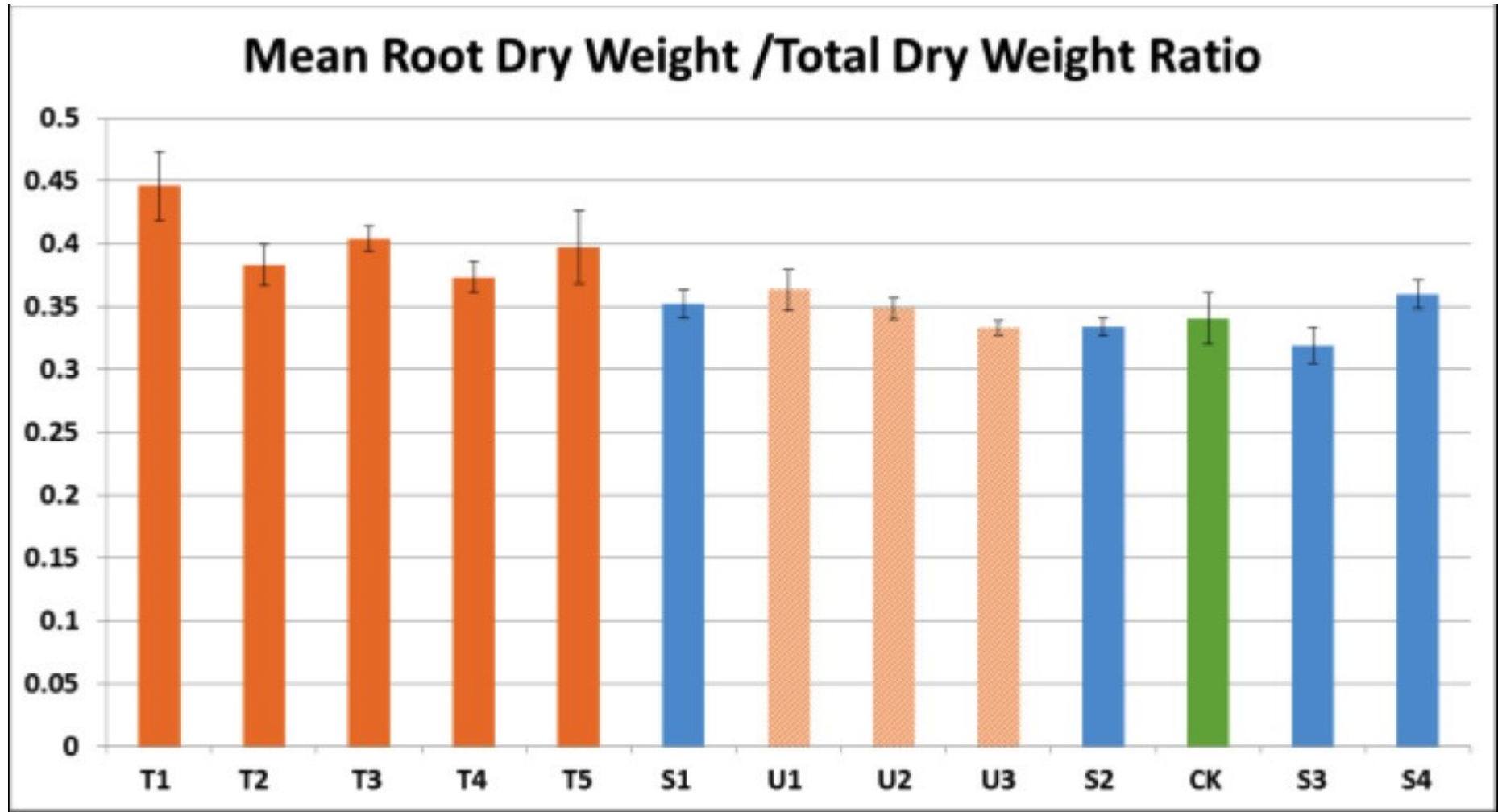
Each symbol represents one plant. This graph shows trajectory through time for each plant. Fast plants stay fast; slow plants stay slow. Useful for evaluating treatments that affect early development.

Effect of Treatment on Total Root Length



This Graph Shows trajectory through time for total root length of plant. Effect of Treatment Dose is shown.

Root to Total Mass



Note: this graph uses end of experiment data, not x-ray data

Other Applications

- Maize Stalk and Ear Damage Assessment
- Ear Shoot Development
- Nematode Infestations
- Chlorophyll Content of Leaves
- Surface Tension Characterization for Foliar Spray

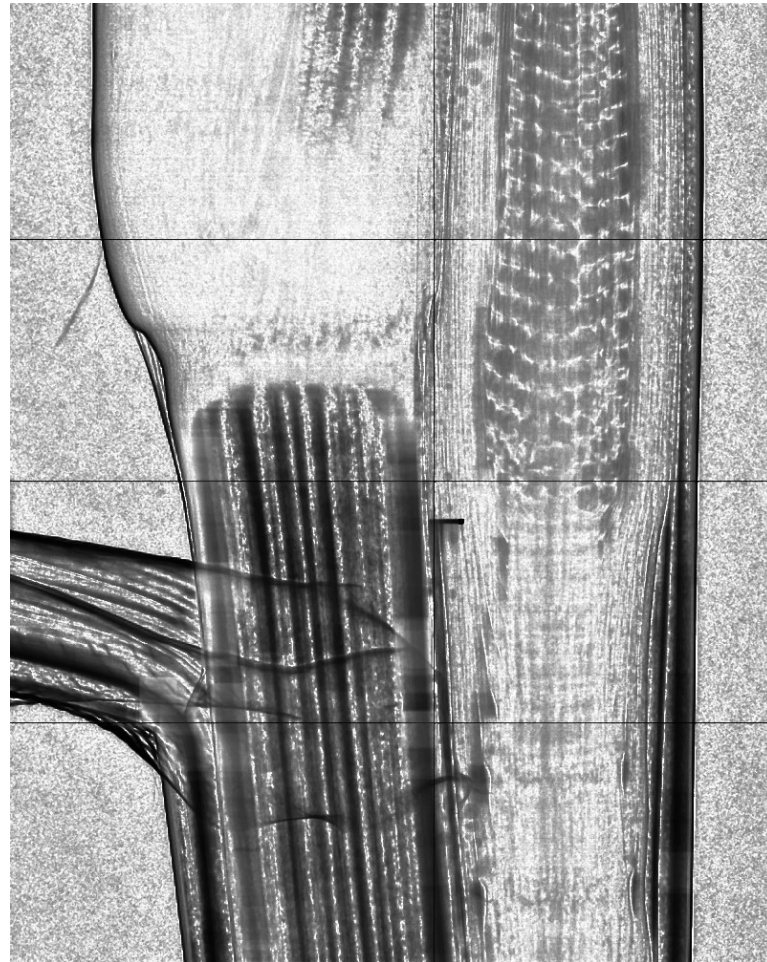
Damage to Maize Ear and Stalk



Ear Shoot Early Development

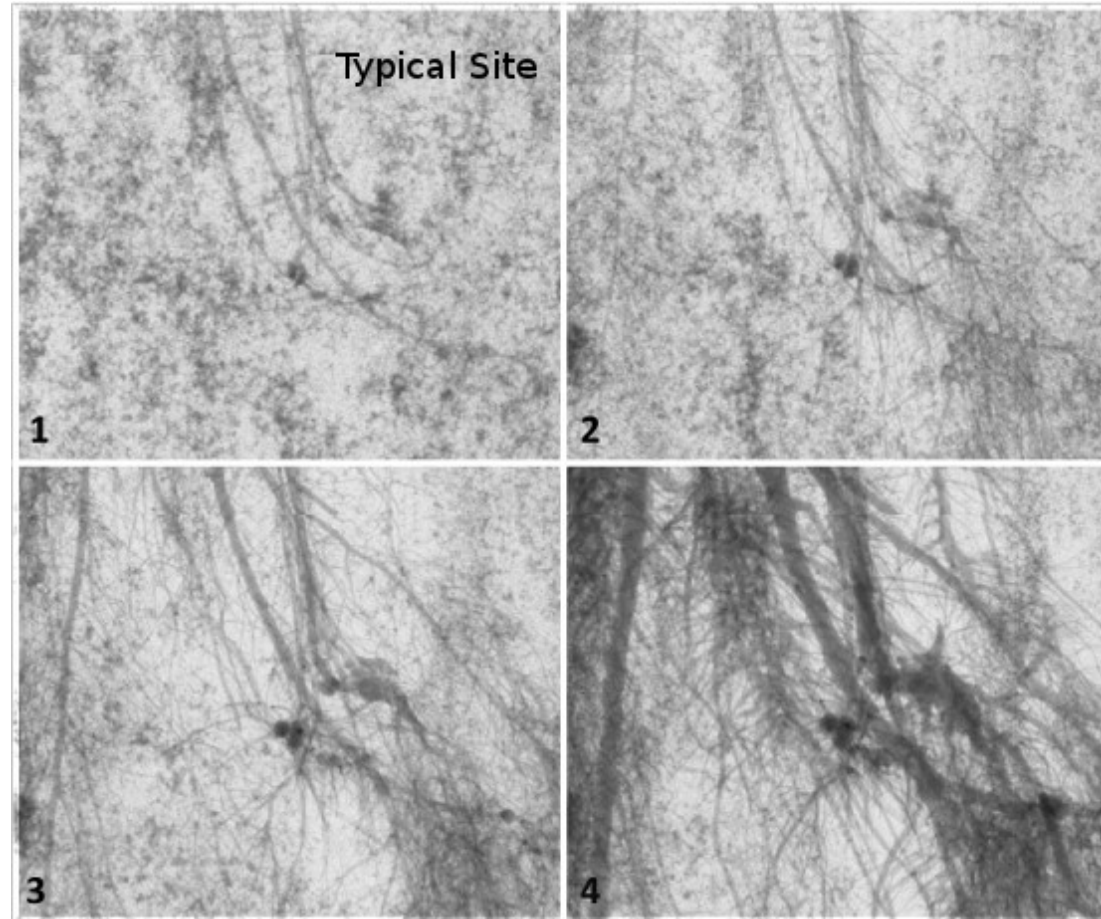


photograph



x-ray image

Root Knot Nematode Infestation in Sunflower



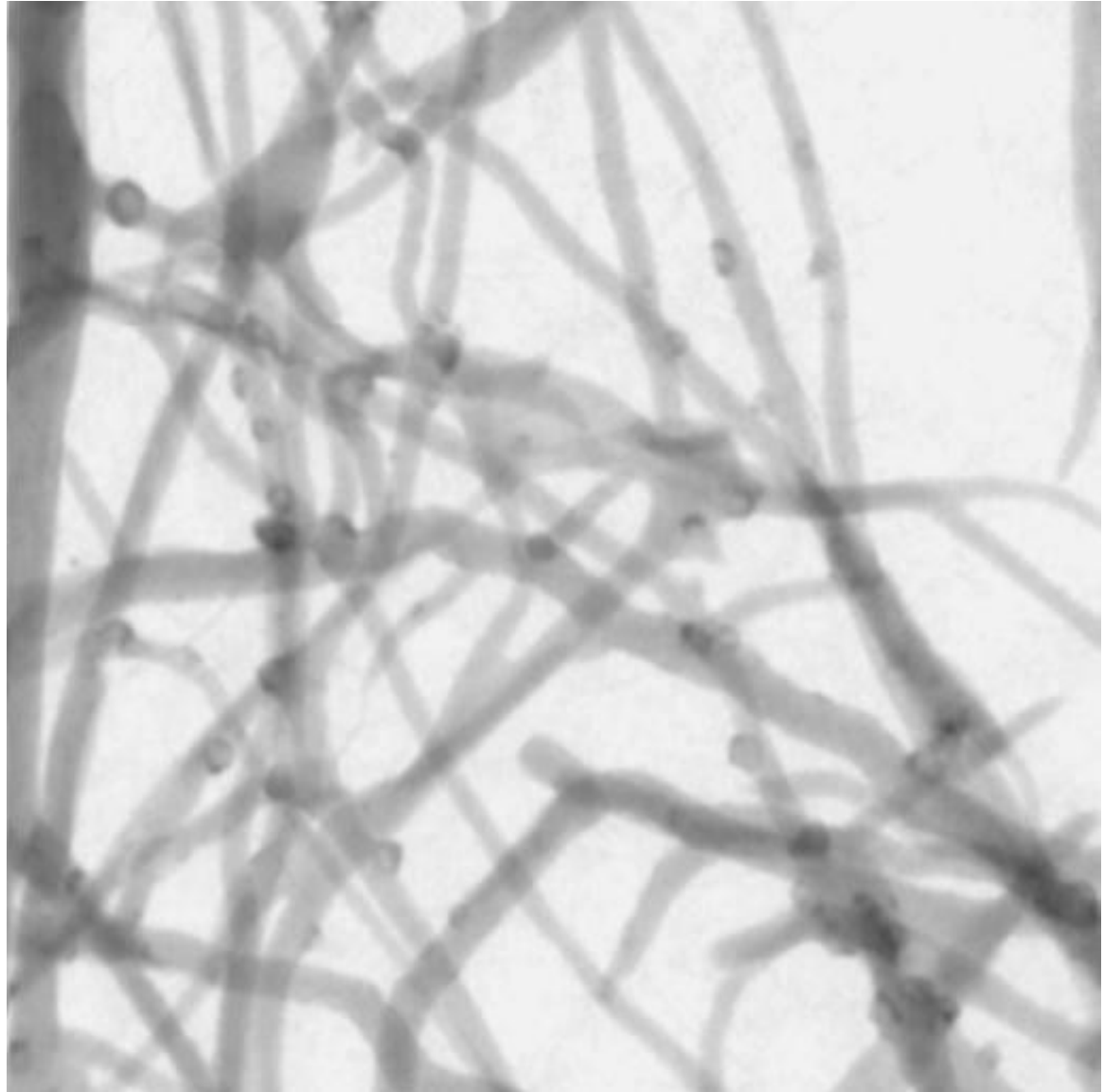
Weekly development of nematode galls grown in Artificial Substrate

Reniform Nematode Infestation

Cotton grown in soil and washed prior to x-ray image



photograph of a Reniform Nematode egg mass

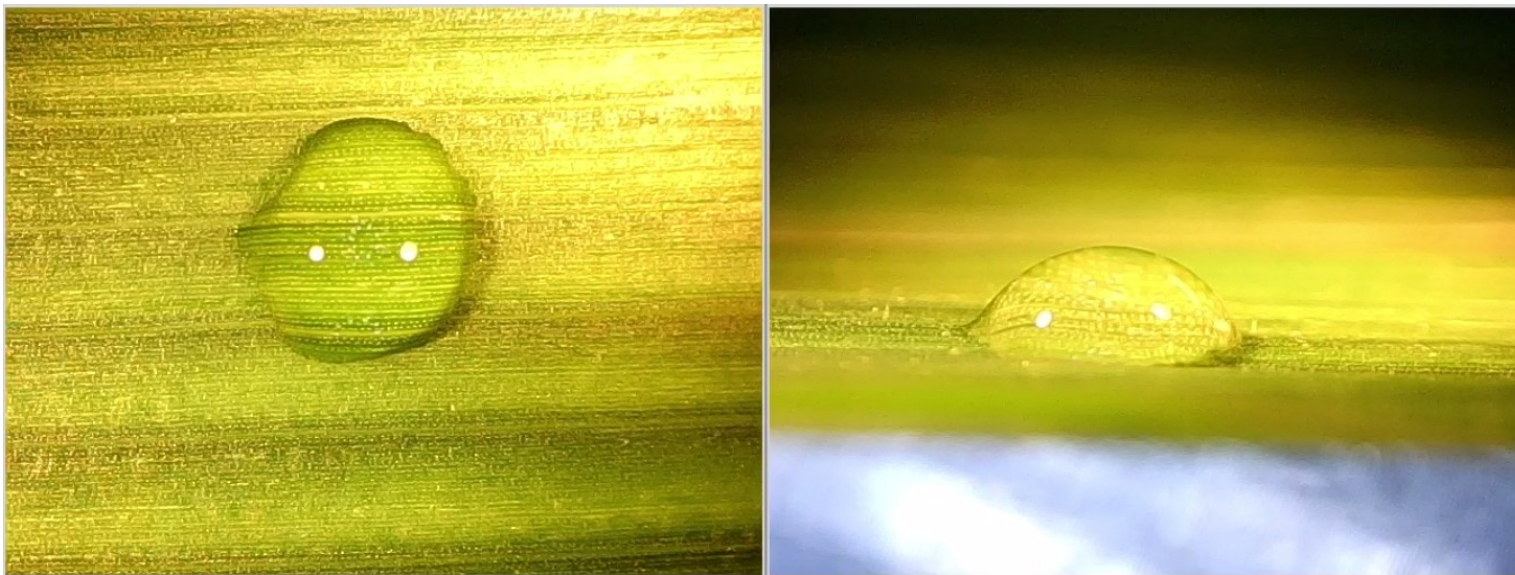


X-ray image of many Reniform Nematode egg masses

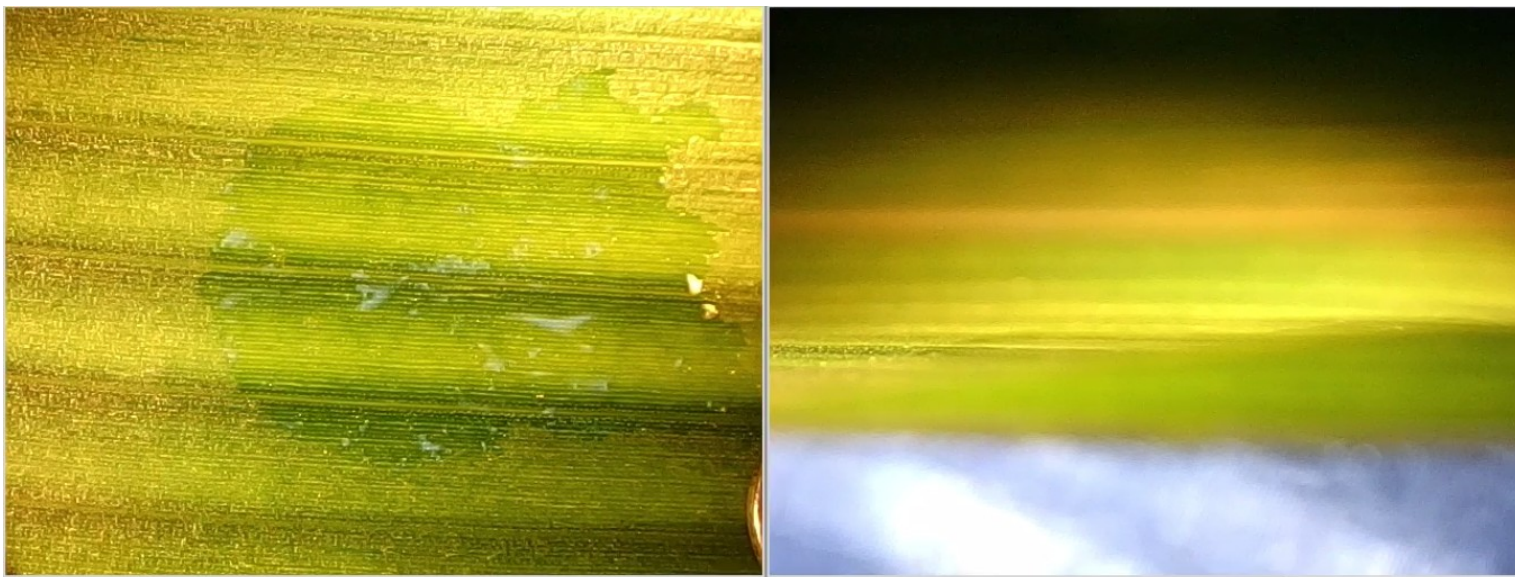
Chlorophyll Content of Leaves

- Hypothesis: leaf area x chlorophyll content = constant
- Higher chlorophyll content gives possibility of greater photosynthetic efficiency
- Less leaf area per plant gives possibility of higher plant density per hectare

Foliar Spray and Surfactant



treatment with no surfactant



treatment with surfactant (note spreading)

Conclusions

- Plant growth evaluation requires consideration of both above and below surface plant characteristics.
- A simplified, uniform plant growth environment allows the evaluation of genetics and treatment without the complicating effects of weather, soil properties, pests, and other variables.
- Software can analyze root system images and provide measures of root architecture.
- Conventional methods are used for above surface characteristics.
- Integrated analysis of above and below surface plant characteristics provide the whole picture of plant performance

The Power of the Whole Picture

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- ✓ Plant Height
- ✓ Stalk Diameter
- ✓ Leaf Area
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- ✓ Total Dry Weight Biomass
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- ✓ Root Dry Weight Biomass
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