



The University of Georgia

De-bugging soybean

A story of nano-spears and QTLs

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Leaf-chewing insects in Soybean

Lepidoptera



Coleoptera



Defoliation Thresholds in Soybean



30%



15%

>20% defoliation
during pod formation and filling
Yield reduction



Vegetative

Reproductive

Resistance Mechanisms: **Antixenosis**

Experiment: choice

Resistance: non preference

Phenotype: reduced plant defoliation



Greenhouse plots

Susceptible

Resistant



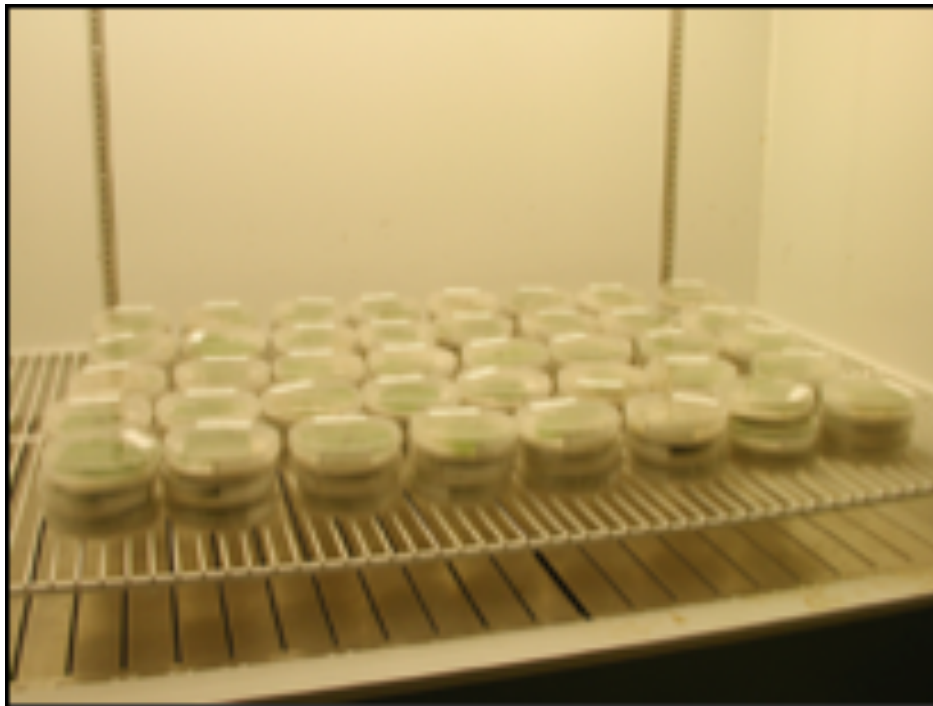
Visual rating: % defoliation

Resistance Mechanisms: **Antibiosis**

Experiment: non choice

Phenotype: larval weight

Growth chamber

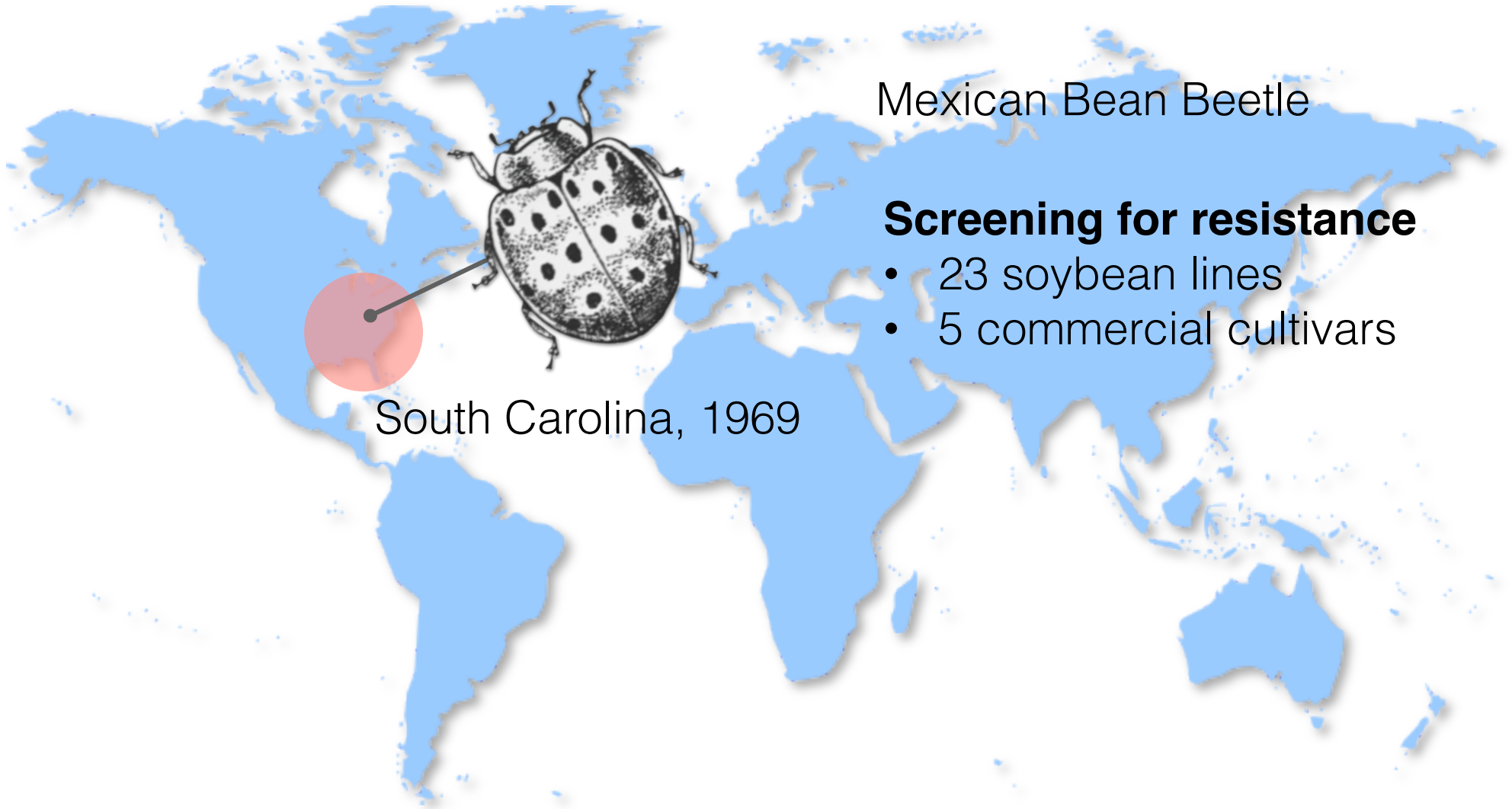


Susceptible

Resistant



Insect Resistant Soybean



Mexican Bean Beetle

Screening for resistance

- 23 soybean lines
- 5 commercial cultivars

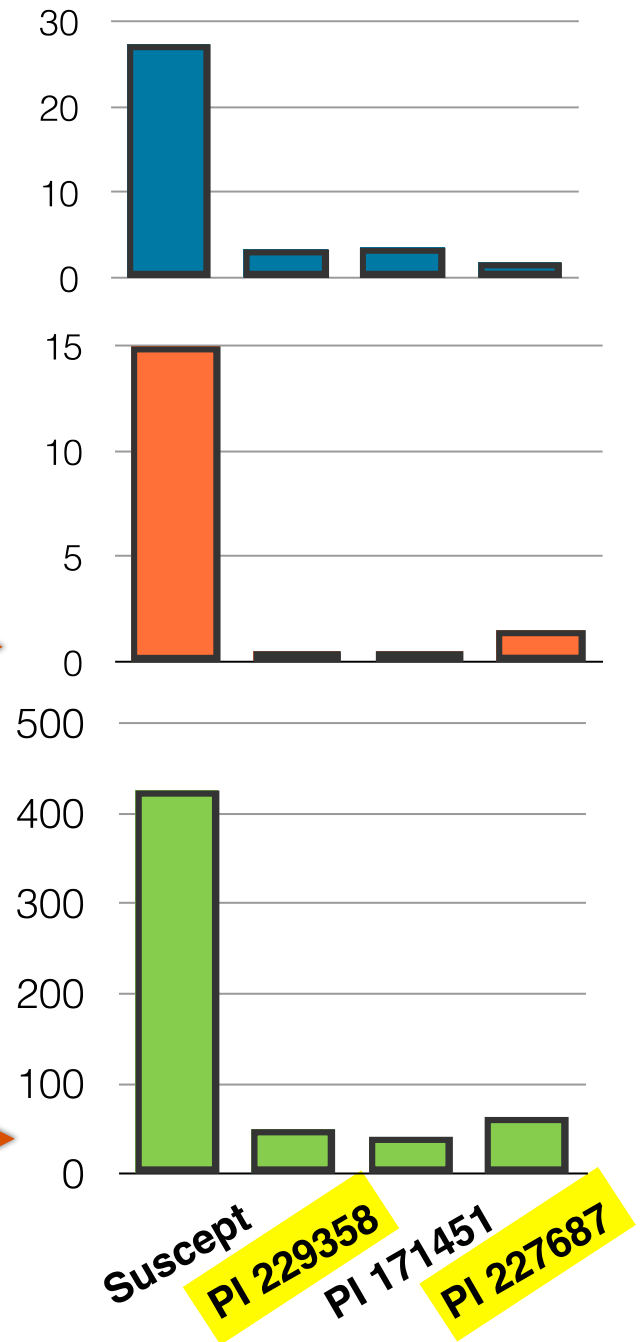
Insect Resistant Soybean



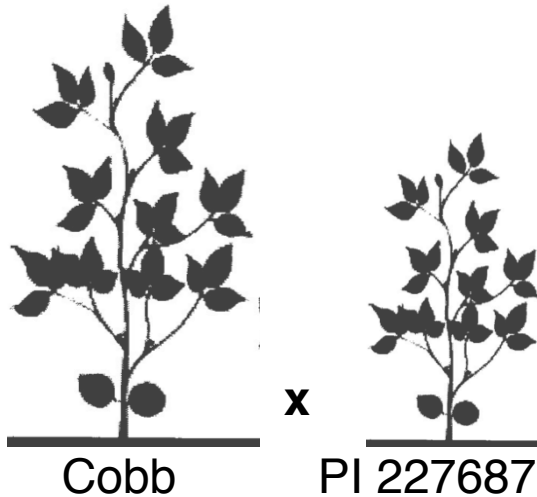
beetles / plant

egg masses / plant

Larvae weight / plant



Resistance in PI 227687



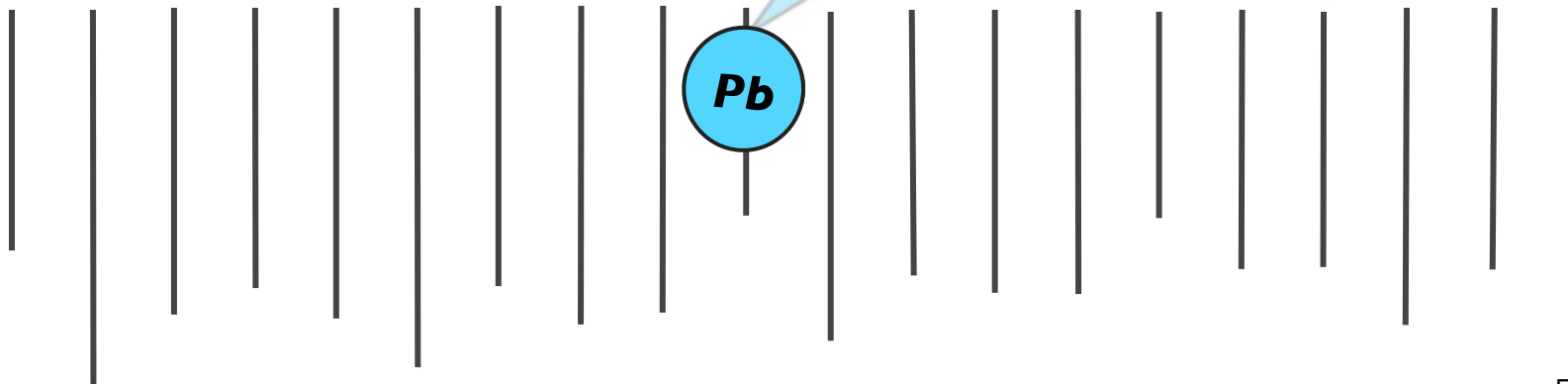
F2:3 population



Corn earworm

Soybean Linkage Groups

RFLP map



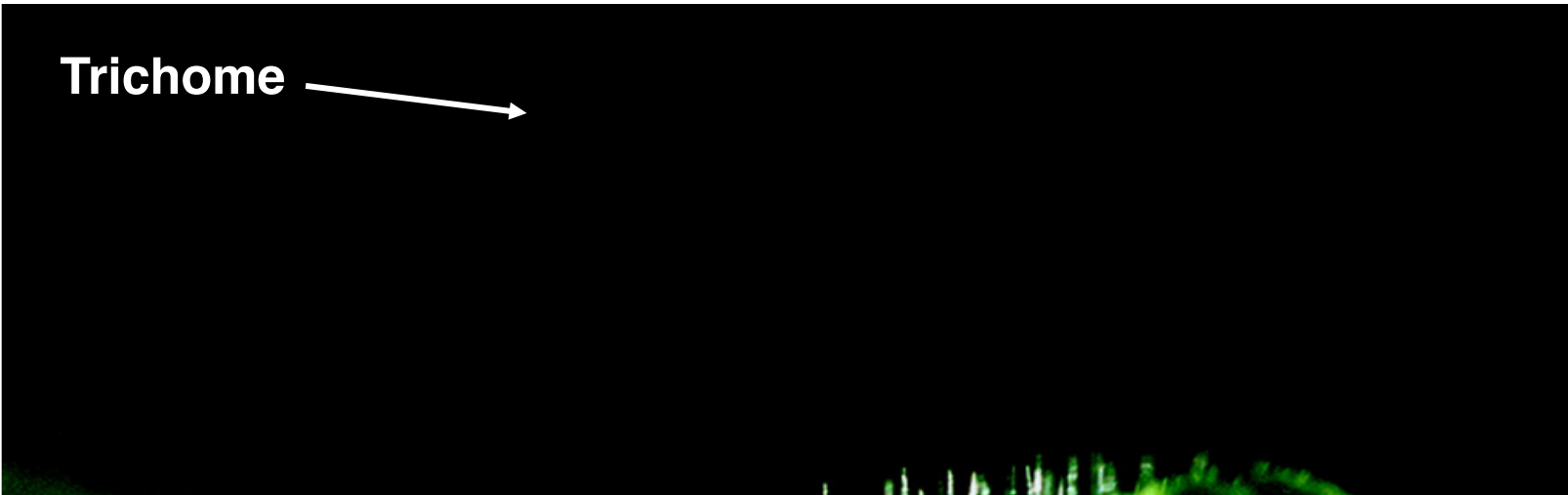
QTL-E explained

26% Antibiosis

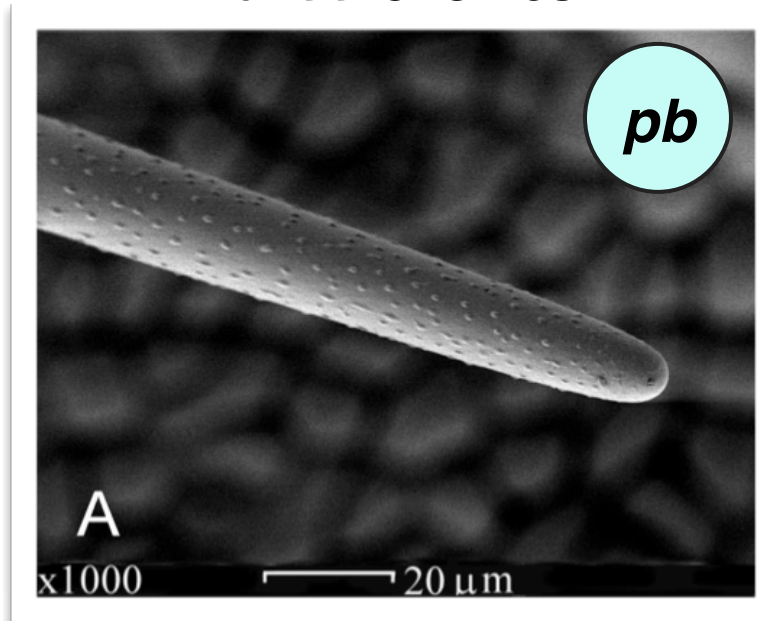
20% Antixenosis

Rector et al., 1998
Hulburt et al., 2004
Boerma & Walker, 2005
Palmer, 2008

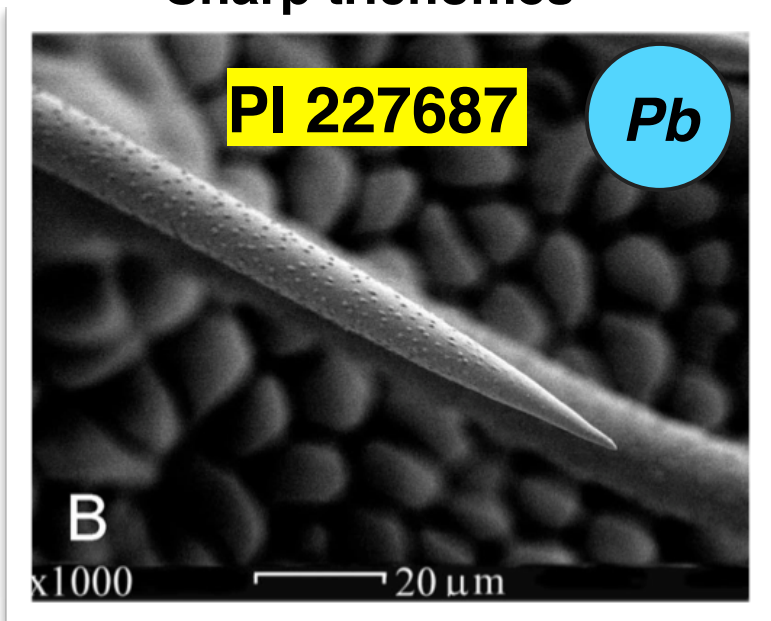
Sharp Pubescence locus: *Pb*



Blunt trichomes



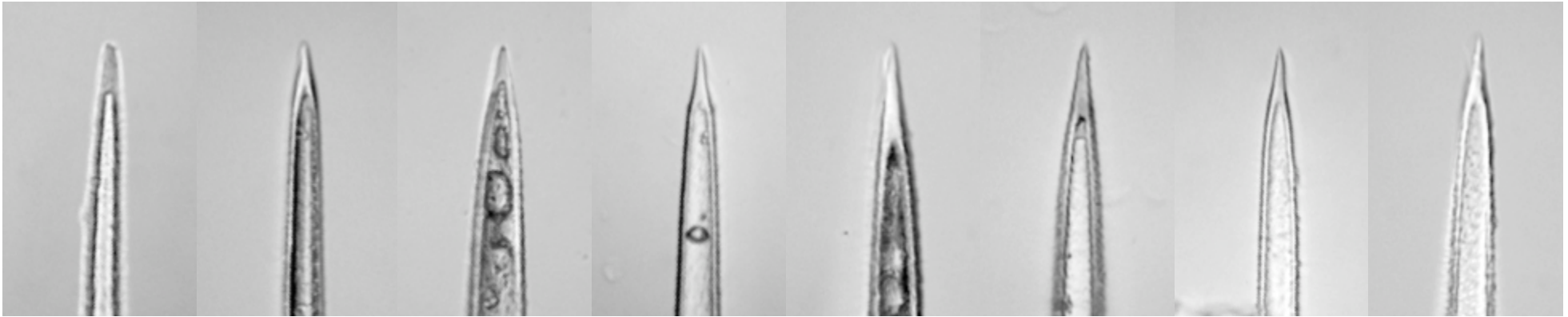
Sharp trichomes



Sharp vs. Blunt Pubescence

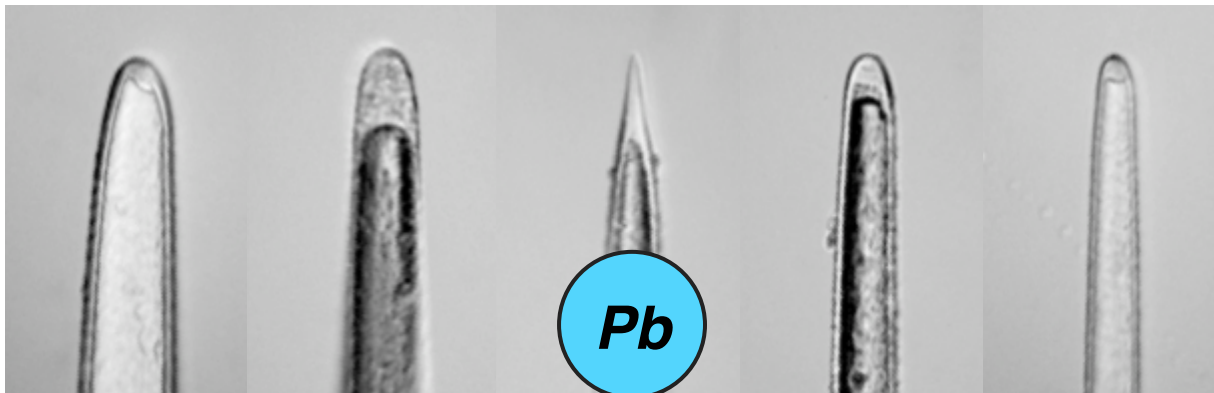
Pb

Wild soybean



pb

Cultivated soybean



CHOICE IOWA SEEDS

56 ANNUAL
CATALOGUE

1921

ONLY ONE
THE BEST
QUALITY

Soy
Beans

Iowa
Gold Mine
Corn

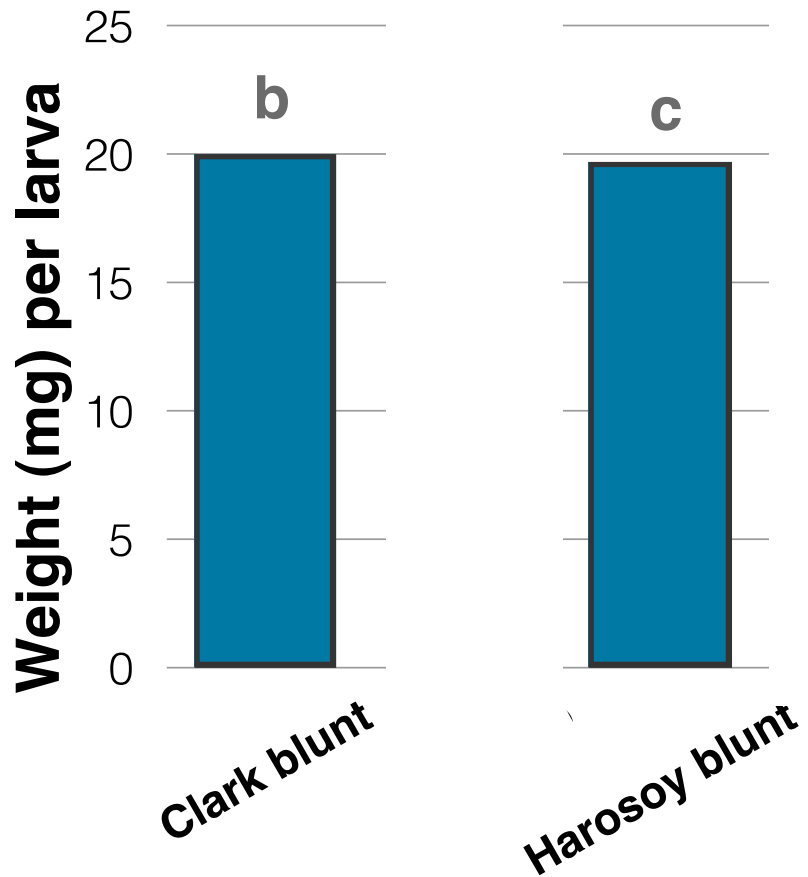


IOWA SEED CO. DES MOINES
IOWA U.S.A.

Smithsonian Museum
Catalog Collection

Sharp vs. Blunt Pubescence

Phenotype: larval weight



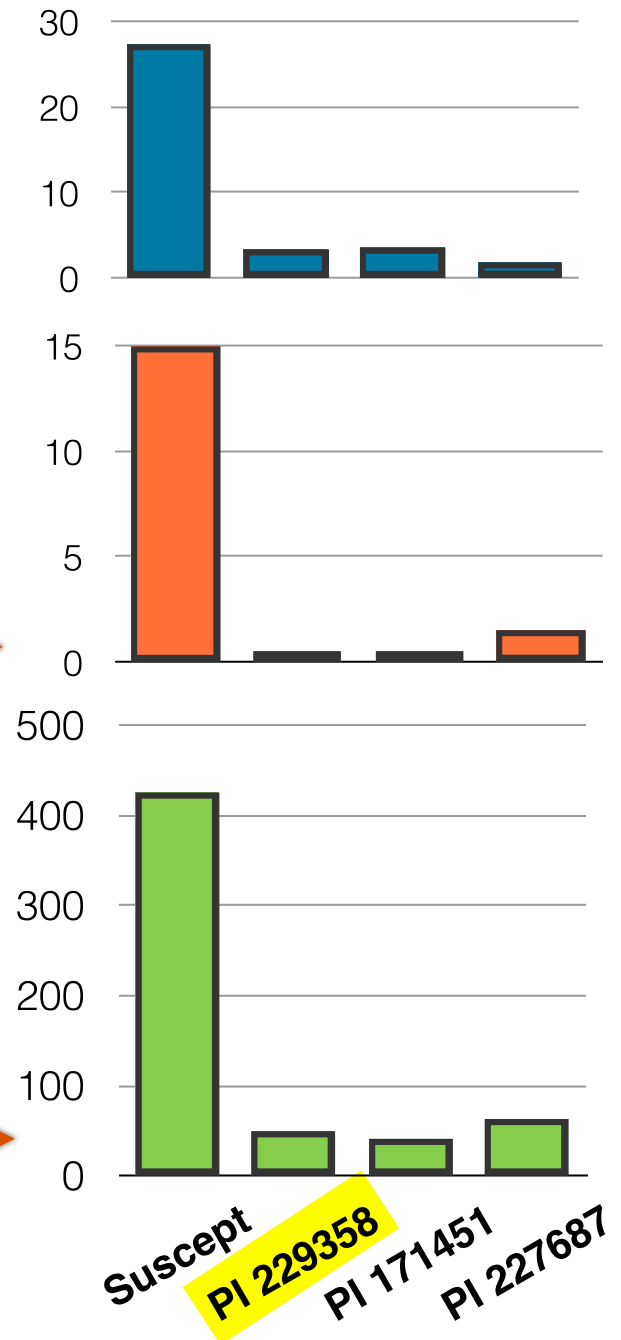
Insect Resistant Soybean



beetles / plant

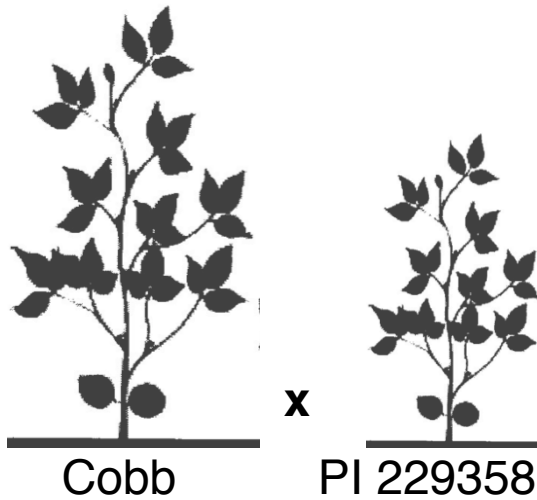
egg masses / plant

Larvae weight / plant



Van Duyn et al., 1971

QTLs in PI 229358 - Antixenosis

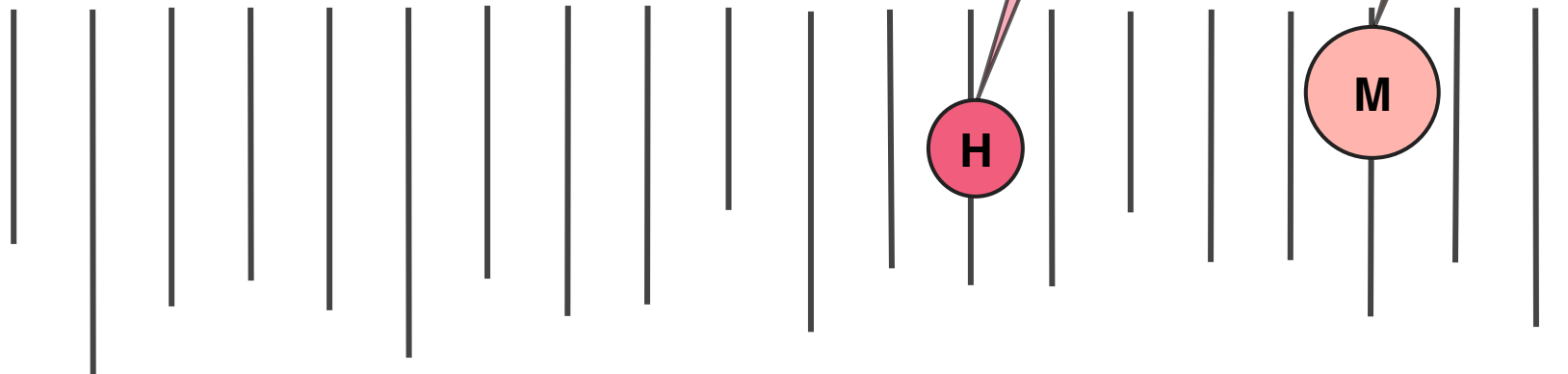


F2:3 population

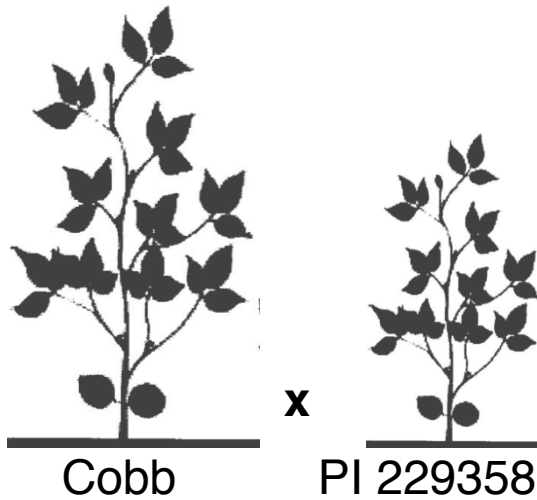


Defoliation

**Soybean Linkage Groups
RFLP map**



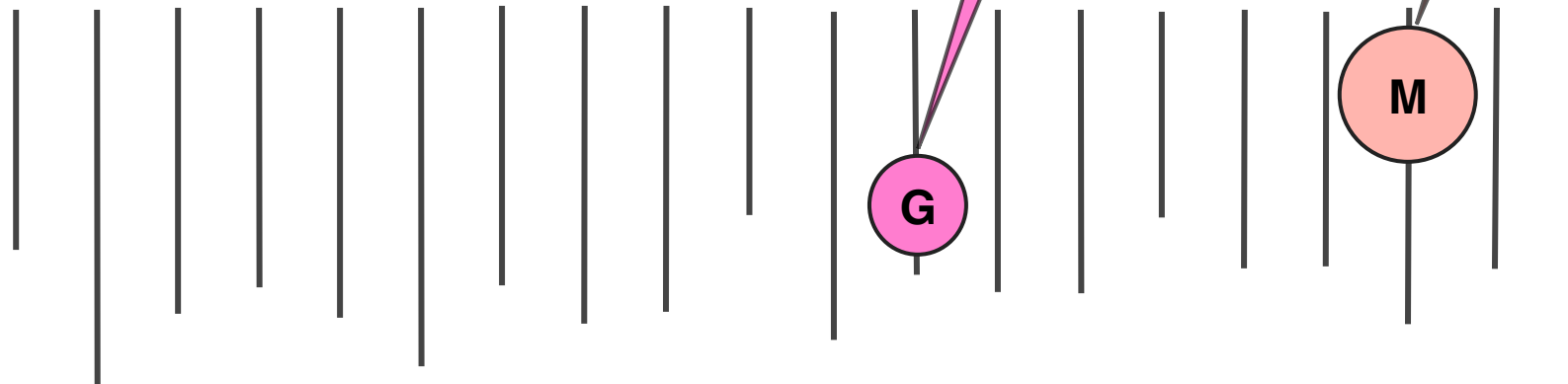
QTLs in PI 229358 - Antibiosis



F2:3 population



Soybean Linkage Groups



QTL-M **activates** the other QTLs

Benning BC6F2:3 Near Isogenic Lines

% Defoliation

	- M	+ M
- H	31.4 ^a	23.4 ^b
+ H	30.1 ^a	19.2 ^c

Larval weight (mg)

	- M	+ M
- G	131.3 ^a	80.0 ^b
+ G	128.4 ^a	49.3 ^c

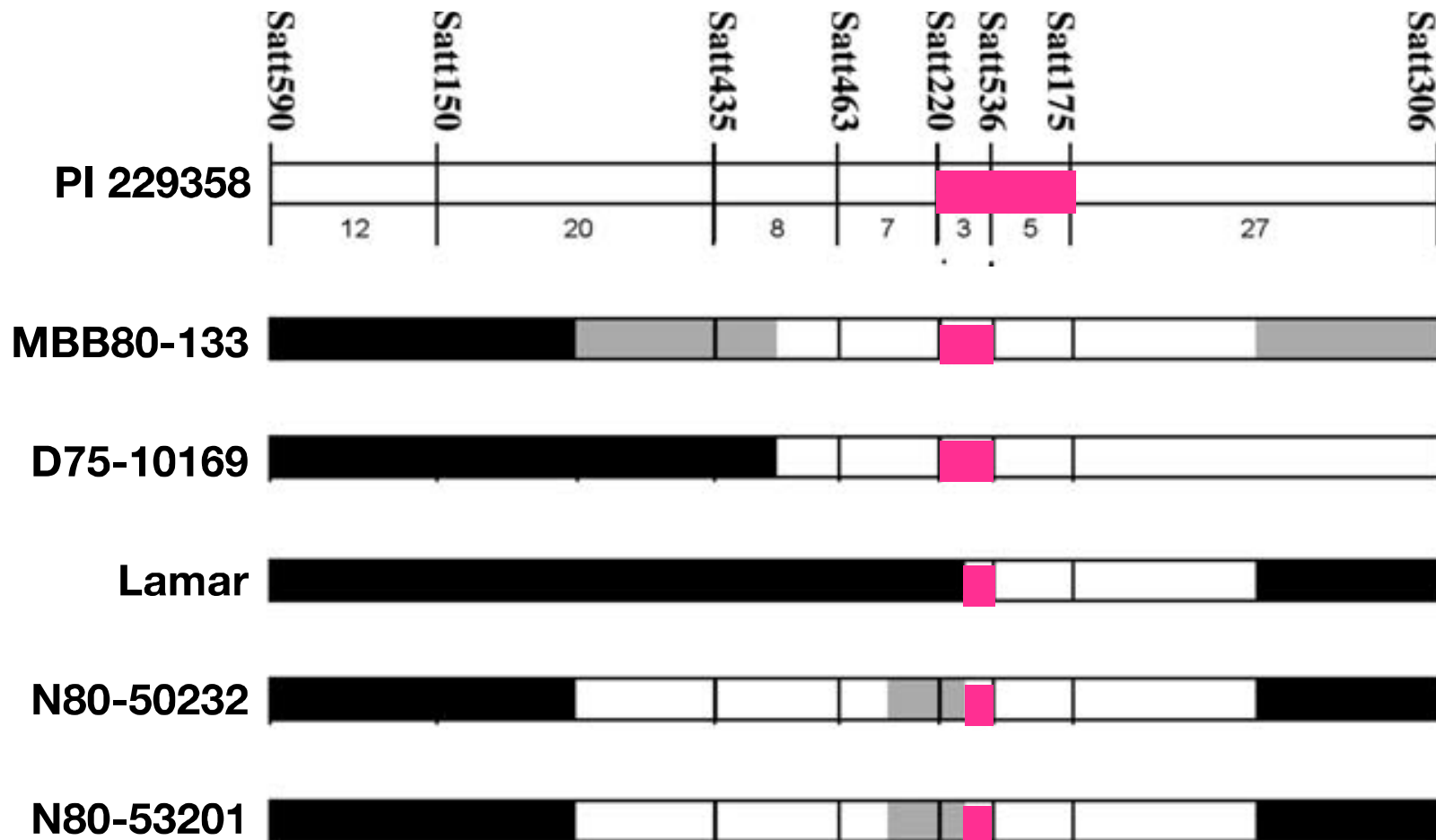
Retrospective study of QTL-M

15 soybean breeding lines

Phenotypic selection
Insect Resistance

Graphical genotype

82 cM of Chromosome 7
SSR flanking QTL-M

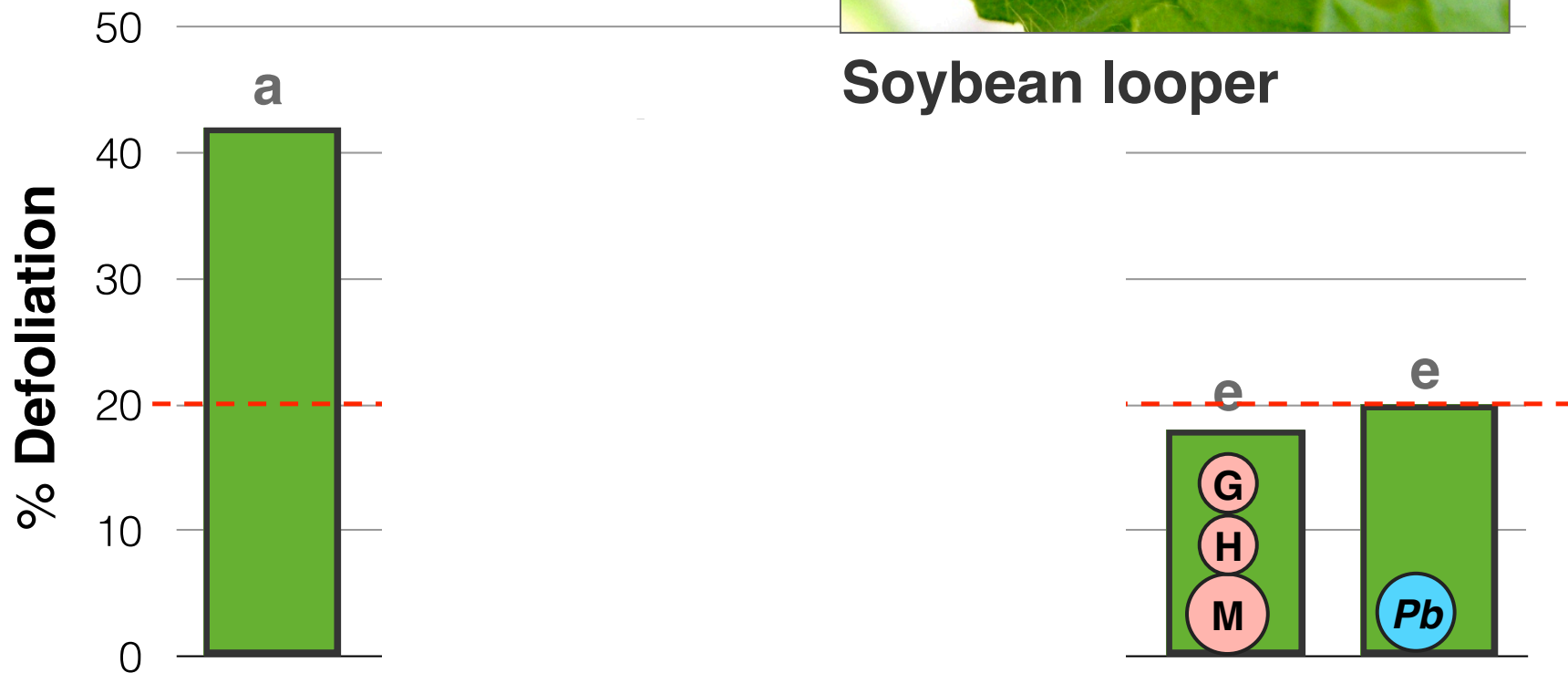


QTL-M + Sharp Pubescence

Benning BC₆F_{2:3}
Greenhouse



Soybean looper



ANOVA: Bars represent means from fifteen replicates using RCB design ($p < 0.001$)

QTL-M + Sharp Pubescence

Benning BC₆F₂:3
Choice test

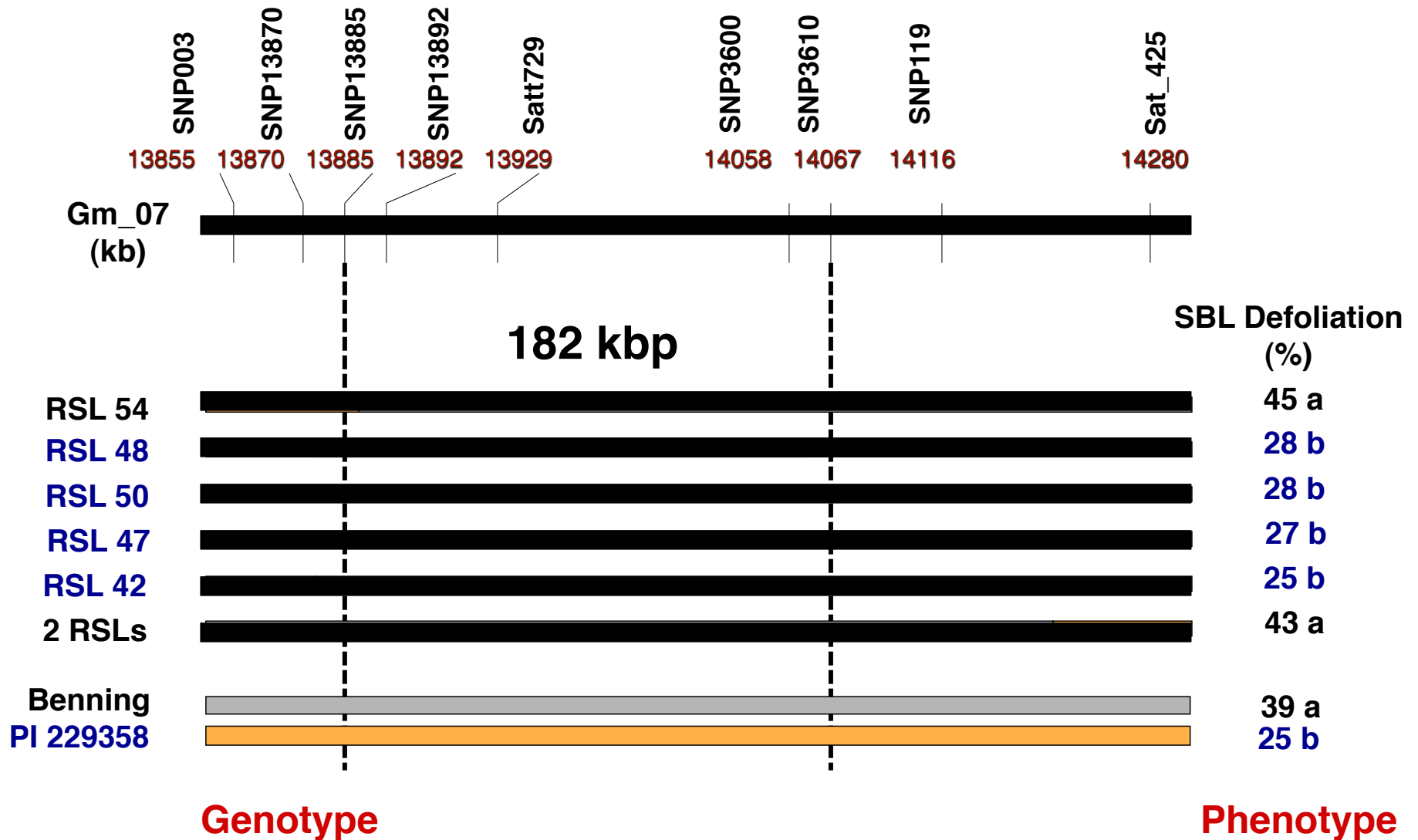


ANOVA: Bars represent means from fifteen replicates using RCB design ($p < 0.001$)

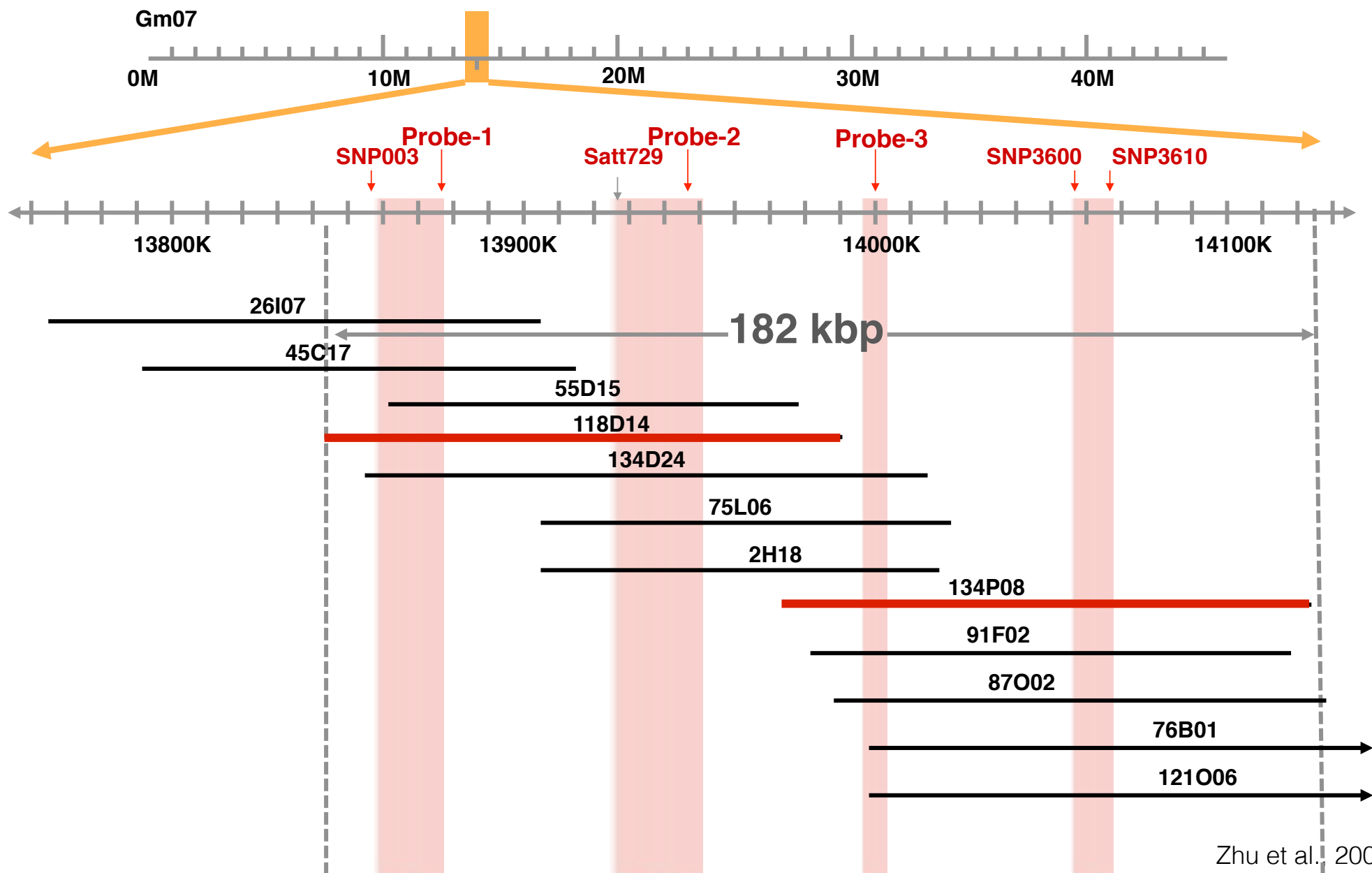
Cloning and Characterizing **QTL**

1. Confers **antibiosis** and **antixenosis** resistance
2. Activates **QTL-G** and **QTL-H**
3. Enhances the effectiveness of **Bt**
4. Effective across **multiple** insect herbivores

QTL-M: Recombinant Substitution Lines

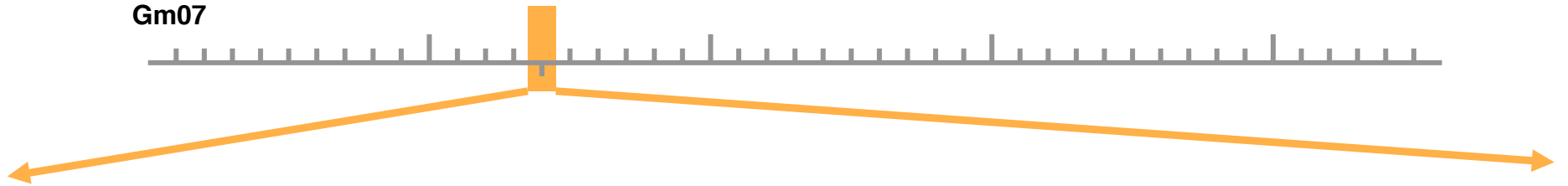


QTL-M: BAC Contig – PI 229358



BAC Sequence Aligned to the Soybean Genome

Gm07



13900K

14000K

14100K

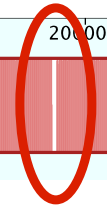
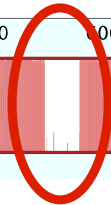
182 kbp

20000 40000 60000 80000 100000 120000 140000 160000 180000 200000 220000 240000 260000 280000

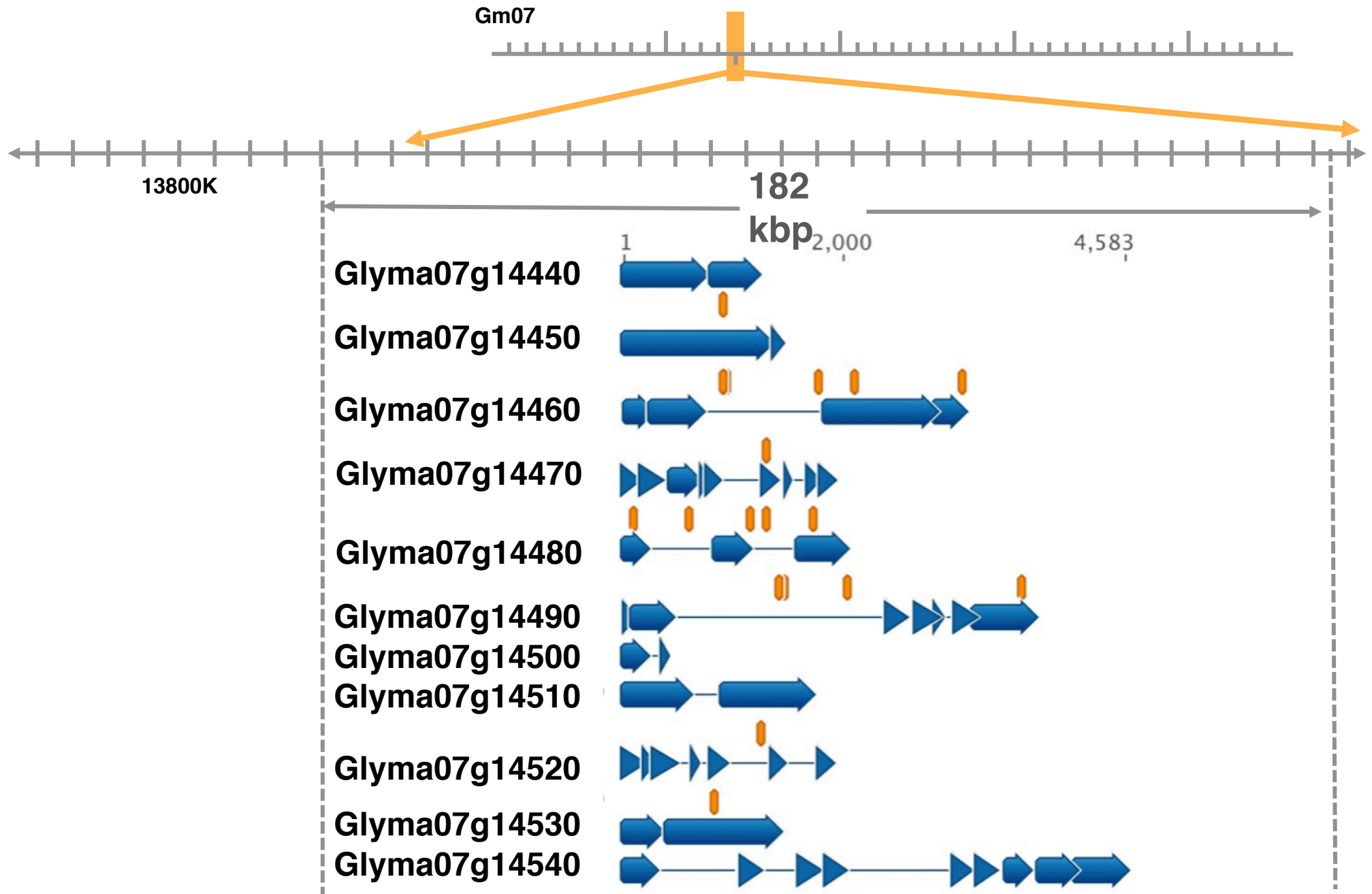
178 kbp – PI 229358

20000 40000 60000 80000 100000 120000 140000 160000 180000 200000 220000 240000 260000

182 kbp – Williams 82



SNPs between Williams 82 and PI 229358



Polymorphisms with **Susceptible** Accessions



32 Soybean Genotypes Ancestral to Modern Varieties

SNPs **Unique** to Insect Resistant Soybeans Accession

Glyma07g14440 Protein of unknown function



Glyma07g14450 Protein of unknown function



Glyma07g14460 Cytochrome P450



Glyma07g14470 Ploop-NTPase



Glyma07g14480 MYB related protein



Glyma07g14490 Phosphoglycerate mutase



Glyma07g14500 Protein of unknown function



Glyma07g14510 Glucosyl/Glucuronosyl transferase



Glyma07g14520 Protein of unknown function



Glyma07g14530 Glucosyl/Glucuronosyl transferase

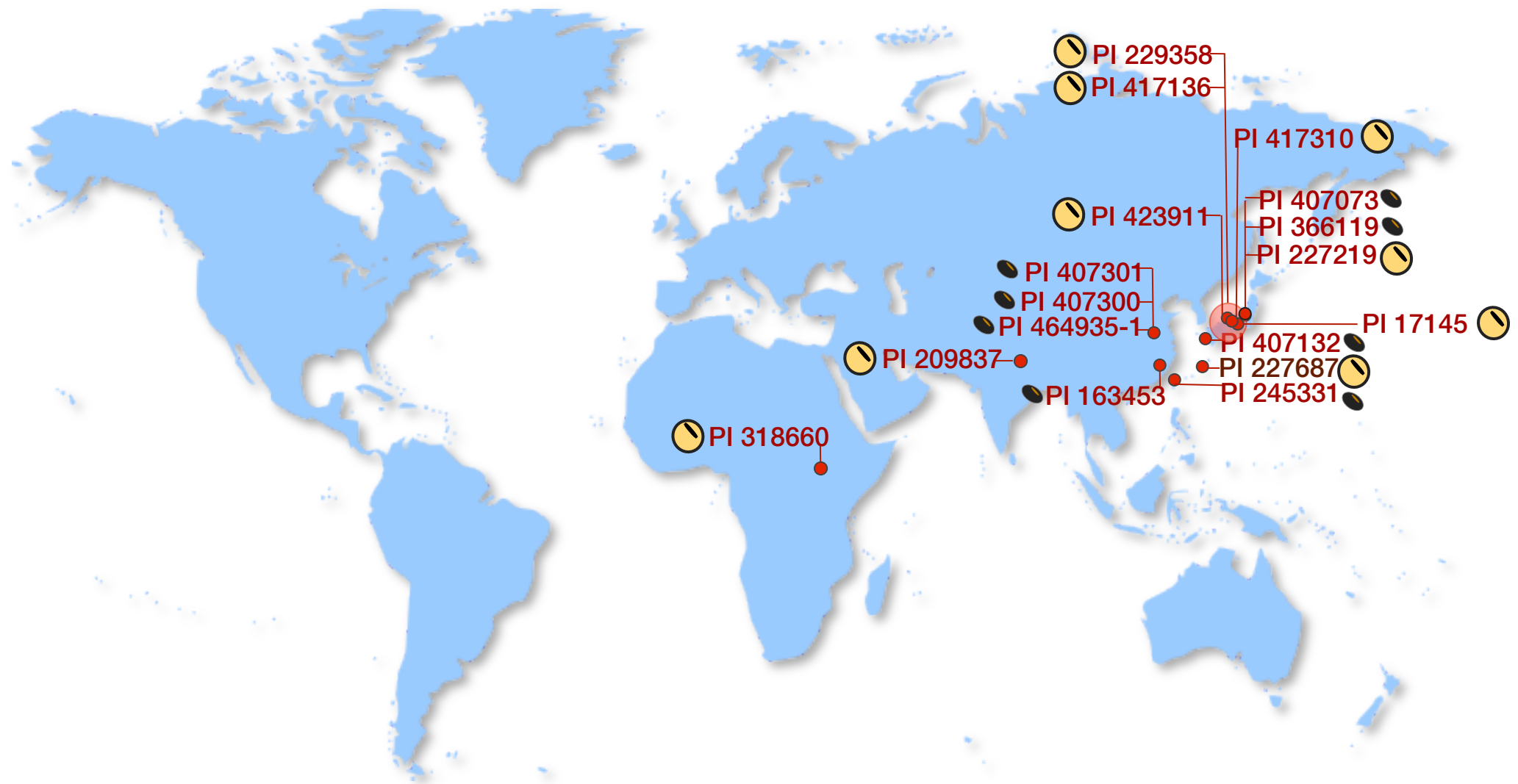


Glyma07g14540 DNAJ/HSP40



■ Polymorphism between PI 229358 and 32 Ancestors

Shared SNPs with Insect Resistant



16 Reported Insect-Resistant Soybean Genotypes

SNPs **Unique** to Insect Resistant Accessions

Glyma07g14440 Protein of unknown function



Glyma07g14450 Protein of unknown function



Glyma07g14460 Cytochrome P450



Glyma07g14470 Ploop-NTPase



Glyma07g14480 MYB related protein



Glyma07g14490 Phosphoglycerate mutase



Glyma07g14500 Protein of unknown function



Glyma07g14510 Glucosyl/Glucuronosyl transferase



Glyma07g14520 Protein of unknown function



Glyma07g14530 Glucosyl/Glucuronosyl transferase

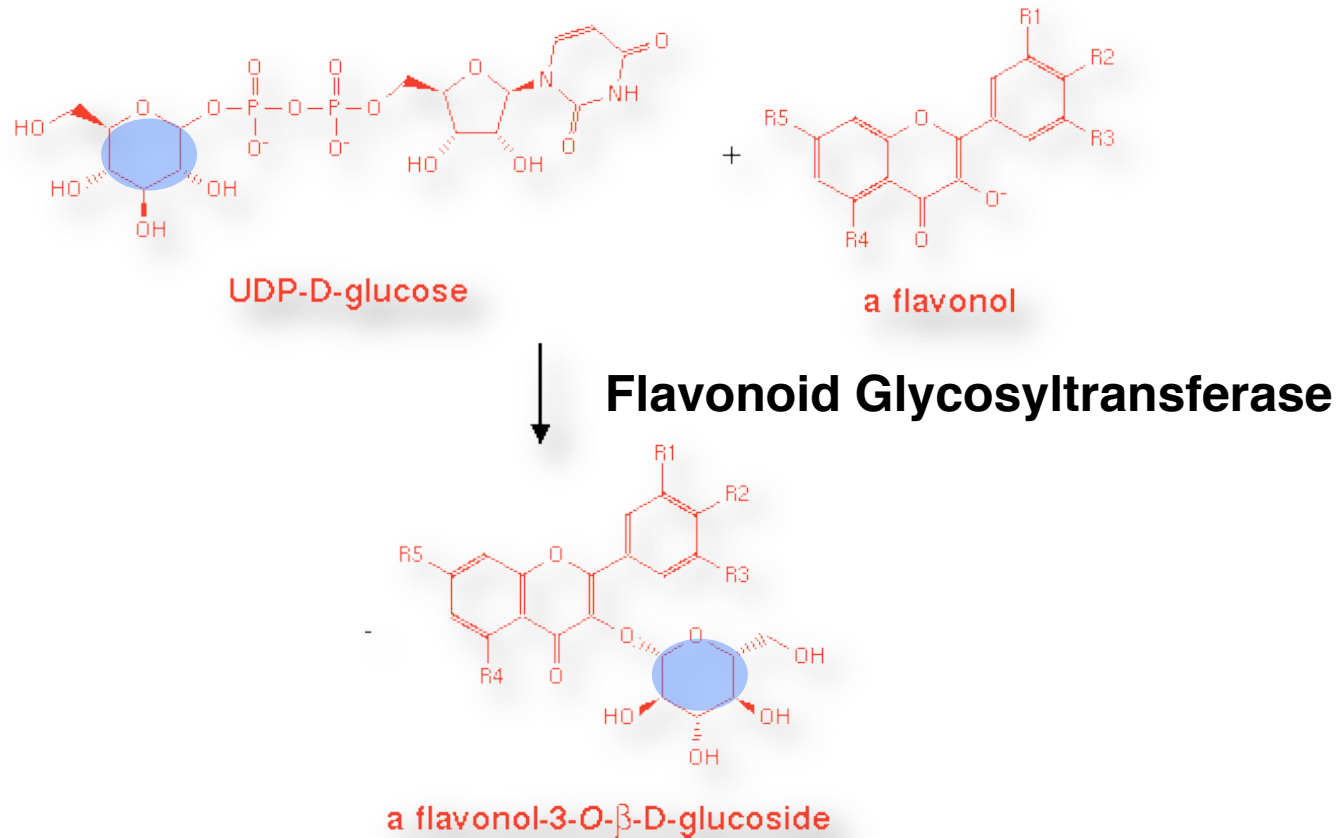


Glyma07g14540 DNAJ/HSP40



■ SNP shared with insect resistant genotypes, excluding **PI 227687**

Glyma07g14530 – Putative Flavonoid Glycosyltransferase



Some insects **can sequester** plant flavonoids

Other insects **are deterred** by flavonoids:

Corn earworm - **maize**

Cabbage looper – **soybean**

Bark beetle – **spruce**

Fenny et al., 1976
Berhow and Vaughn, 1999
Hoffmann-Campo et al., 2001
Widstrom and Snook, 2001

Glyma07g14530 Expression in Leaves

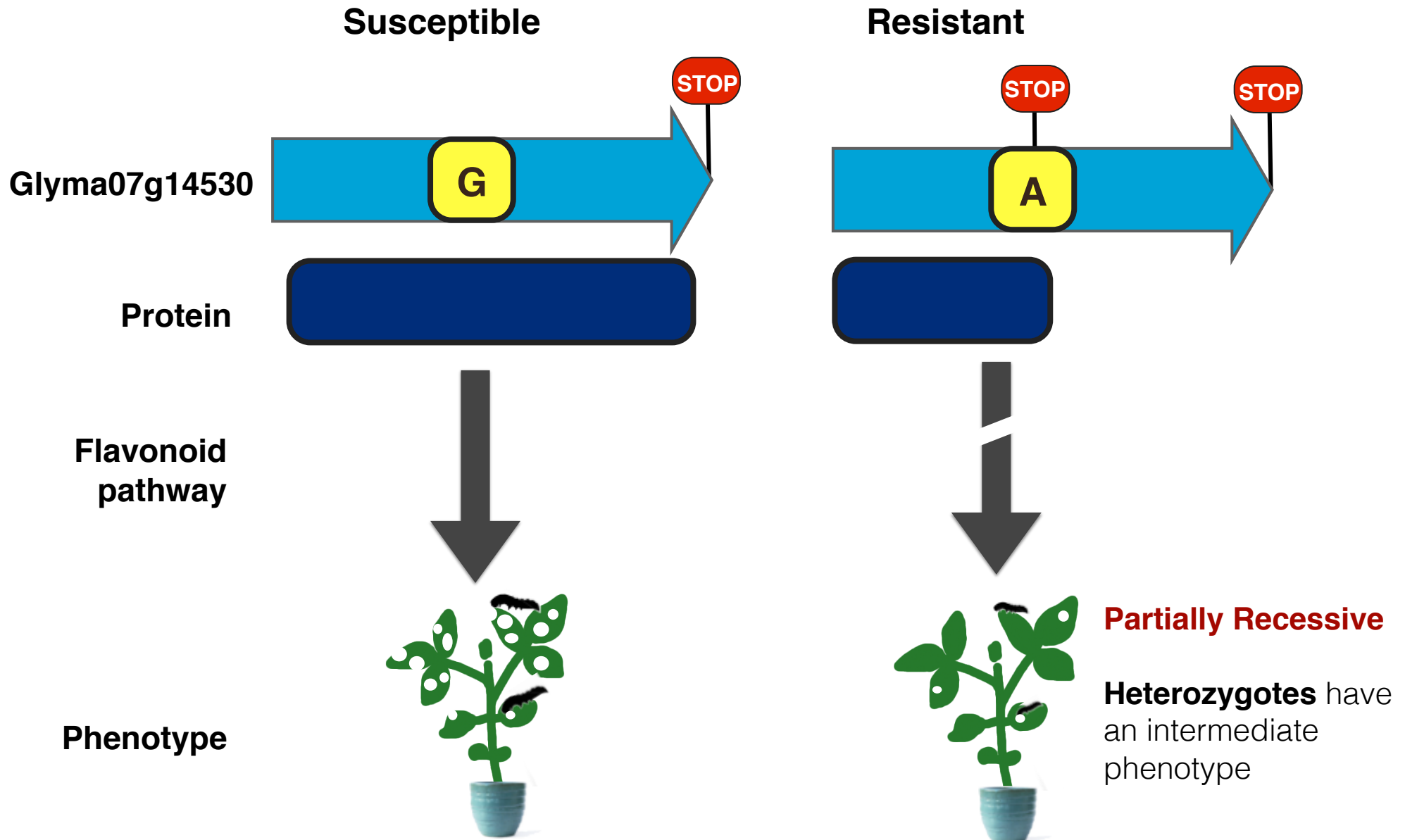
Glyma07g14530

Resistant
1,416 bp

Susceptible
229 bp



Susceptible vs Resistant Soybeans



Work in Progress

Characterization of **Glyma07g14530**

- Complementation tests in transgenic plants
- Determine which leaf flavonoids are different between QTL-M and qtl-m plants

Summary

- **PI 227687 resistance** is associated with sharp pubescence
- **PI 229358 resistance** is conferred by 3 QTLs:
 - **M**: antibiosis and antixenosis
 - **H**: antixenosis
 - **G**: antibiosis
- **QTL-M** activates **QTL-H** and **QTL-G**

Summary

- **QTL-M** enhances **Bt**
 - Pyramiding these genes may provide a more durable resistance
- **QTL-M + Pb** plants are more resistant to defoliation, and caterpillars are smaller
 - Non-transgenic alternative for insect resistance
- A putative **flavonoid glycosyltransferase** is the candidate gene for QTL-M:
 - Modification of the flavonoid pathway provides a possibility to obtain insect resistance in other crops

The De-bugging Team through the Years



Neal Stewart

Soybean transformation
Bt

David Hulburt

*Pb*studies - PI 227687

Brian Rector

QTL discovery
PI 229358

Joe Zhou

PI 229358 BAC library
Gene pyramiding

Jim Narvel

Retrospective study QTL-
M

Caleb Warrington

Seed yield
Insect Resistant
near isogenic lines

David Walker

Jack near isolines

Bo-Keun Ha



QTL-M
SNP discovery
Candidate genes

Thanks:



Tissue culture, Transformation

- Noah Lawler
- Donna Tucker

Entomology

- Dean Kemp
- John All
- Michael Strand

Genotyping

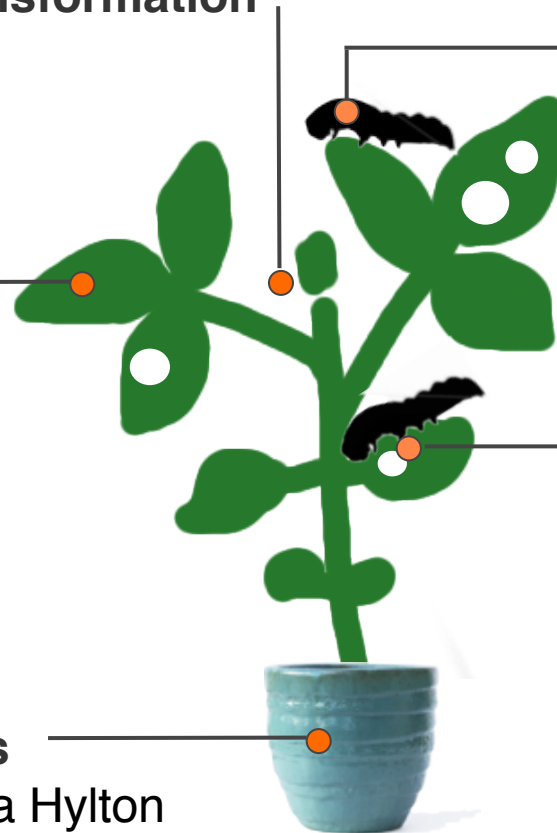
- Noah Lawler

Gene Expression, Cloning

- Pete LaFayette

Bioassays

- Christina Hylton



SAVE THE DATE!

“Forty years with soybeans”

A great

Symposium & Banquet

Celebrating the career of

DR. H. ROGER BOERMA

Distinguished Research Professor

Director of the Center for Applied Genetic Technologies

**Thursday afternoon, June 28th - Friday noon, June 29th, 2012
Athens, GA**



**Information regarding RSVP & hotel accommodations
coming soon.**