

# NEMATODE SUSCEPTIBILITY RANKINGS FROM SOFT-TISSUE X-RAY IMAGING

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Phenotype Screening  
CORPORATION  
*enabling discovery*

THE UNIVERSITY of  
TENNESSEE **UT**  
KNOXVILLE



Cotton  
Incorporated

# A New Method for Nematode Studies is Being Developed

- The method is based upon soft-tissue X-ray imaging.
- The entire root system of the cotton plant under study is captured in an X-ray image.
- The resolution of the X-ray imaging system allows for reniform nematode egg mass site identification and counting.
- Egg mass location can be tied to specific attributes of the plant's root system architecture.

# Six Cotton Lines from the Auburn Breeding Program, and Two USDA Germplasm Lines Were Evaluated

- Resistant lines: A107, B103, and LONREN-2, BARBREN-713.
- Susceptible lines: A118, A209, B124, B227
- Infection density: 1500 nematodes per 100 cc of soil.
- Plants were grown for six weeks in containers at the UT greenhouse.

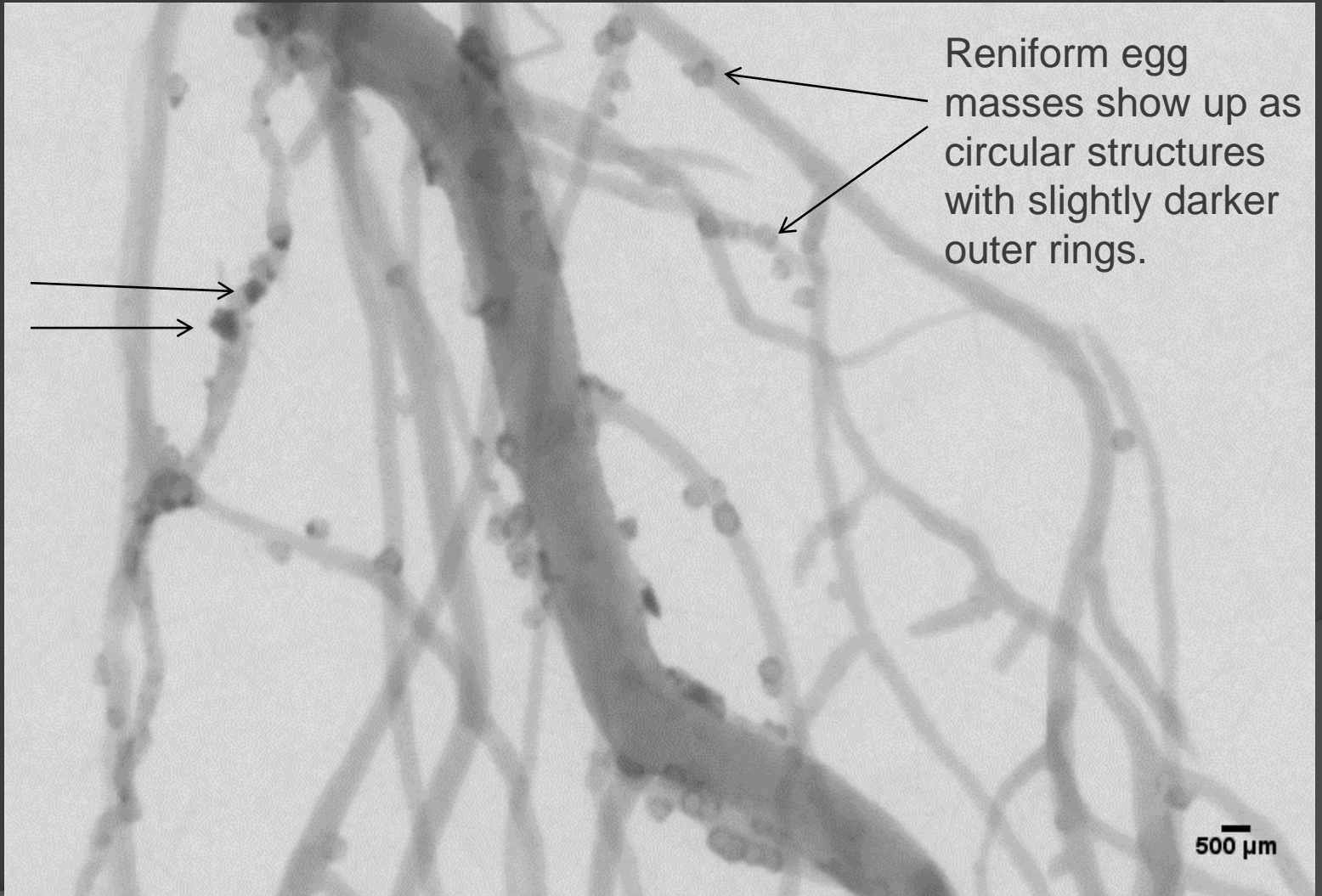


# Root Systems Were Carefully Washed and Prepared for X-ray Imaging



# Soft-tissue X-ray Image of B227 Cotton Root System With Reniform Egg Masses

Soil particles are uniformly dark.



Reniform egg masses show up as circular structures with slightly darker outer rings.

500 μm



# An Analyst Clicks on the Egg Mass Locations Which are Then Logged

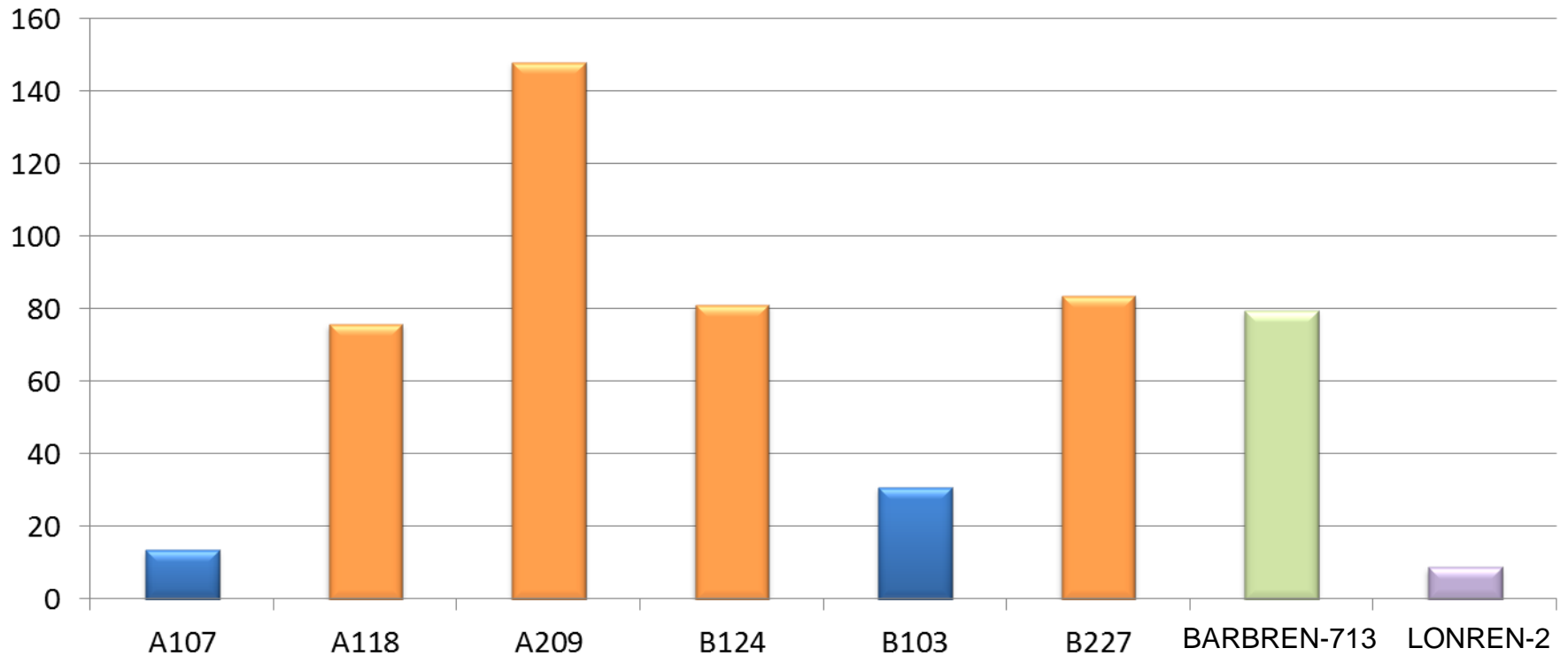
The screenshot shows the ImageJ software interface. The main window displays a grayscale image of a biological specimen, likely a branch or stem, with several colored crosshair markers (red, green, blue, yellow, orange) placed on it. The image is titled "B227\_120507\_cyan\_00Cropped8.tif (200%)". The status bar at the bottom left indicates "770x527 pixels; 8-bit; 396K".

An adjacent text window titled "B227\_120507\_cyan\_00Cropped8.txt" displays a log of the markers. The log contains the following data:

point	x	y	slice	color	ID
0	451	503	1	0	1
1	440	500	1	1	2
2	430	498	1	2	3
3	420	492	1	3	4
4	414	474	1	4	5
5	405	469	1	5	6
6	382	475	1	6	7
7	393	455	1	7	8
8	365	449	1	8	9
9	452	404	1	9	10
10	448	392	1	10	11
11	426	305	1	11	12
12	407	290	1	12	13
13	492	339	1	13	14
14	153	133	1	15	16
15	416	412	1	16	17
16	310	360	1	17	18
17	303	331	1	18	19
18	304	323	1	19	20
19	287	310	1	20	21
20	301	308	1	21	22
21	302	298	1	22	23
22	227	330	1	23	24
23	295	262	1	24	25
24	472	173	1	25	26
25	459	158	1	26	27
26	446	135	1	27	28
27	431	135	1	28	29
28	414	132	1	29	30
29	286	181	1	30	31
30	309	131	1	31	32
31	228	162	1	32	33
32	279	98	1	33	34

# Egg Mass Count by Line

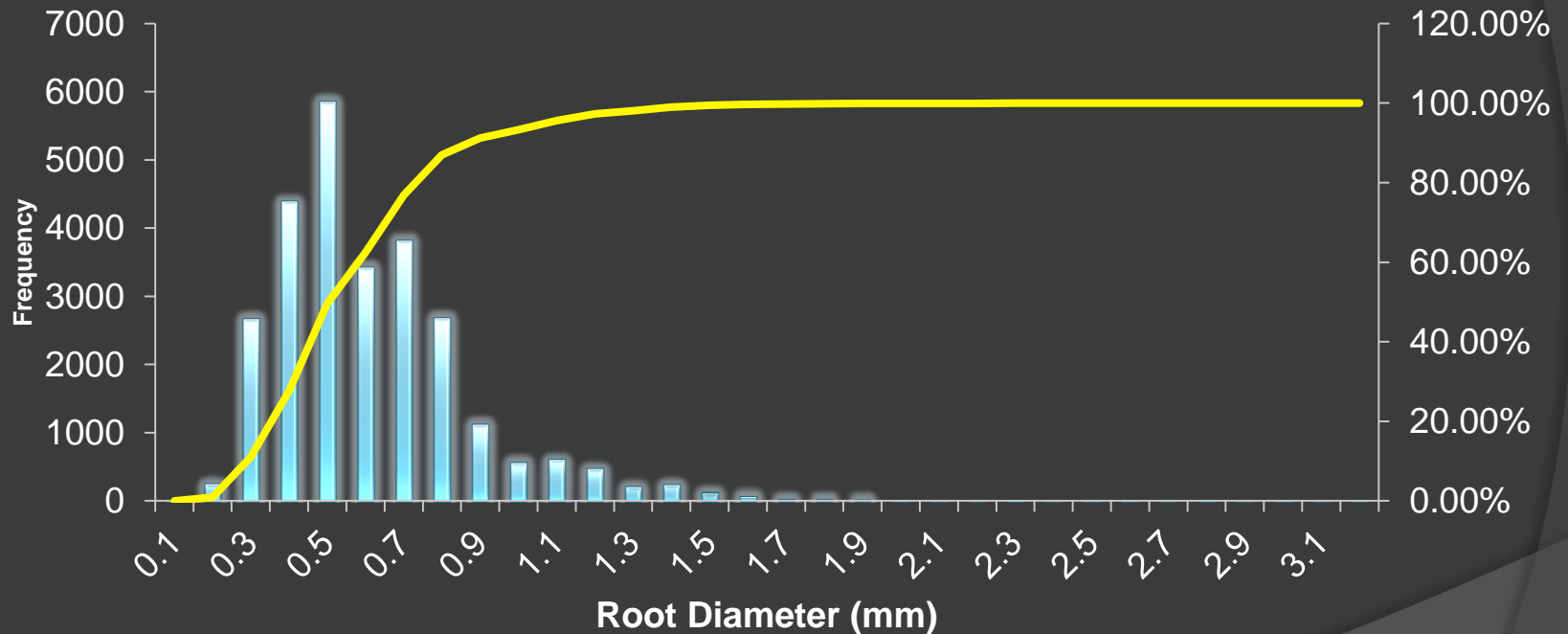
Mean Egg Mass Count by Line



Resistant  
Susceptible

# At Six Weeks the Cotton Plants Tended Towards Smaller Root Diameters

Root Diameter and Cumulative % Distribution of all Plants





# The Root System Is Analyzed by Root Diameter Ranges (Size Classes)



Original X-ray  
of root system



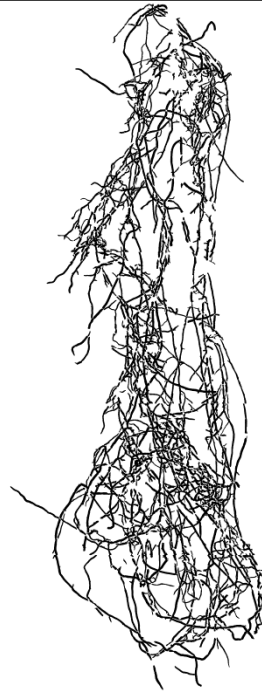
1,392u – 2,900u  
diameter roots

**Size Class 3**



696u – 1,450u  
diameter roots

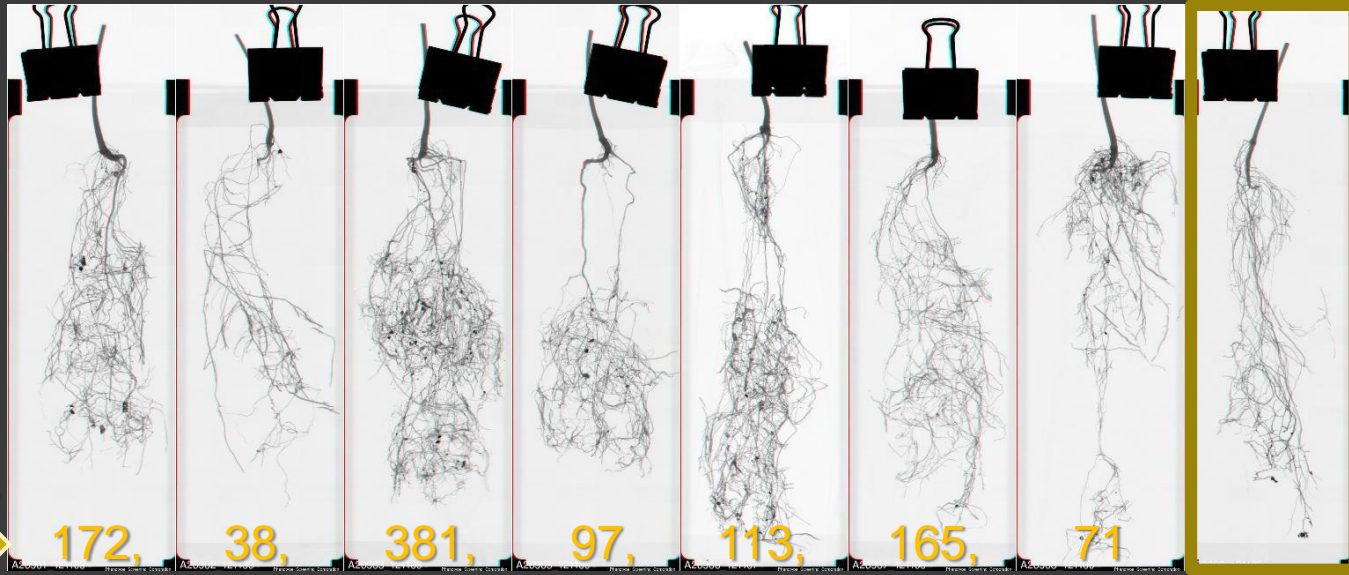
**Size Class 4**



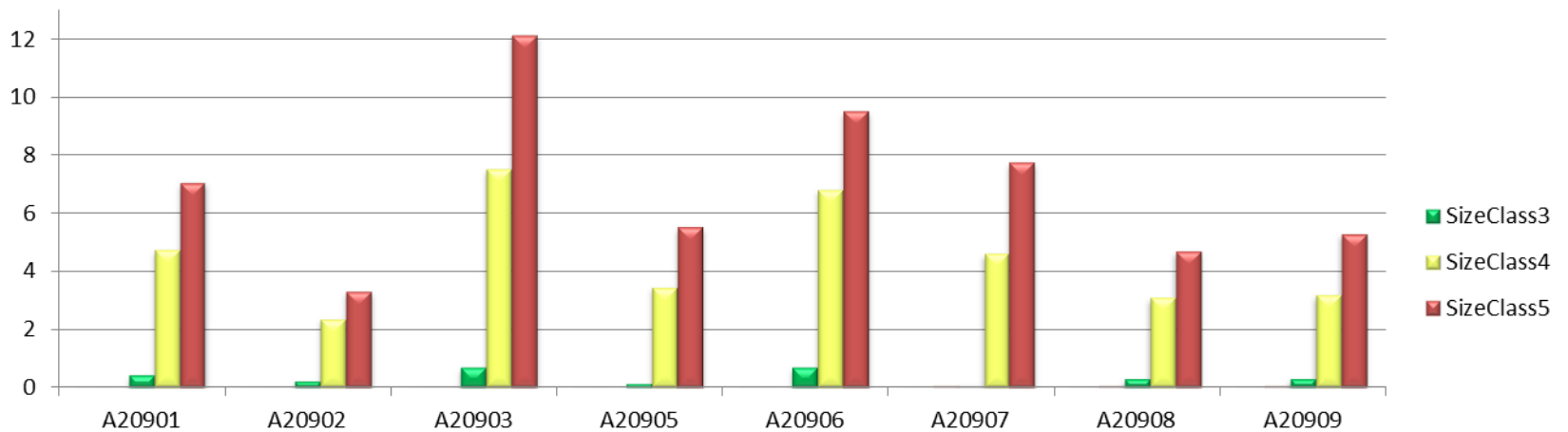
348u – 725u  
diameter roots

**Size Class Five**

# Susceptible Line A209

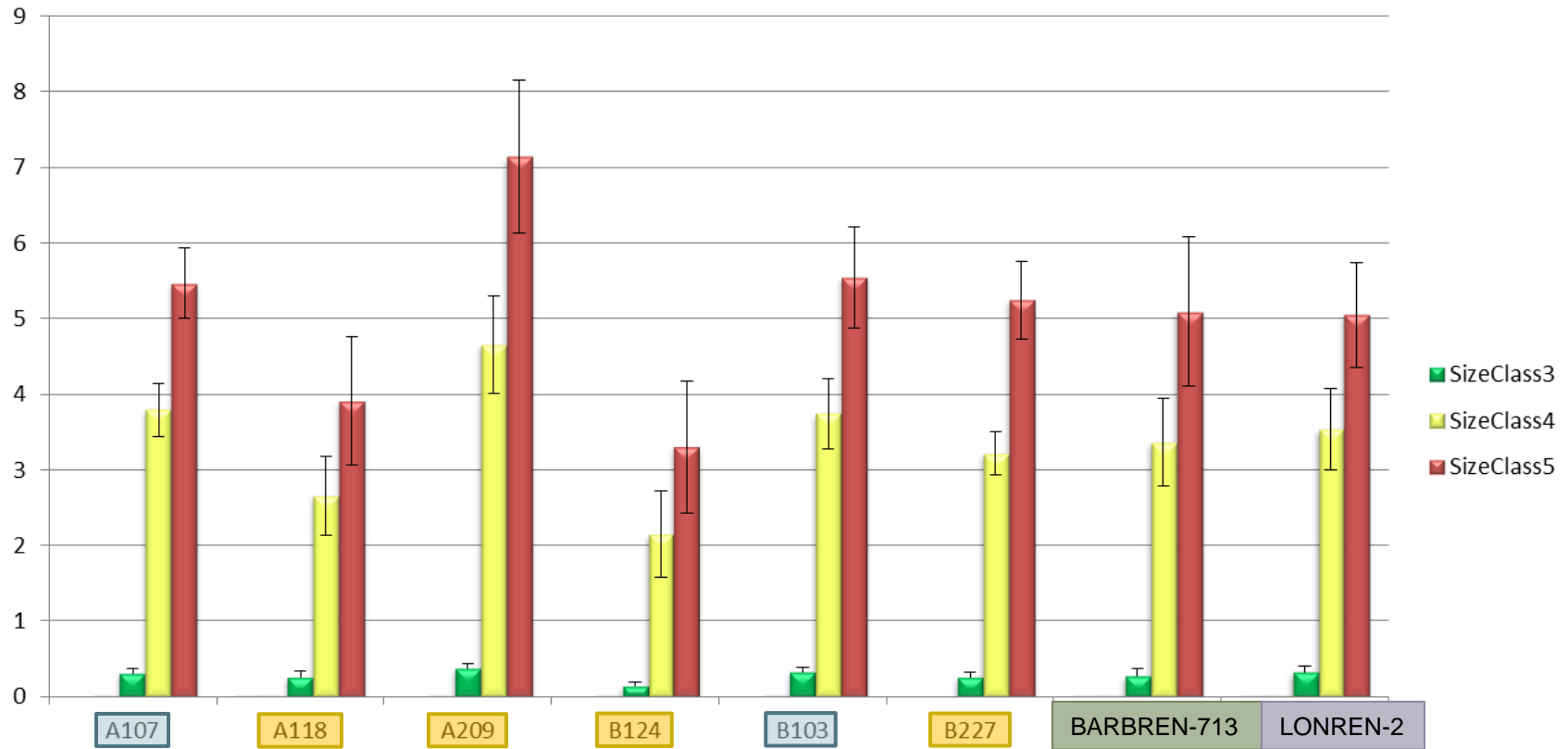


Total Root Length (m) of A209 Line by Root Size Class



# Total Root Length Line Signatures

Mean Total Root Length (m) of Cotton Lines by Root Size Classes  
(all infected plants) w/Standard Error bars



Shown with standard error bars

Resistant Line

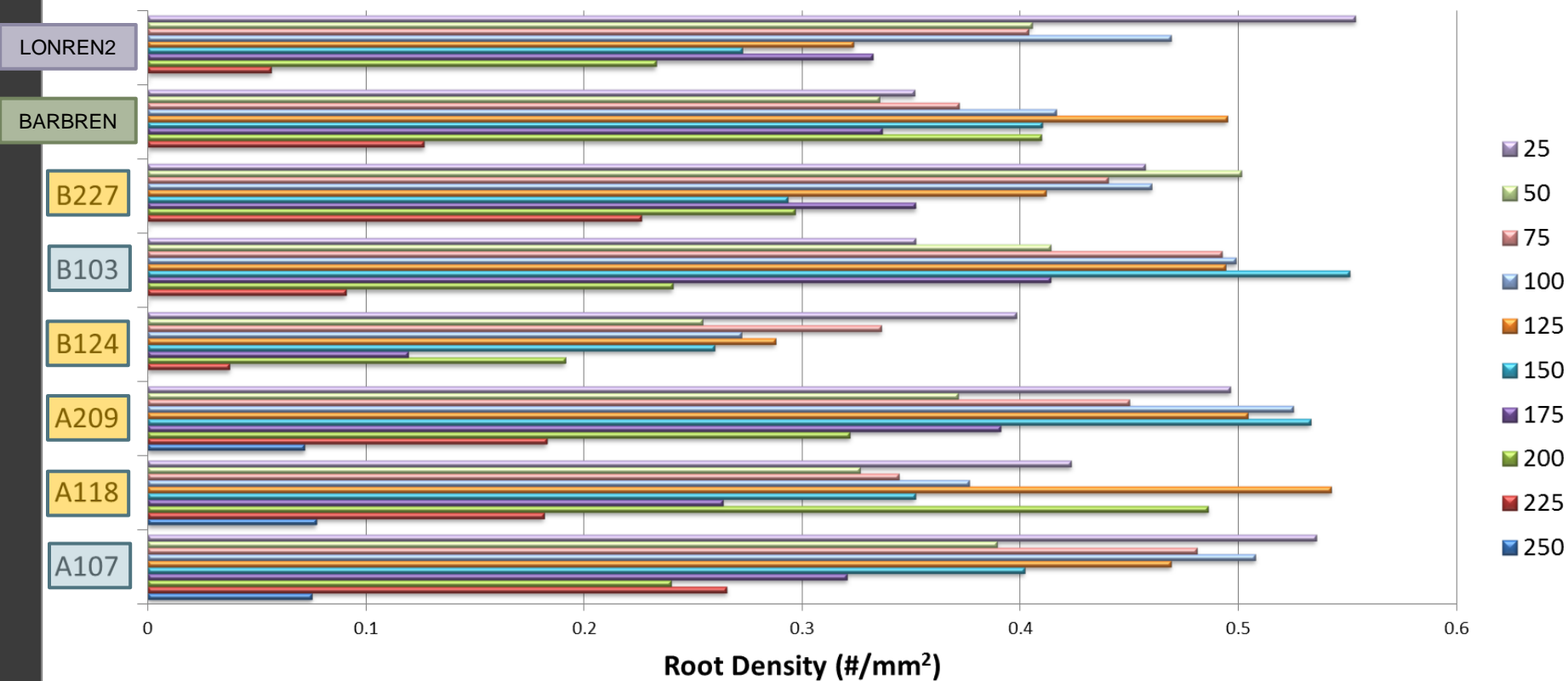
Susceptible Line

Resistant Line

Resistant Line

# Size Class 5 (348u - 725u) Root System Distribution by Depth

Mean Root Density (#/mm<sup>2</sup>) of Size Class 5 Roots for Each Line by Root Depth (mm)  
(infected plants only)



Resistant Line

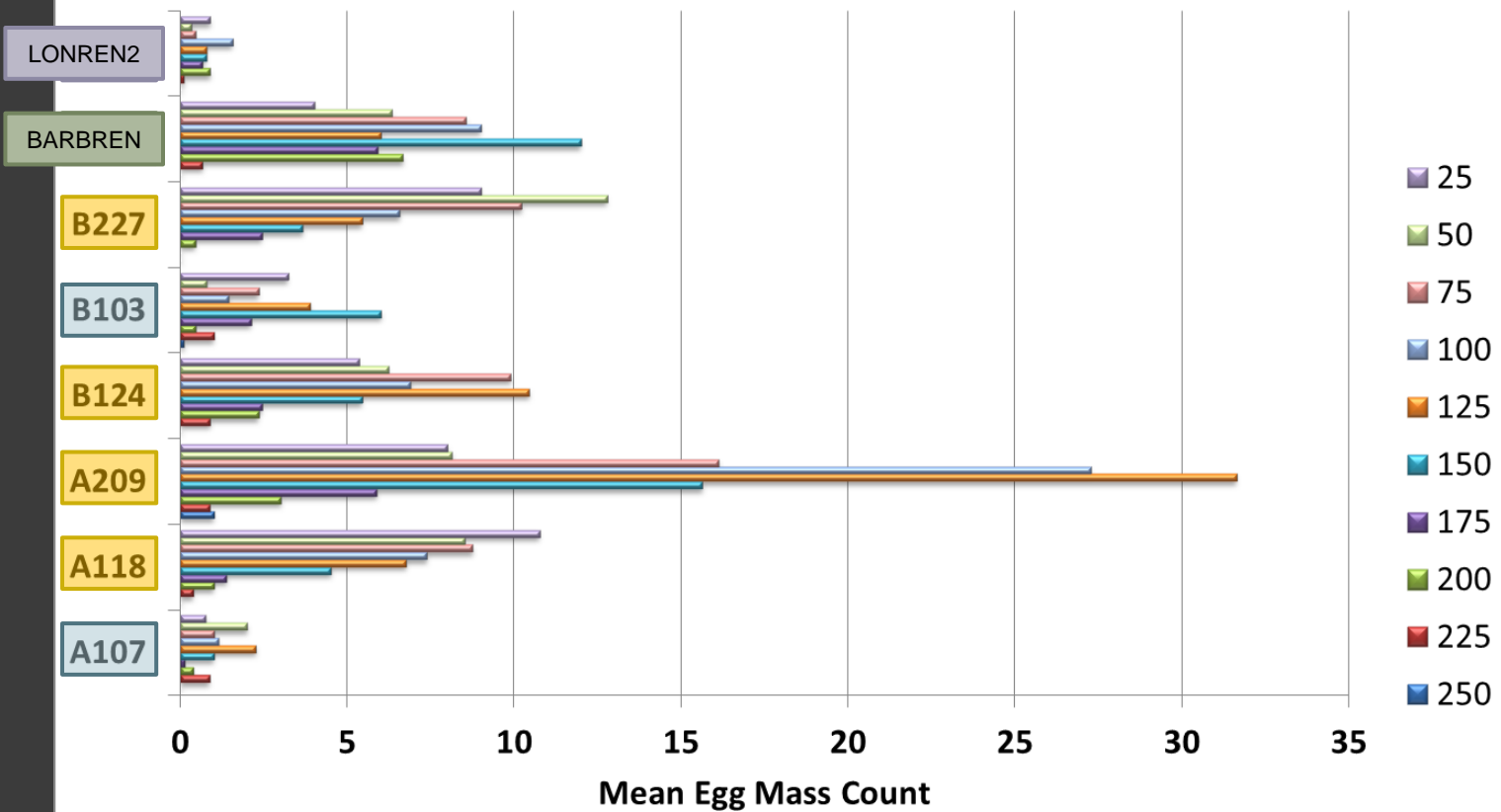
Resistant Line

Resistant Line

Susceptible Line

# Mean Egg Mass Count of Each Line by Root Depth

Mean Egg Mass Count of Each Line by Root Depth (mm)  
(infected plants only)



Resistant Line

Resistant Line

Resistant Line

Susceptible Line

# Normalizing Counts to Root Length

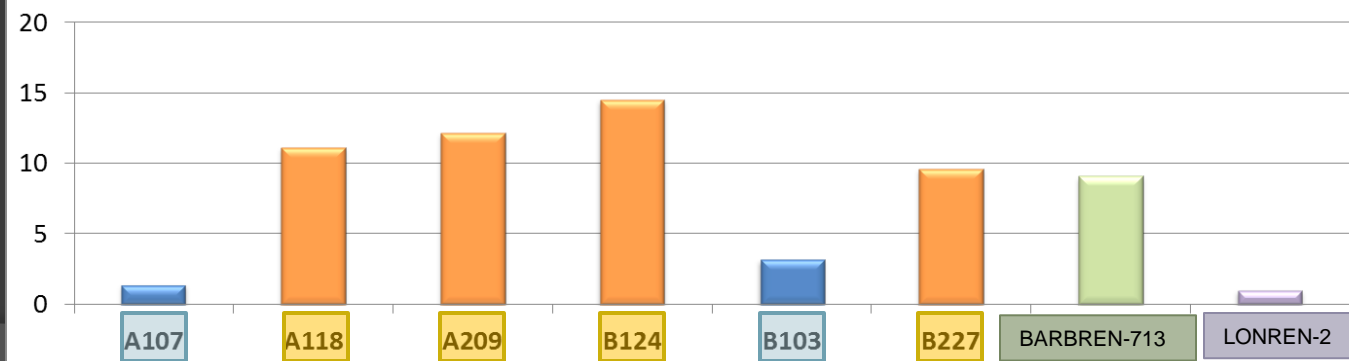
Mean Total Root Length (m) of Cotton Lines by Root Size Classes (all infected plants)



Mean egg mass count

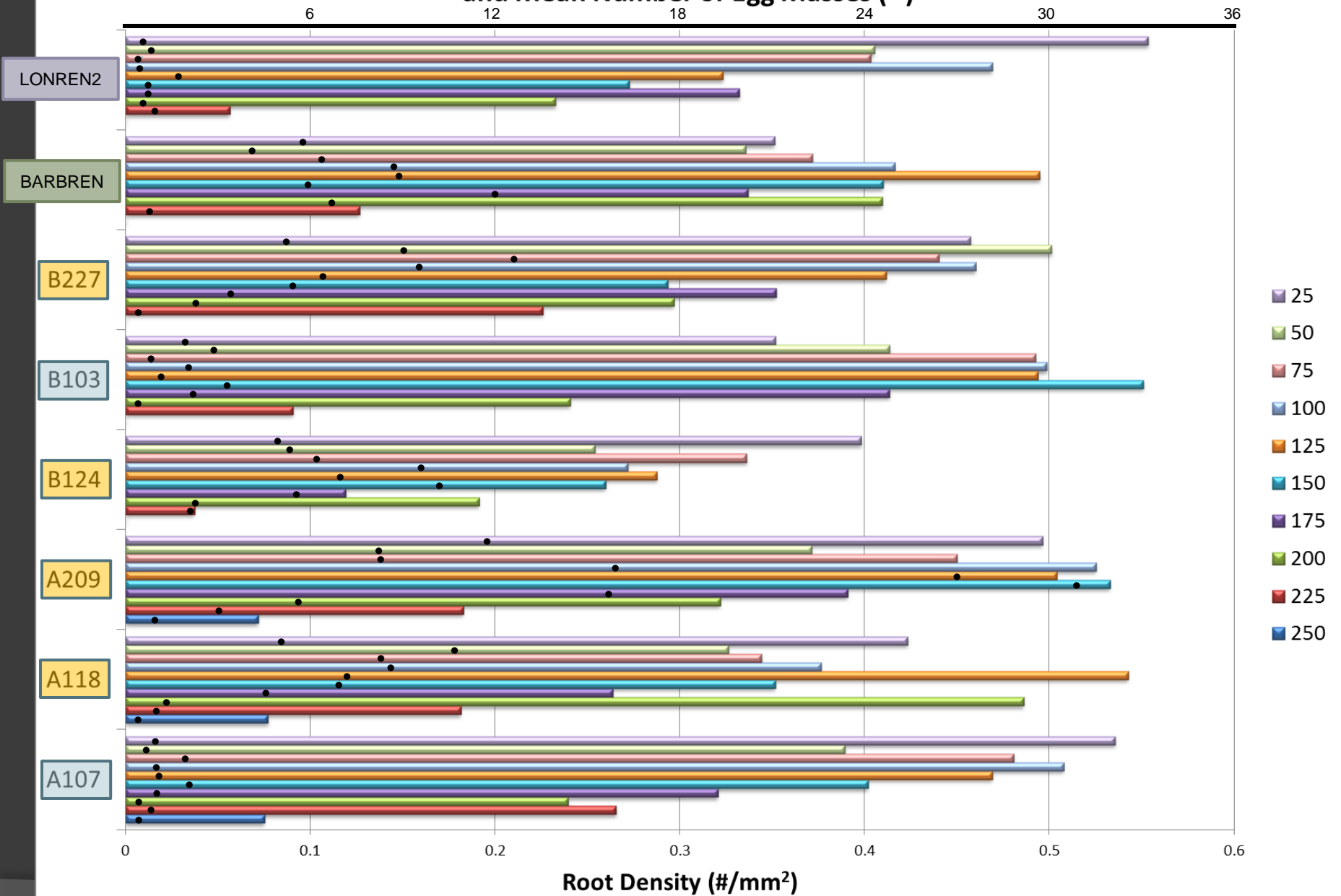
➔ 13.6, 76, 148.1, 81.2, 30.9, 83.7, 79.6, 8.9

Egg Mass Count per Unit Root Length





# Mean Root Density (#/mm<sup>2</sup>) of Size Class 5 Roots for Each Line by Root Depth (mm) and Mean Number of Egg Masses (●)



# Study Conclusions

- The resistant lines A107 and B103 supported low nematode reproduction.
- The resistant germplasm line BARBREN-713 supported higher nematode reproduction.
- All lines had unique root system architecture signatures, supported varying degrees of nematode reproduction and had unique nematode feeding site distributions.
- When normalized for total root length, of the susceptible varieties B124 ranked the most susceptible and B227 the least.

# Method Summary

- X-ray based method captures root architecture and reniform nematode egg mass distribution in a single image.
- Screening costs are reduced due to elimination of egg extraction step and to automated root architecture analysis. (Egg mass counting can be automated, further reducing costs.)
- Root development and nematode distributions can be studied in an integrated manner.