

AUTOMATED X-RAY IMAGE ANALYSIS METHODS FOR INVESTIGATING ROOT ARCHITECTURE OF CORN ROOTWORM RESISTANT AND SUSCEPTIBLE MAIZE VARIETIES.

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Corn Rootworms (Genus *Diabrotica*) Are A Major Insect Pest Of Maize



Non-transgenic Corn Rootworm Resistance Includes Three Mechanisms

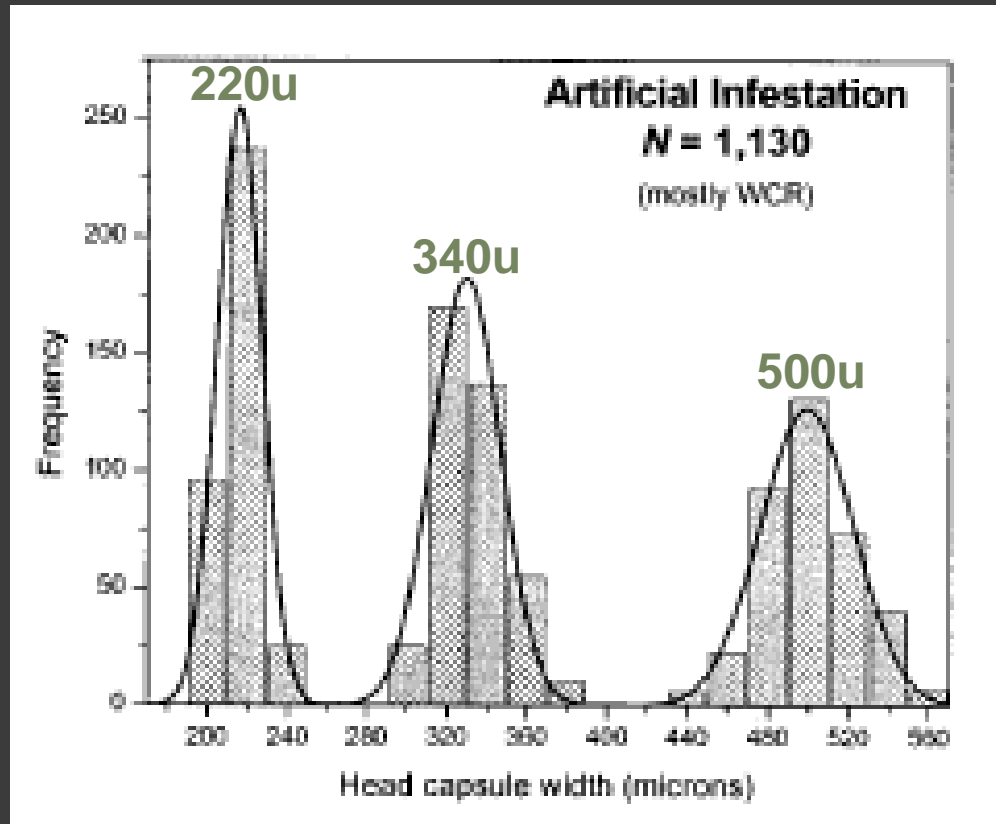
- Non-preference
 - A lack of attractiveness as an oviposition or feeding site
- Antibiosis
 - Increased mortality or impeded feeding and growth of larvae
- Tolerance
 - Ability to withstand a level of feeding without compromising yield or quality

(Painter, 1951)

Tolerance is the Main Resistance Mechanism to Larval Feeding Screened for in Maize

- ◎ Large, densely branched root systems
- ◎ Improved root re-growth
 - may negatively impact yield when adequate moisture is present

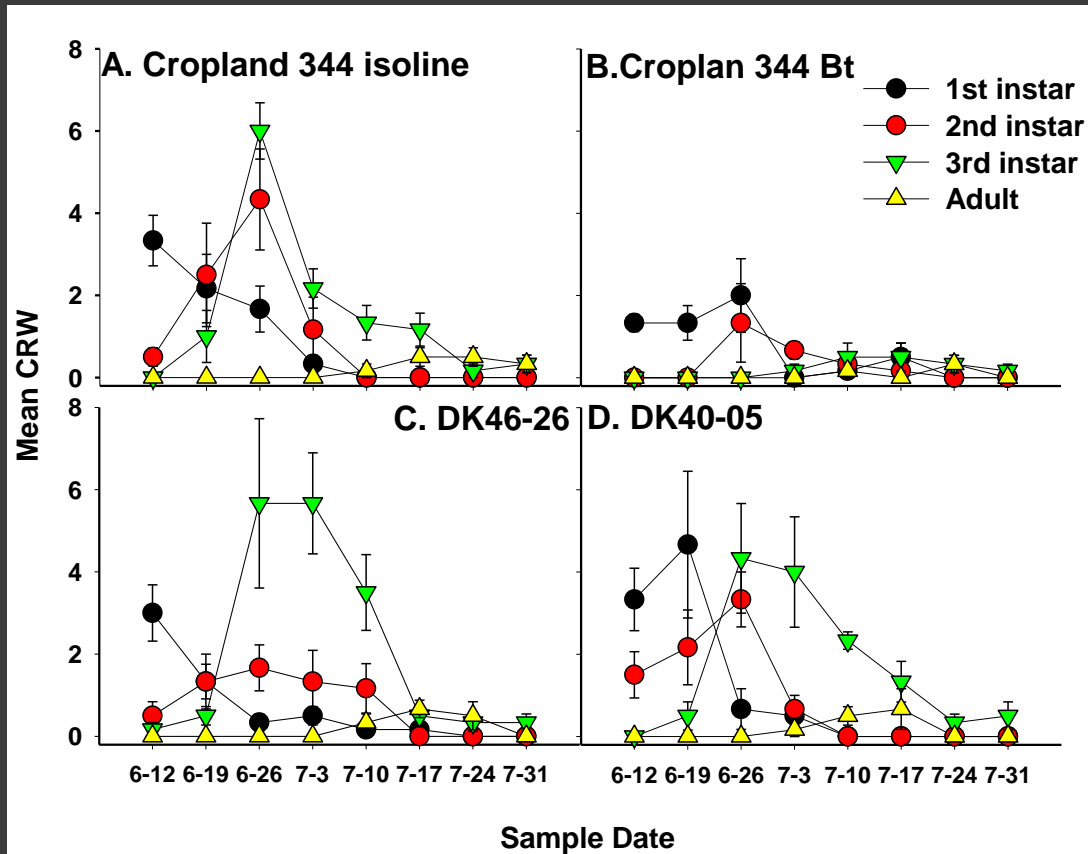
Maize Phenology Affects Corn Rootworm Establishment, Damage and Development



Larvae increase in size as they develop and their feeding preference changes as they grow.

Hibbard 2008,
Hammack 2003

Corn Rootworm Larvae Develop Through Three Larval Feeding Stages



Each instar lasts approximately seven to ten days for an individual insect.

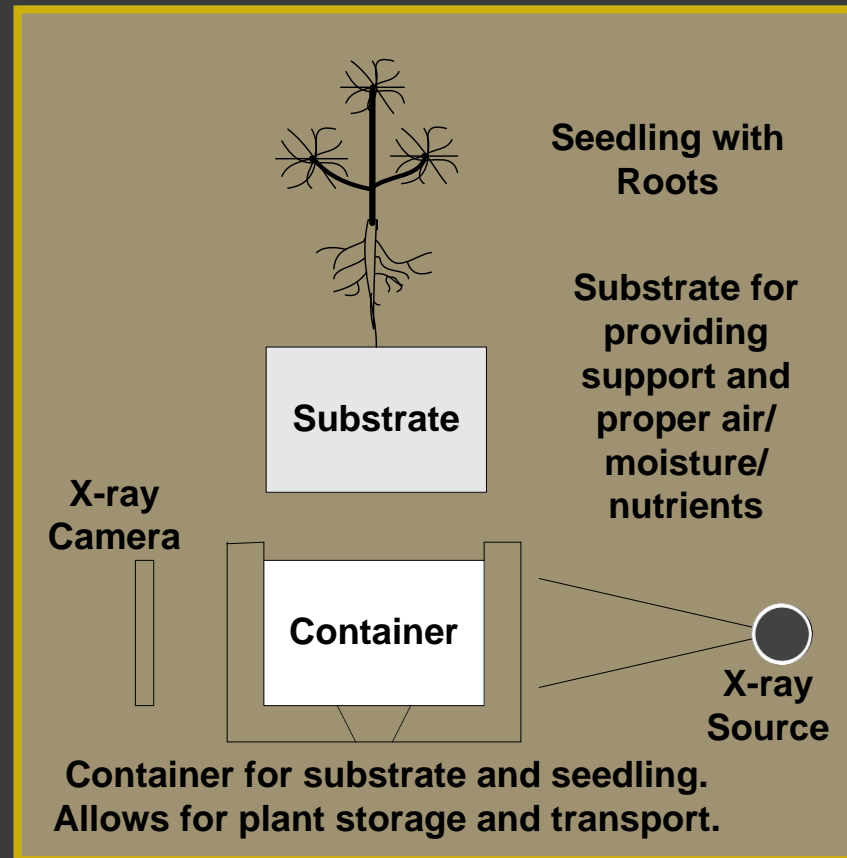
Lehman et al, 2010

We Selected Synthetic Maize Populations Previously Evaluated in 2006

Variety	Source	Comment
BS29-11-1	Iowa State University	susceptible in MO, derivative of BS29
BS29-07-1	Iowa State University	susceptible in MO, derivative of BS29
CRW8-3	USDA ARS, Columbia, MO	high resistance in MO, derived from BS19/20
CRW3(C6);	USDA ARS, Columbia, MO	high resistance derived from Hibbard et al 1999

Resistance / susceptibility assessed in MO trials using root damage ratings.

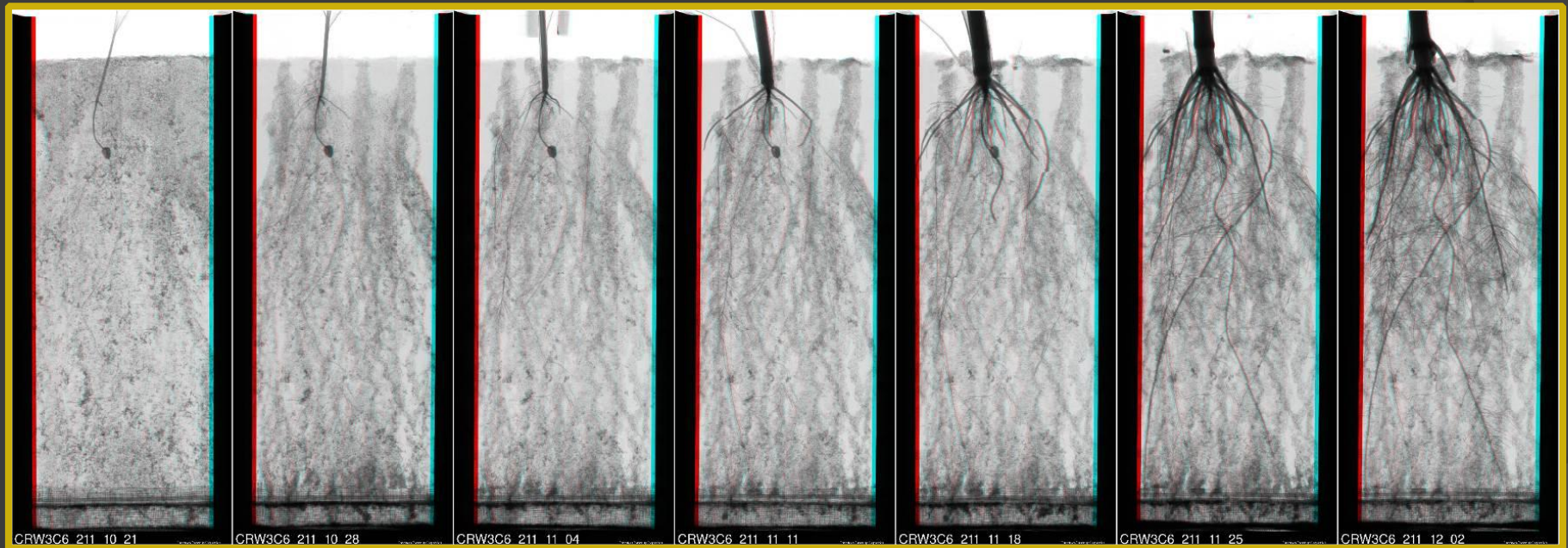
We Used “Soft-tissue” X-ray Imaging to Study Root Development



The Four Selected Maize Varieties Were Grown at PSC Using Their Plant Growth System

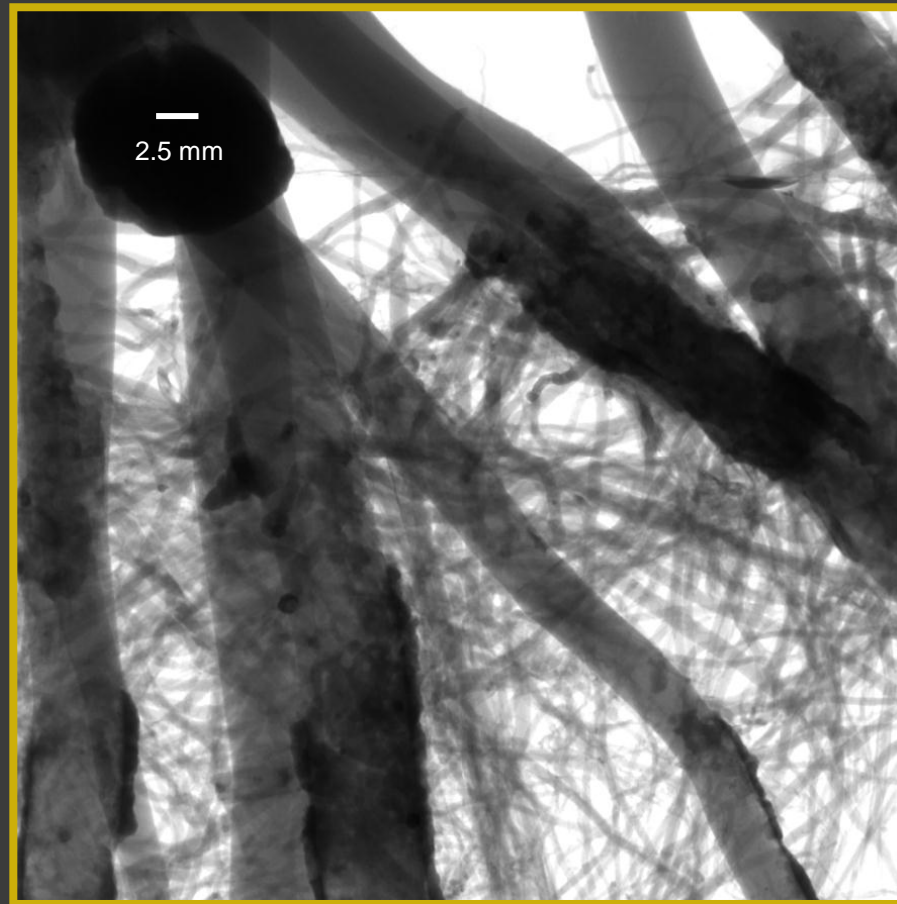


Soft-tissue X-ray Imaging Allows For Non-Destructive Root Characterization

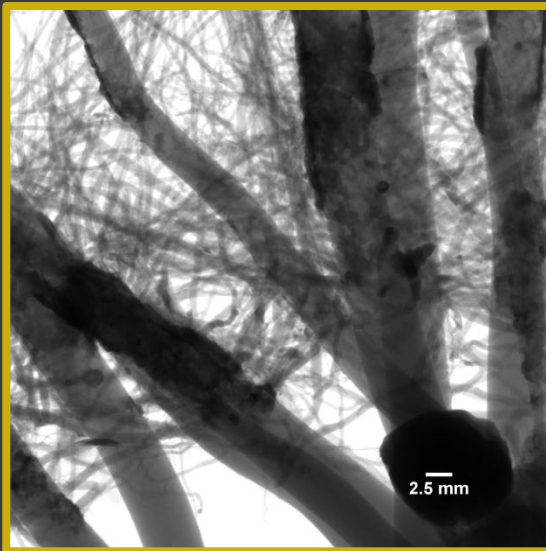


Our analysis was conducted on the plants imaged during these weeks.

Manually Characterizing the Root System Architecture of Mature Plants Is Tiresome



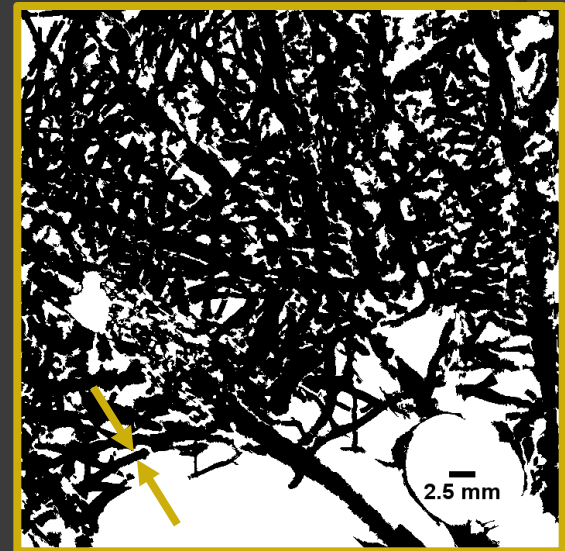
Automated Methods Were Developed to Characterize the RSA From X-ray Images



Original X-ray image



Binary image of roots
from 1.5mm to 10mm



Binary image of roots
from 0.4mm to 2.5mm

These Automated Methods Were Applied to Our Maize Varieties

Global Traits

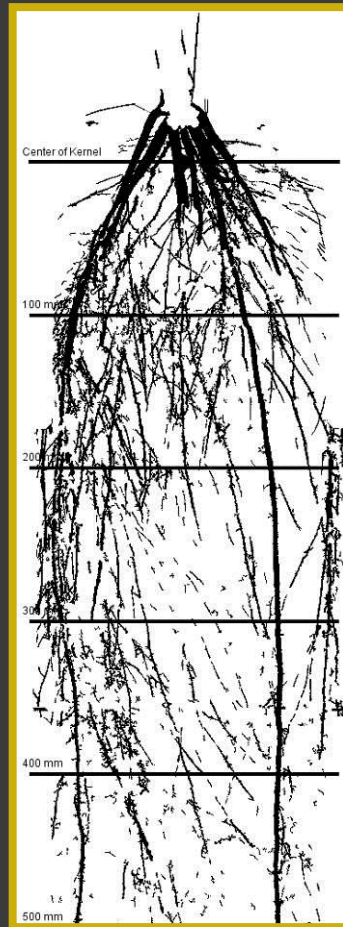
- Projected Root Area,
- Total-Root-Length,
- Total-Root-Length Density,
- Traits at Specific Depths
- Root location,
- Root diameter,
- Root cross-sectional area (~mass),
- Root Count Density

The screenshot shows a Microsoft Excel spreadsheet titled 'Master Weekly datashaken5x17.xls'. The data is organized into columns representing different root system characteristics. Key columns include:

- Root System Class (Coarse, Medium, Fine)**: Categorized by Root Length (TL), Root Diameter (TD), Root Density (RD), and Root Volume (RV).
- Characterization of Specific Root Crossing**: Includes Root Length (TL), Root Diameter (TD), Root Density (RD), Root Volume (RV), and Root Mass (RM).
- Characterization of Root System at Specific Depths**: Includes Root Length (TL), Root Diameter (TD), Root Density (RD), Root Volume (RV), and Root Mass (RM).
- Key Experimental Discriminators**: Includes Root System Class, Root System at Specific Depths, and Root Mass.

The table contains approximately 60 rows of data, each representing a different maize variety or treatment. The data is presented in a structured format with clear column headers and numerical values.

Automated Analysis: Spatial Traits Were Determined at Predefined Transects



We used the center of the corn kernel as our reference point in our transect analysis.

For Phenology Study Purposes We Analyzed All Images By Root Size Class

- Five root diameter size classes were investigated.

• Size Class Five	“Fine”	181u - 616u
• Size Class Four		362u - 1,232u
• Size Class Three	“Medium”	725u - 2,465u
• Size Class Two		1,450u - 4,930u
• Size Class One	“Coarse”	2,900u - 9,860u

- Three of the classes were non-overlapping and were used in the following global traits characterization.

Root System Architecture Was Analyzed by Size Classes



Coarse
2,900u - 9,860u

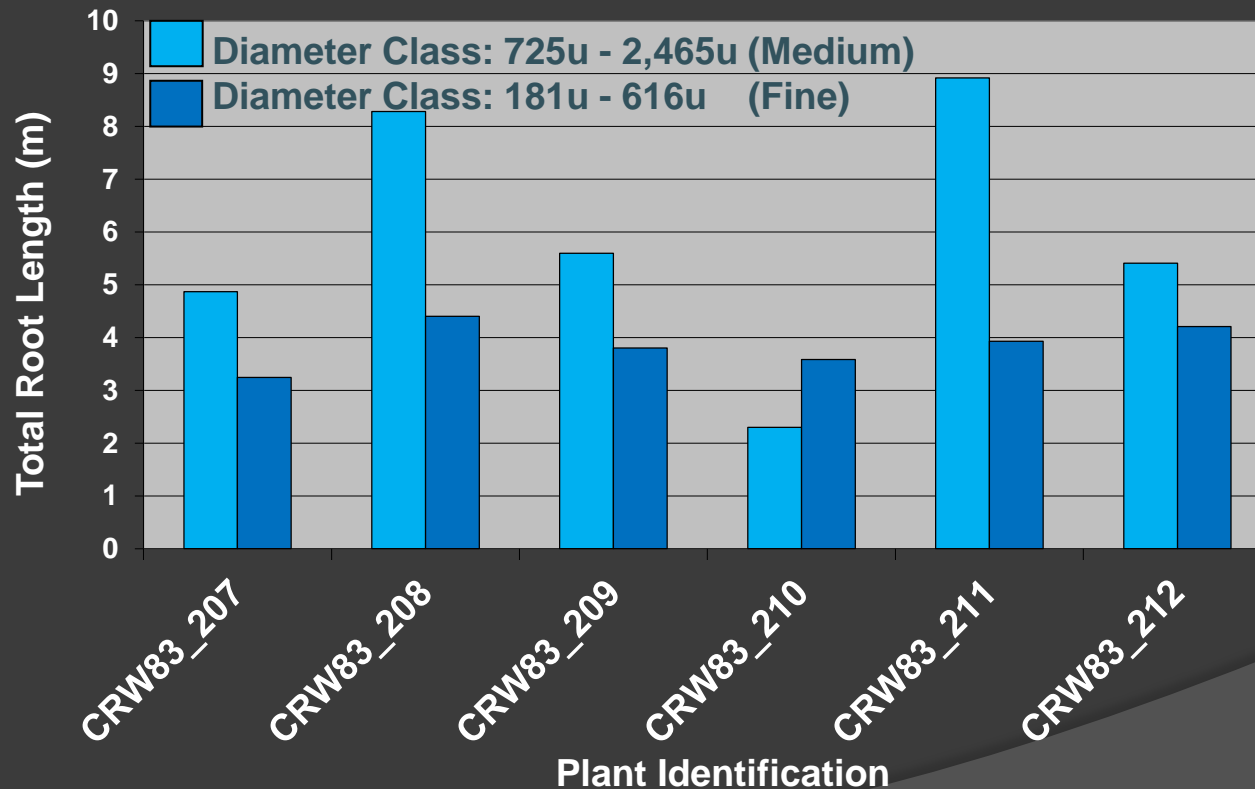
Medium
725u - 2,465u

Fine
181u - 616u

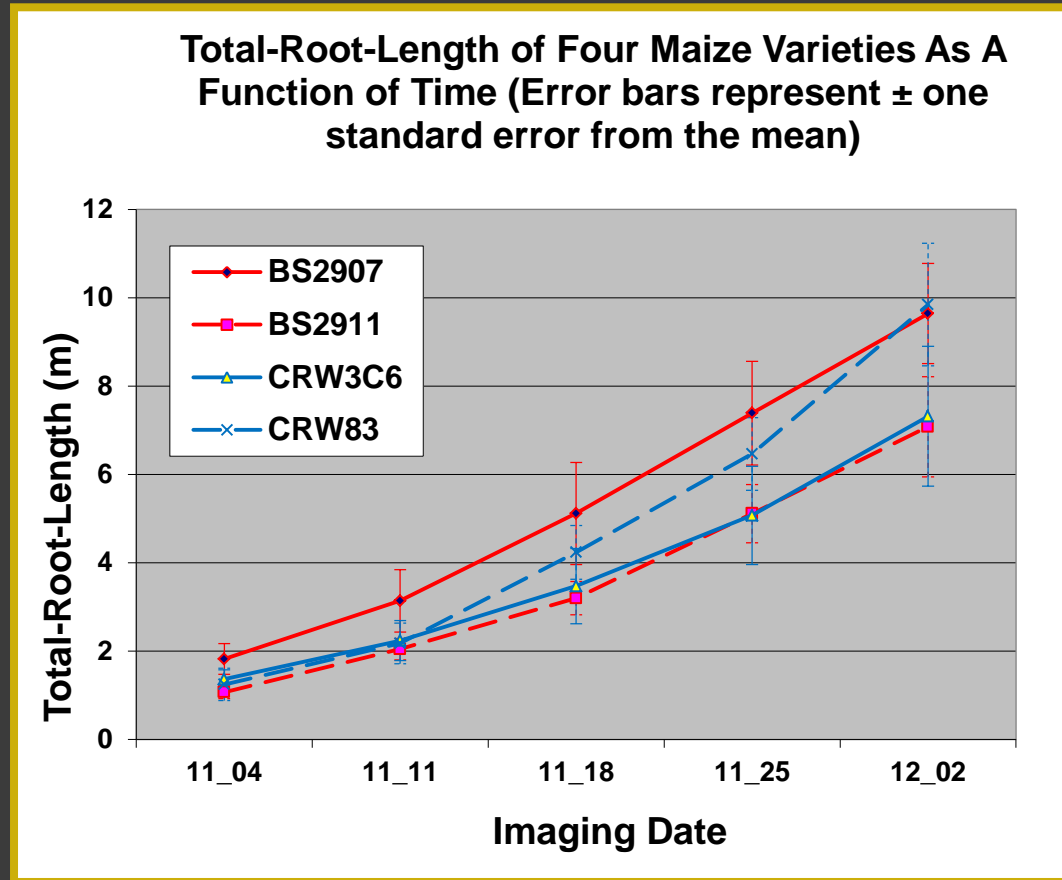
Composite

Total-Root-Length Of Non-overlapping Root Diameter Classes (Intra-Variety Differences)

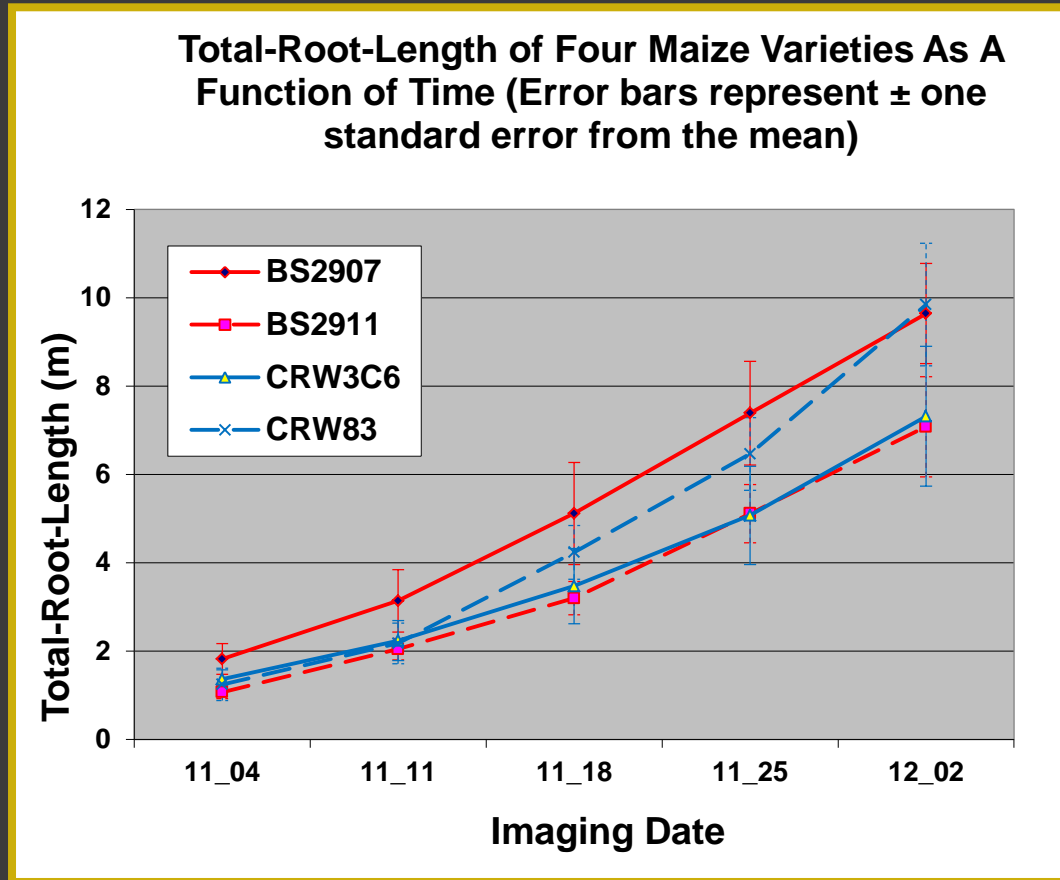
Comparison of Total-Root-Length of Root Diameter Classes for Six Replicates of Maize Variety CRW83 at Week Seven



Total-root-length Of The Four Varieties As A Function Of Time w/ Standard Error

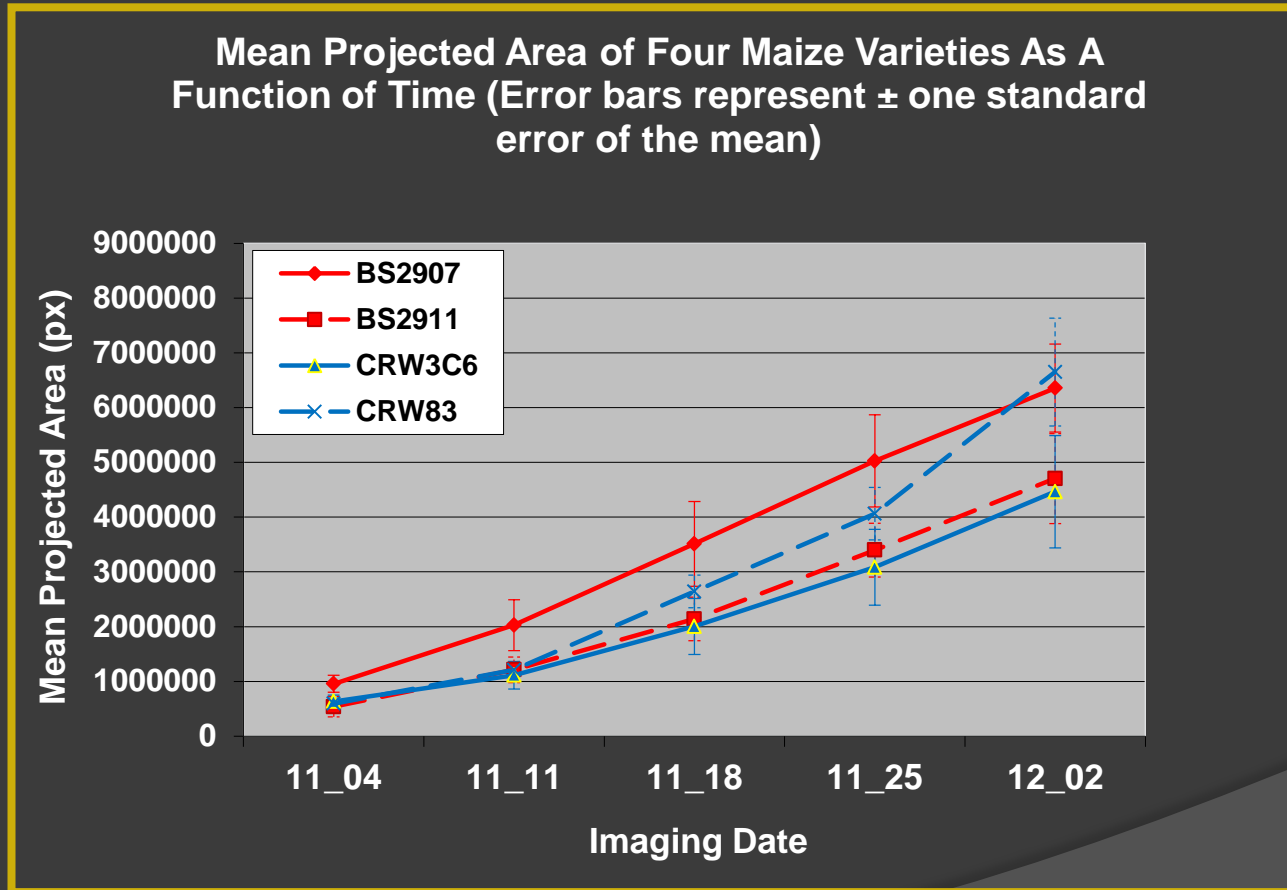


Total-root-length Of The Four Varieties As A Function Of Time w/ Standard Error

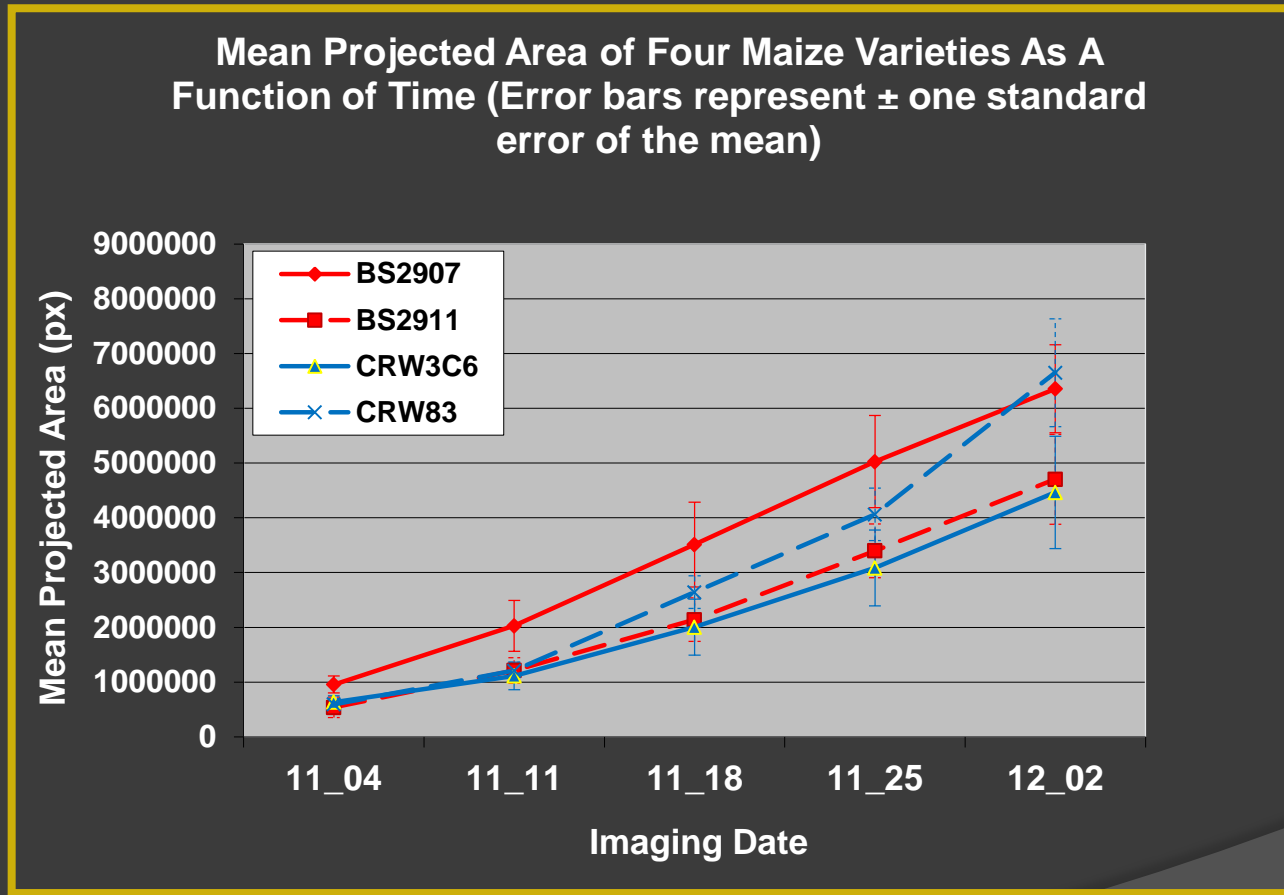


During periods of larval feeding the overall size of the root system is not a discriminator of resistance.

Projected Area Of The Four Varieties As A Function Of Time w/ Standard Error

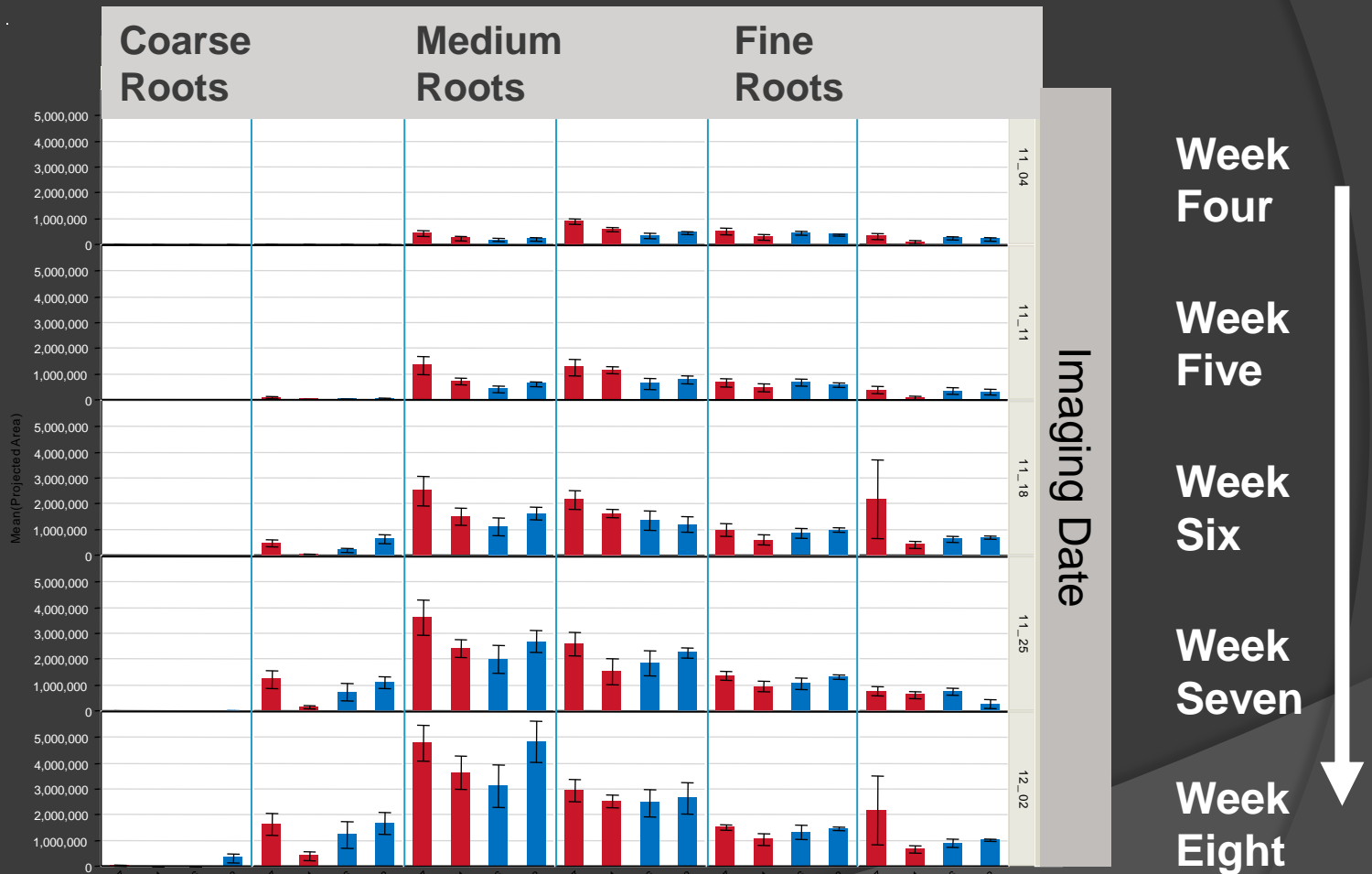


Projected Area Of The Four Varieties As A Function Of Time w/ Standard Error

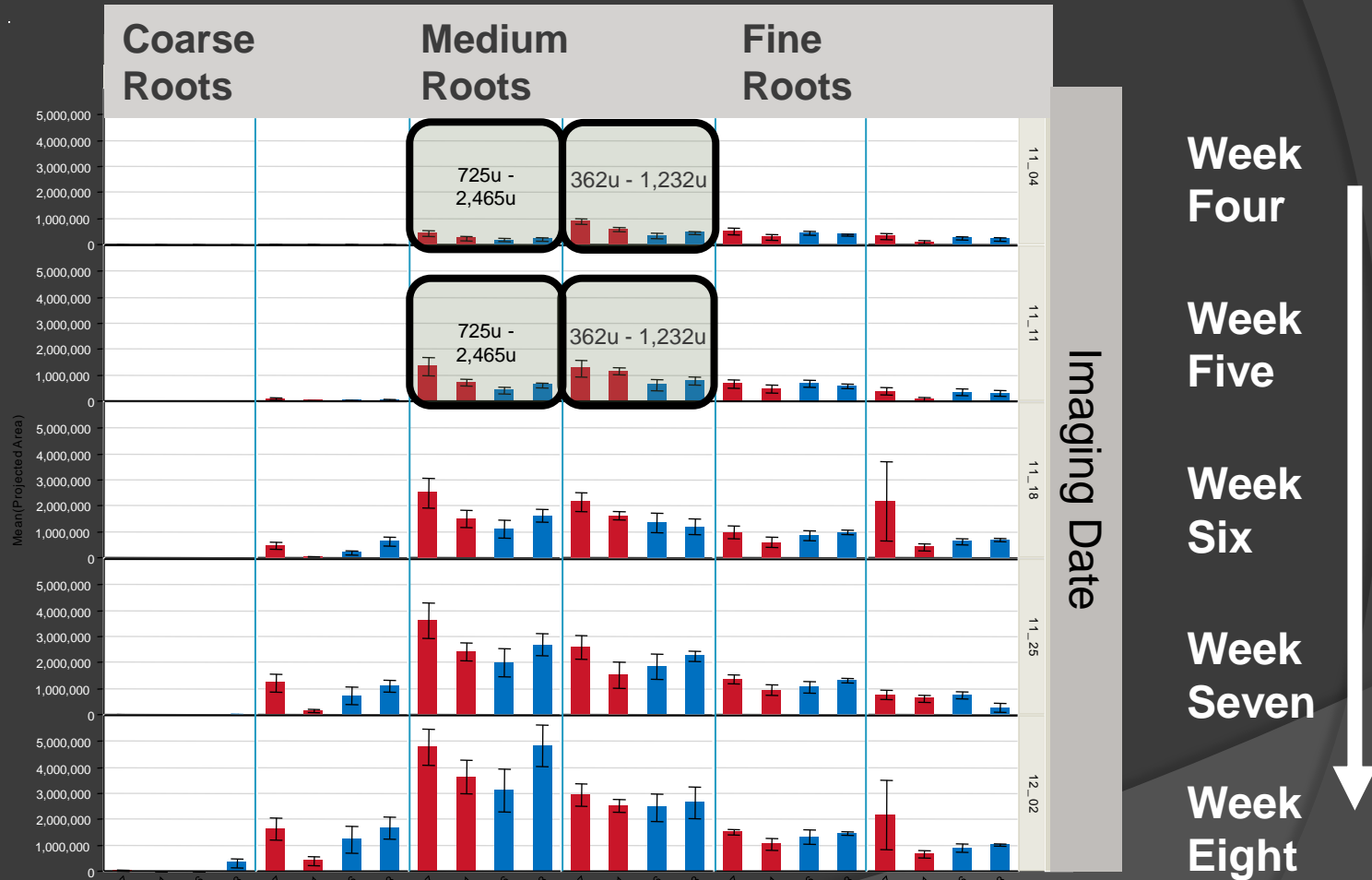


Again, during periods of larval feeding the overall size of the root system is not a discriminator of resistance.

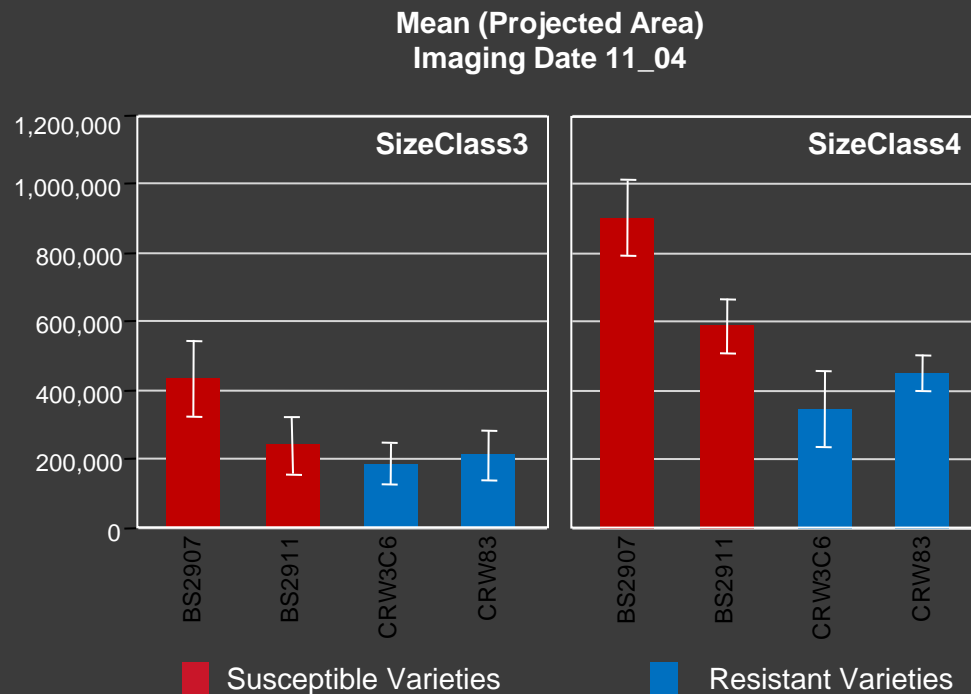
Mean Projected Area by Imaging Date as a Function of Root Size Class



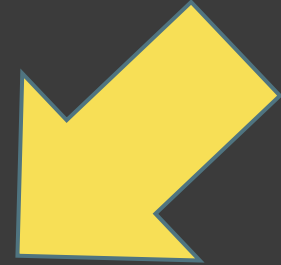
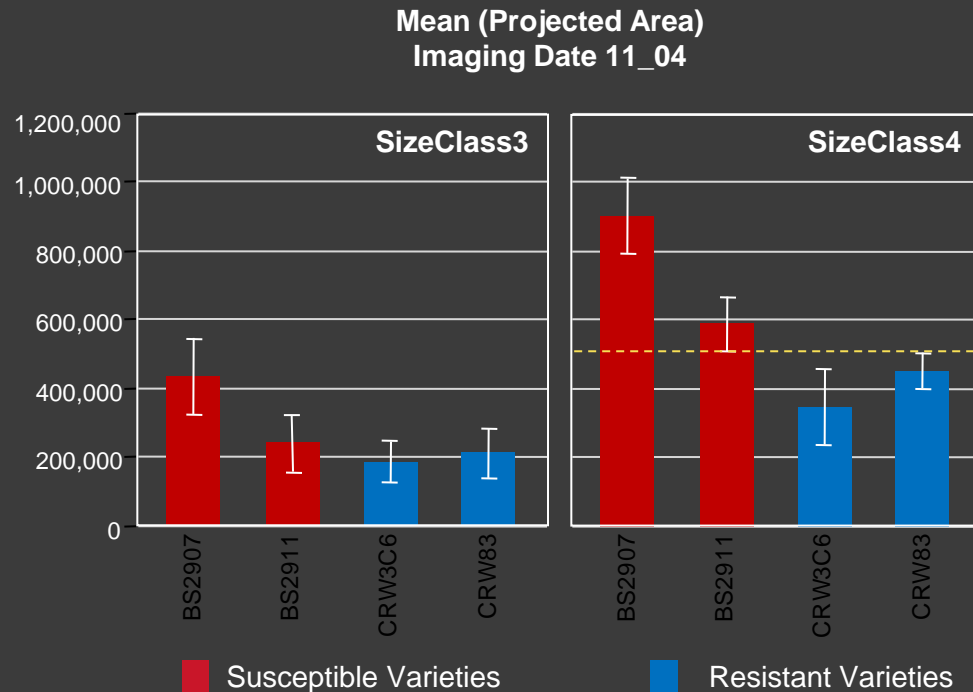
Mean Projected Area by Imaging Date as a Function of Root Size Class



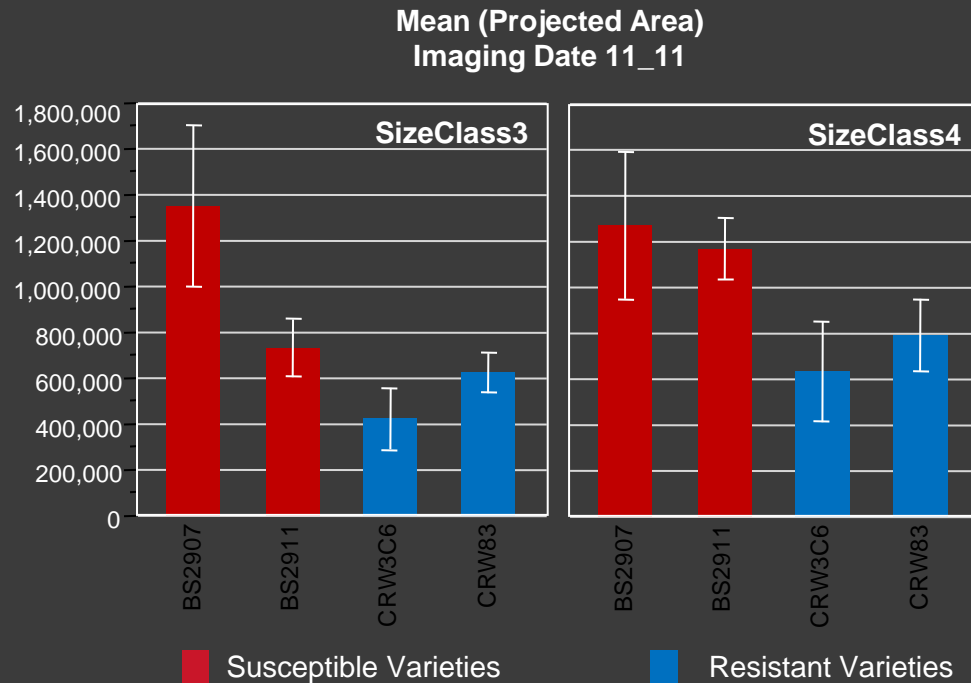
Mean Projected Area by Week as a Function of Root Size Class



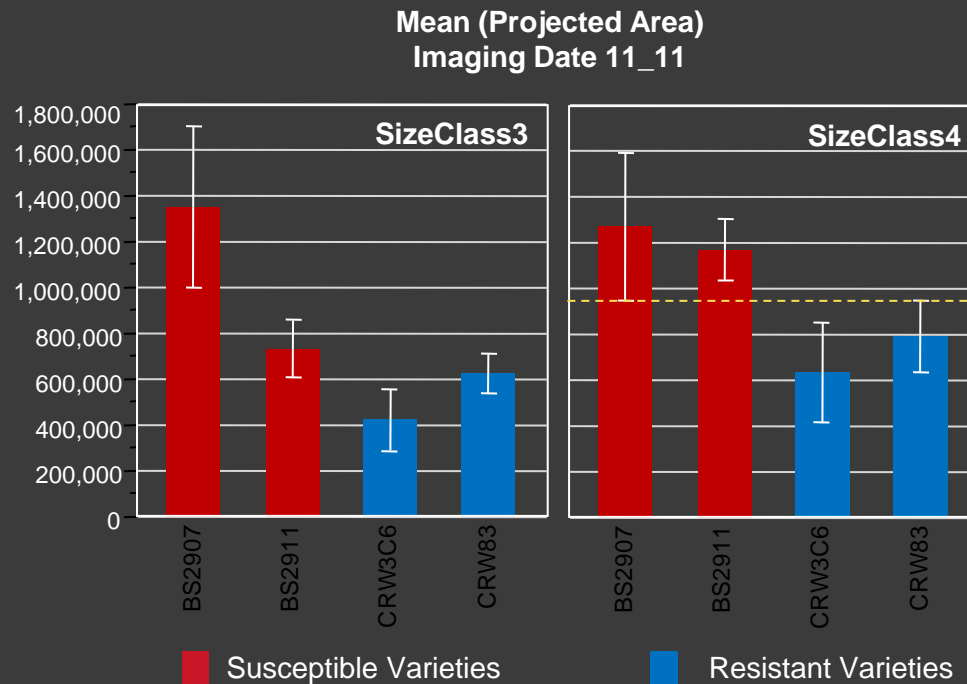
Mean Projected Area by Week as a Function of Root Size Class



Mean Projected Area by Week as a Function of Root Size Class



Mean Projected Area by Week as a Function of Root Size Class

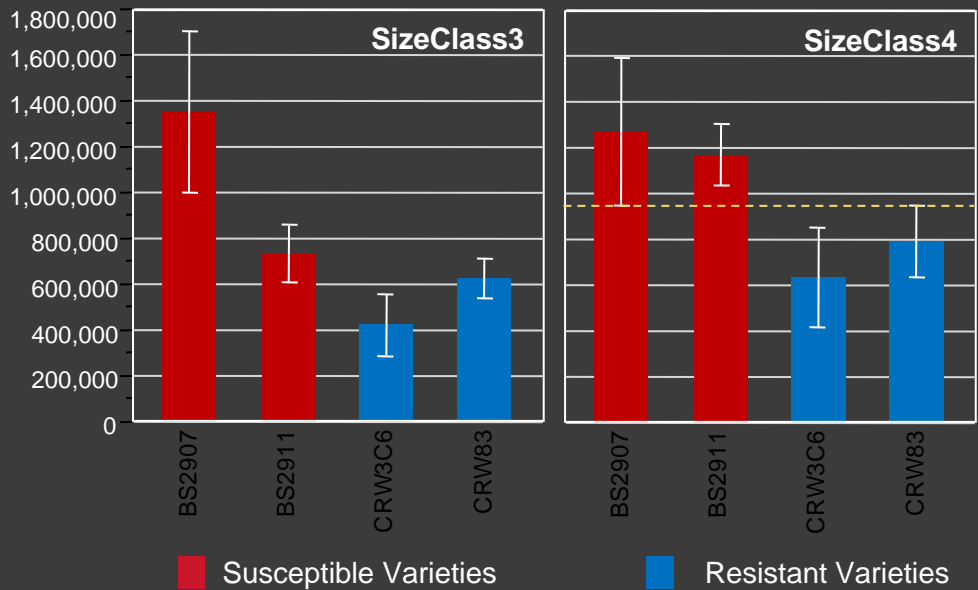


There appear to be more fine to medium roots during the fourth and fifth weeks in the susceptible varieties.
(362u- 1,232u)

Mean Projected Area by Week as a Function of Root Size Class



Mean (Projected Area)
Imaging Date 11_11



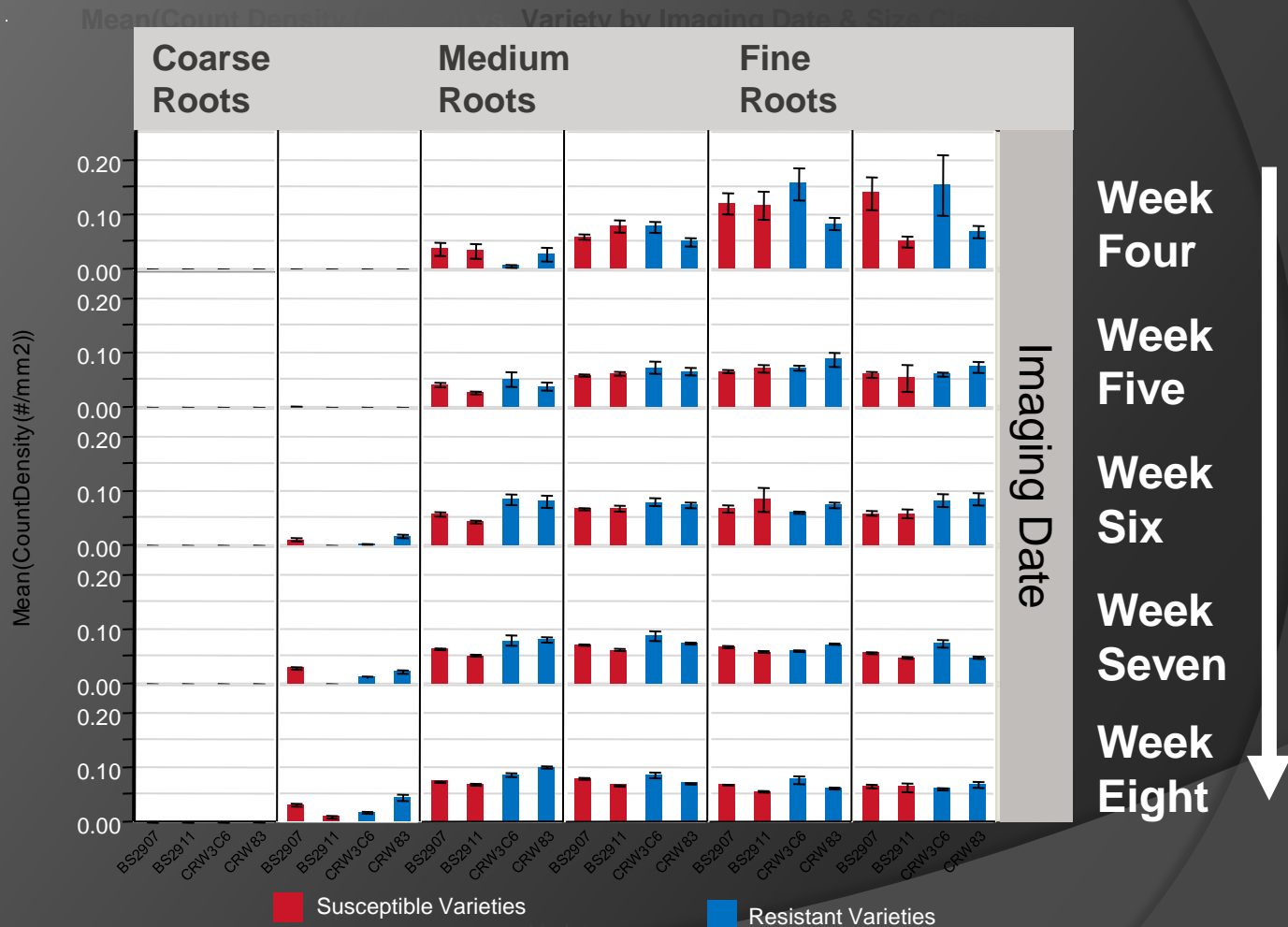
There appear to be more medium to fine roots during the fourth and fifth weeks in the susceptible varieties.

(725u – 2,465u)

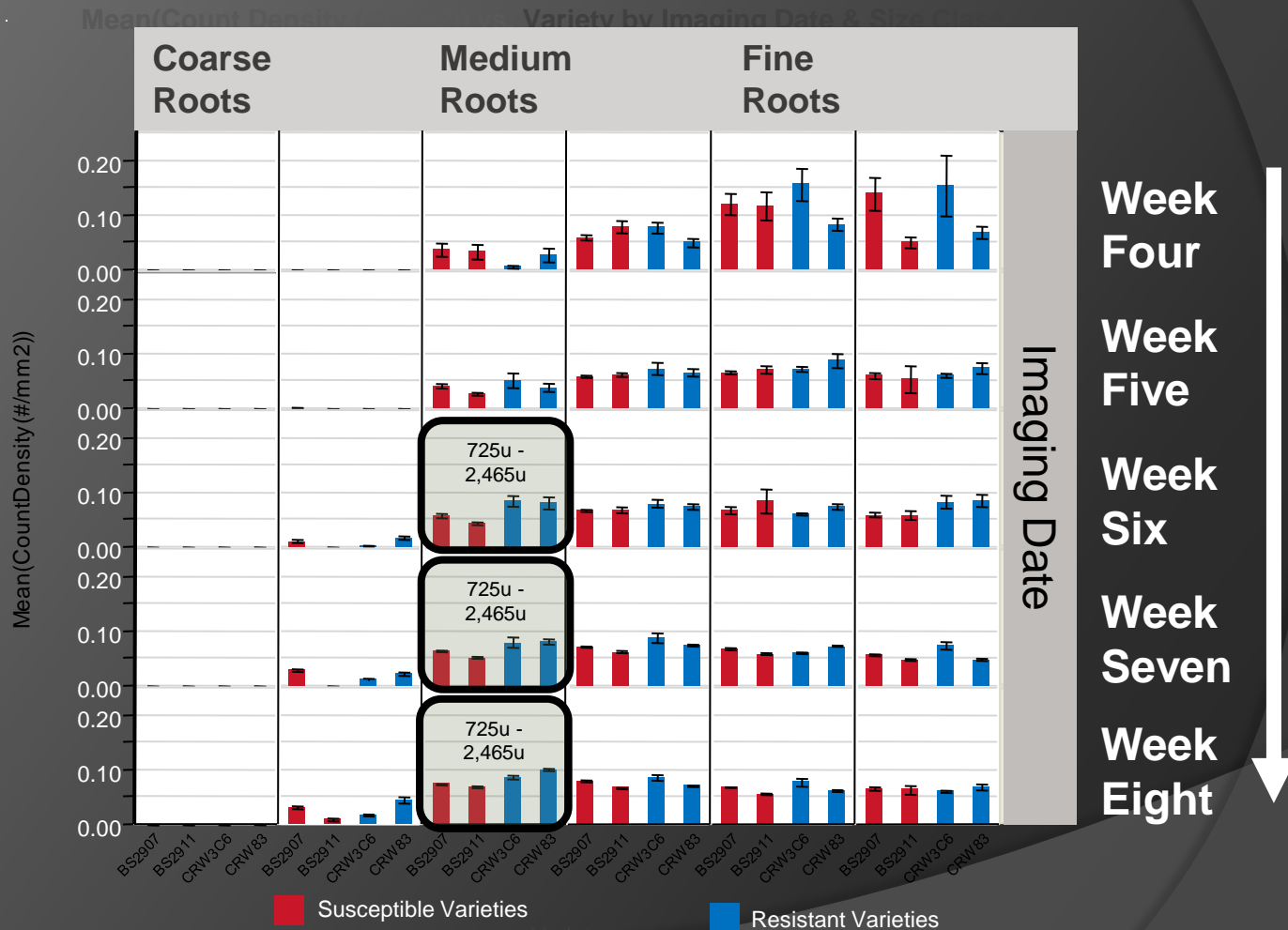
(362u - 1,232u)



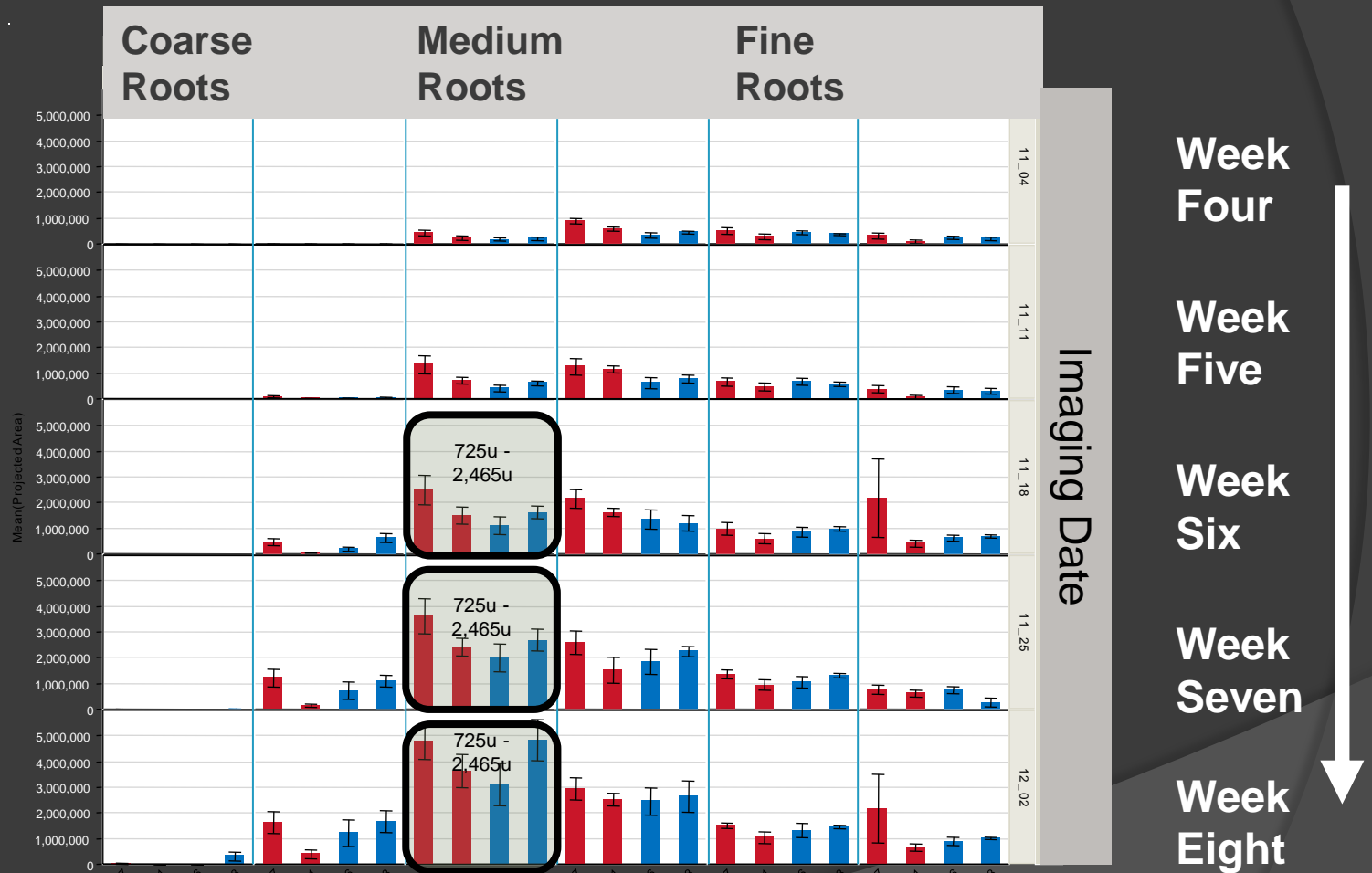
Mean Count Density by Week as a Function of Root Size Class



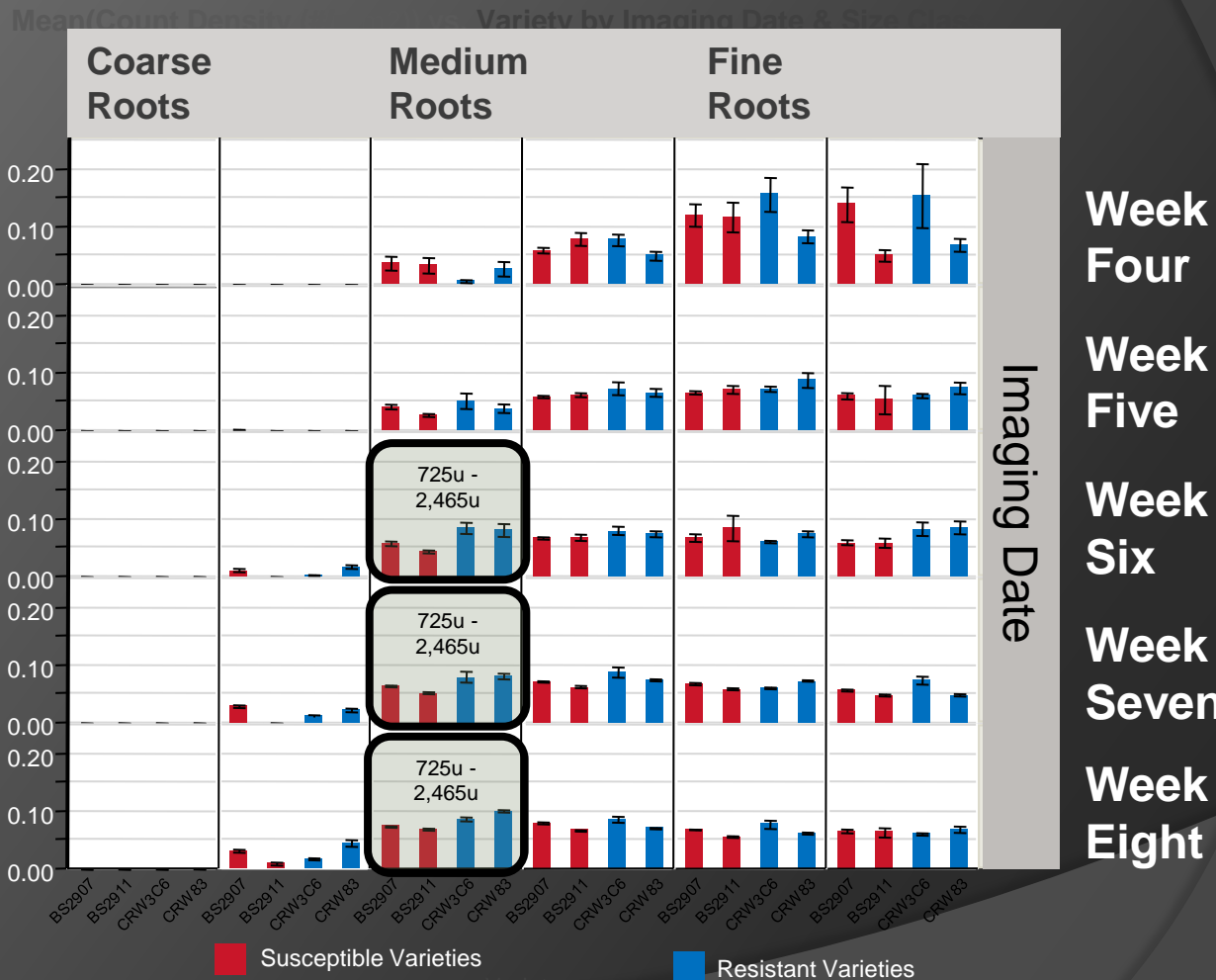
Mean Count Density by Week as a Function of Root Size Class



Mean Projected Area by Imaging Date as a Function of Root Size Class



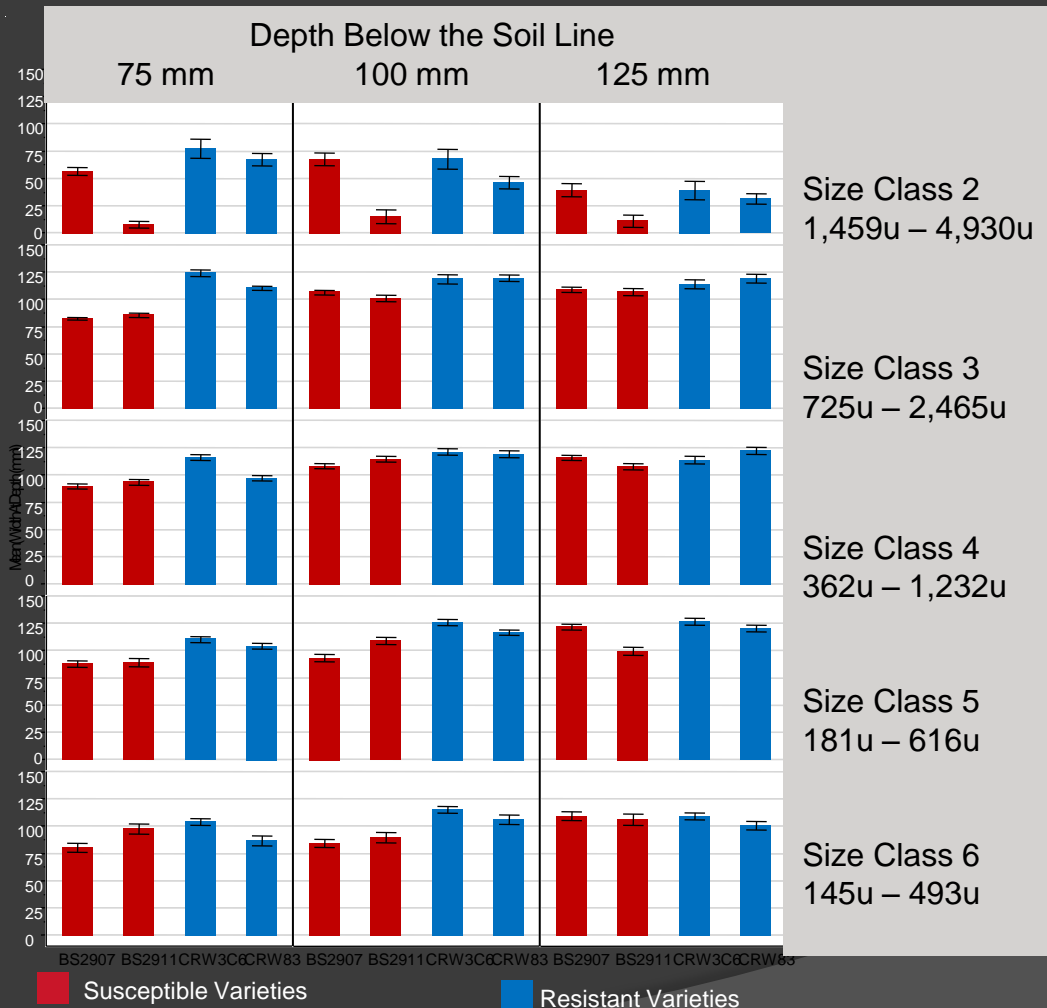
Mean Count Density by Week as a Function of Root Size Class



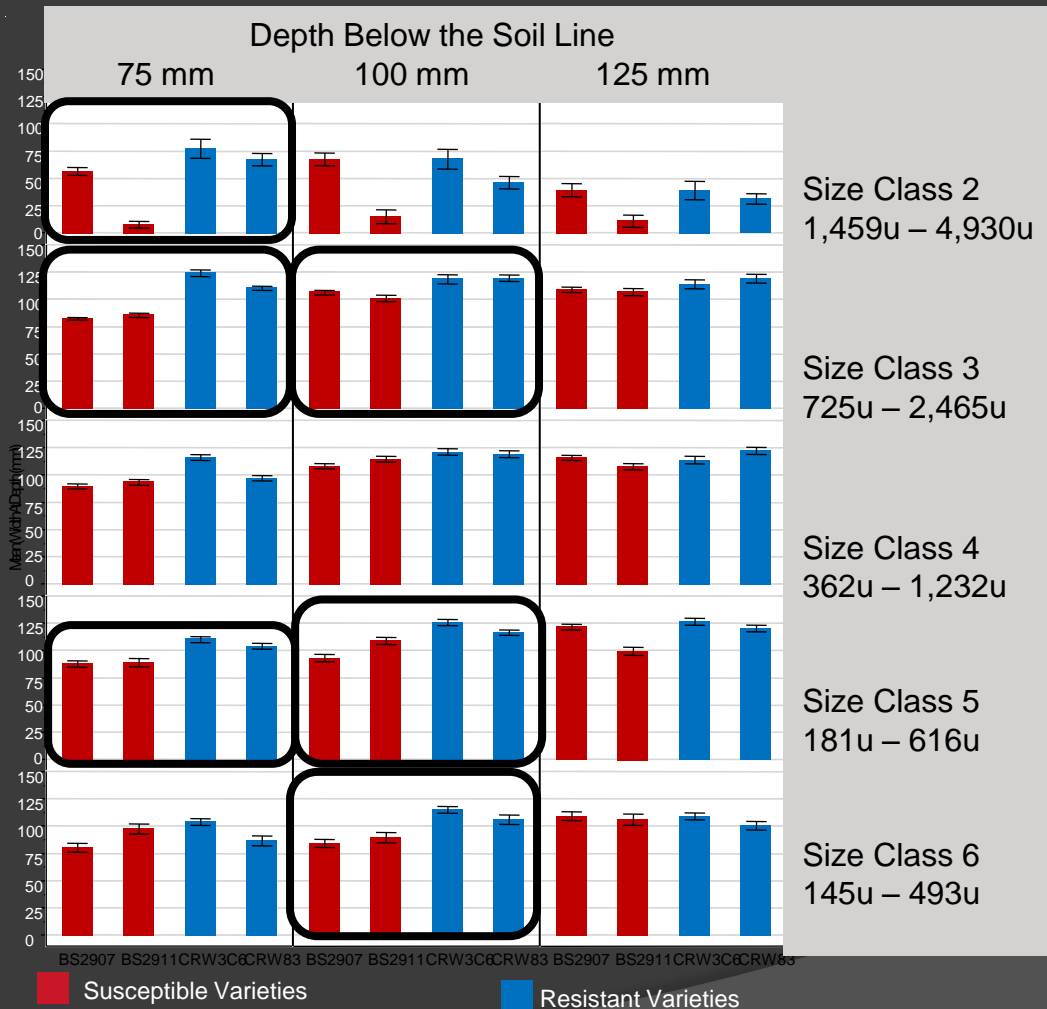
Yikes. I'm lost!



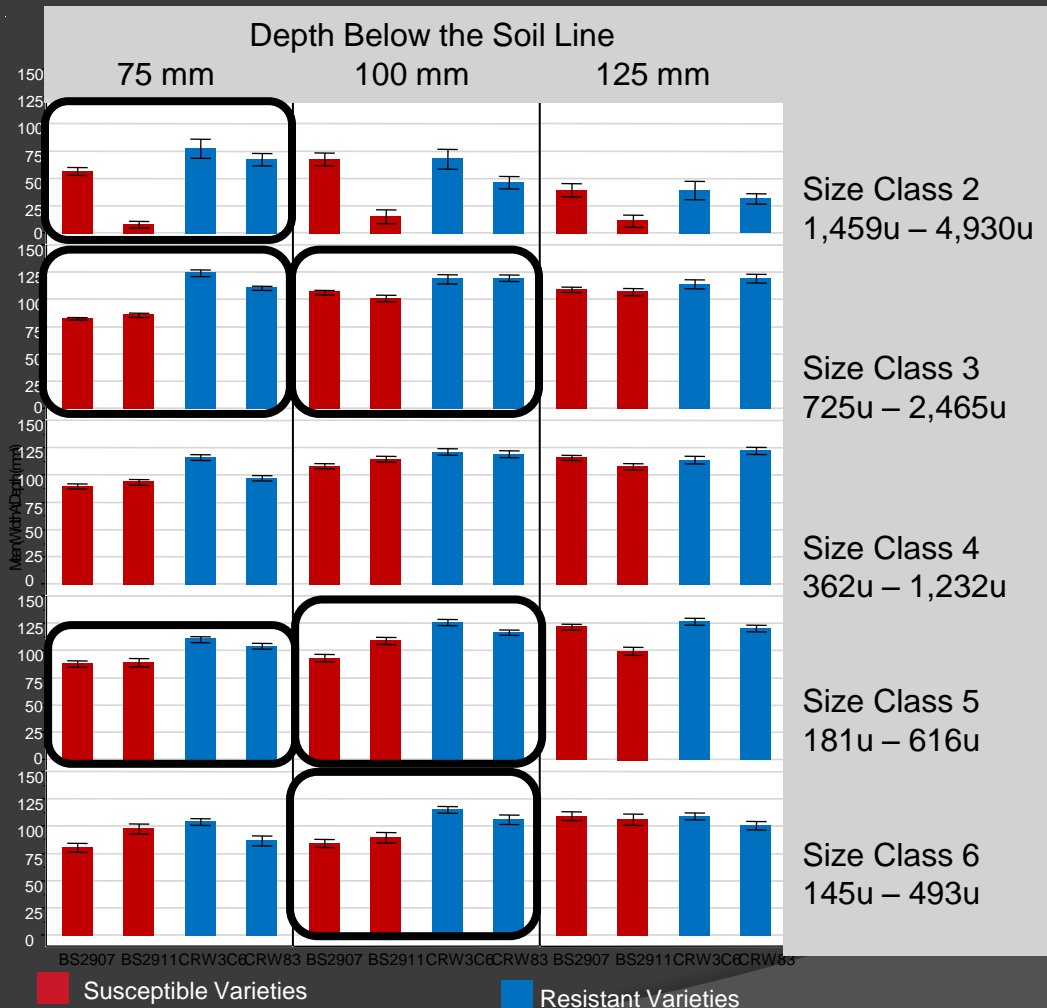
Mean Root Width of Size Classes At Shallow Depths



Mean Root Width of Size Classes At Shallow Depths



Mean Root Width of Size Classes At Shallow Depths



Hey. This is where I hang out.



Maize Root System Architecture Differences Exist During Periods of Potential Larval Feeding

- Resistant and susceptible maize varieties showed statistically significant differences in traits which exist for finite time periods and which occur only for roots of certain sizes at certain depths.
- This suggests that new screening methods may be developed based upon phenology considerations.
- Additional research is required with larger population sizes to validate and expand upon these results.

Acknowledgements



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