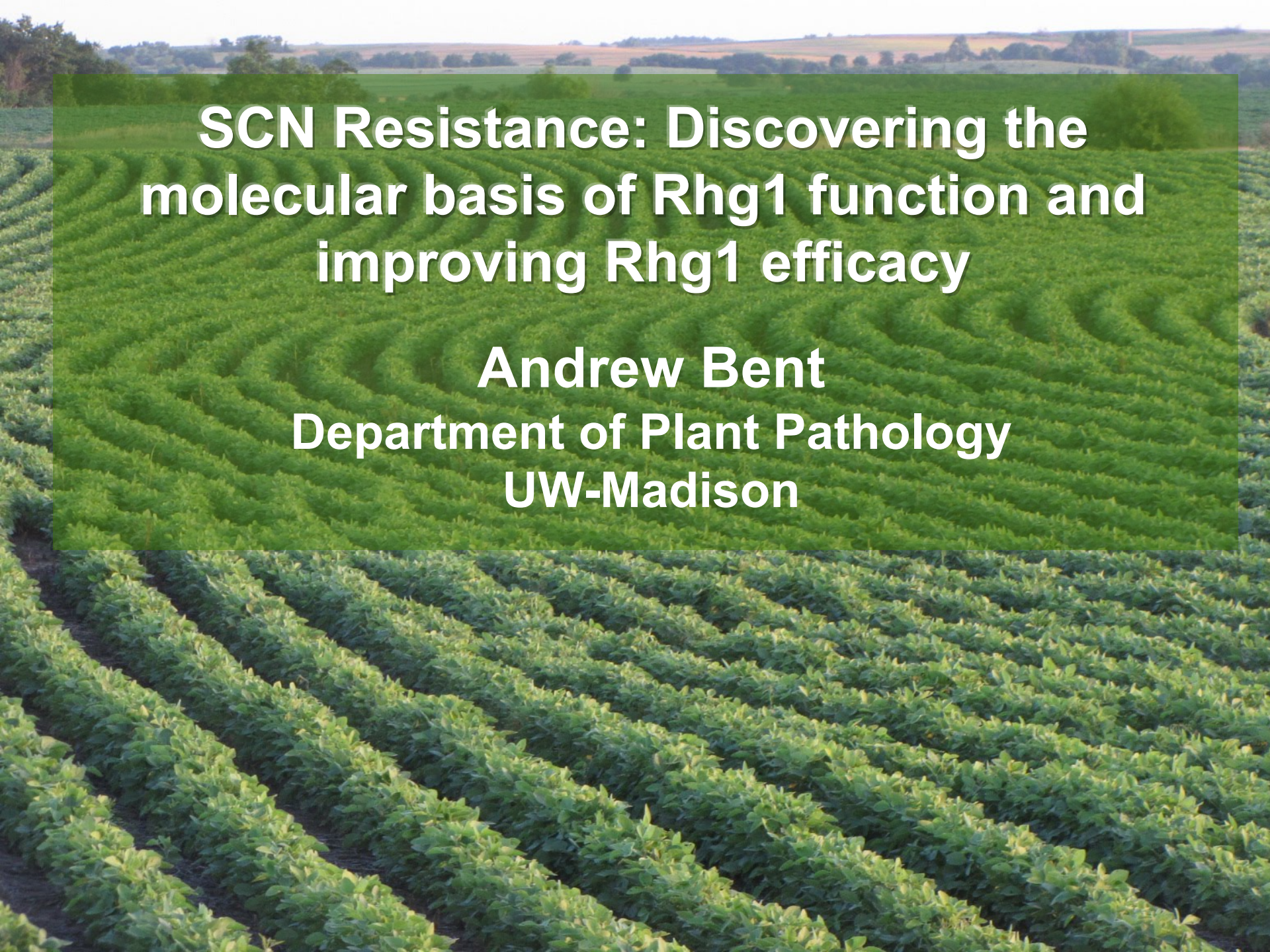

SCN Resistance: Discovering the molecular basis of Rhg1 function and improving Rhg1 efficacy

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Rhg1 locus is the primary means by which growers control the most important disease of soybean

- *Rhg1*-mediated resistance is best control available; used successfully on farms all over the world.
- Marker-assisted selection for *Rhg1* is extremely common in soybean breeding.
- *Rhg1*-mediated resistance is quantitative (partial) and could be improved
- Same *Rhg1* allele (PI 88788 source) is used in over 90% of “SCN-resistant” varieties in central U.S.

What does Rhg1 encode?

REPORTS

Copy Number Variation of Multiple Genes at *Rhg1* Mediates Nematode Resistance in Soybean

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The *rhg1-b* allele of soybean is widely used for resistance against soybean cyst nematode (SCN), the most economically damaging pathogen of soybeans in the United States. Gene silencing showed that genes in a 31-kilobase segment at *rhg1-b*, encoding an amino acid transporter, an α-SNAP protein, and a WI12 (wound-inducible domain) protein, each contribute to resistance. There is one copy of the 31-kilobase segment per haploid genome in susceptible varieties, but 10 tandem copies are present in an *rhg1-b* haplotype. Overexpression of the individual genes in roots was ineffective, but overexpression of the genes together conferred enhanced SCN resistance. Hence, SCN resistance mediated by the soybean quantitative trait locus *Rhg1* is conferred by copy number variation that increases the expression of a set of dissimilar genes in a repeated multigene segment.

Soybean (*Glycine max*) is the world's most widely used legume crop, providing 68% of world protein meal as well as food oil and a renewable source of fuel, with a farm gate value of more than $35 billion in the United States alone (www.soystats.com). Soybean cyst nematode (SCN; *Heterodera glycines*) is the most economically

ly 90% of the commercially cultivated soybean varieties marketed as SCN-resistant in the central United States use the *rhg1-b* allele (haplotype), derived from the soybean line PI 88788, as the main SCN resistance locus. The molecular basis of this SCN resistance has remained unclear.

Genetic mapping has placed *rhg1-b* in an in-

pin (RNA interference) constructs were produced using *Agrobacterium rhizogenes* (*11*–*13*). Soybean resistance to SCN was measured 2 weeks after root inoculation by determining the proportion of the total nematode population that had advanced past the J2 stage in each root (Fig. 1A) relative to known resistant and susceptible controls (*14*). Silencing any one of three closely linked genes at the *rhg1-b* locus of the SCN-resistant soybean variety Fayette significantly reduced SCN resistance (Fig. 1B). Depletion of resistance was dependent on target transcript reduction (fig. S1). Silencing other genes in and around the locus did not affect SCN resistance (e.g., Fig. 1B, genes *Glyma18g02570* and *-2620*) (*10*). The three *Rhg1* genes that were found to contribute to SCN resistance encode a predicted amino acid transporter (*Glyma18g02580*), an α-SNAP protein predicted to participate in disassembly of SNARE membrane trafficking complexes (*Glyma18g02590*), and a protein with a WI12 (wound-inducible protein 12) region but no functionally characterized domains (*Glyma18g02610*) (*15*–*17*).

Concurrent study of the physical structure of the *rhg1-b* locus revealed an unusual genomic configuration. A 31.2-kb genome segment encoding the above-noted genes is present in multiple copies in SCN resistant lines (Figs. 2 and 3). The DNA sequences of fosmid clone inserts con-

Copy number variation of three genes at *Rhg1* mediates SCN resistance in soybean

Gene Silencing: Identified three genes at *rhg1-b* (PI 88788 source) that impact SCN resistance.

qPCR: Transcripts for those three genes are ~10-fold more abundant in Fayette (*rhg1-b*$^{+}$).

Locus Sequencing: Revealed a 31.2 kb repeat at *rhg1-b* and other resistant sources (but not SCN-susceptible varieties).

The repeat encodes the genes implicated in resistance

Fiber-FISH: There are *ten* copies of the repeat in *rhg1-b.*

Complementation: Transgenic roots of Williams 82 overexpressing any one of the three genes did not raise resistance, but *simultaneous overexpression of the three genes increases SCN resistance.*

Predicted products of *Rhg1* genes that impact SCN resistance:

Glyma18g02580: amino acid transporter

Glyma18g02590: α-SNAP (recycling of SNARE apparatus for membrane trafficking)

Glyma18g02610: Mild homology to a wound-induced protein from ice plant with no known functional domains

Many Interesting Aspects:

- *'Rhg1* gene' identified: it is three genes.
- Novel mechanism of disease resistance: Expression polymorphism via copy number expansion.
- Novel type of copy number variation: Multiple repeats of a cluster in which multiple genes contribute to the same phenotype.

- Prospects for improved SCN resistance:
  - Identify lines with more *Rhg1* repeats.
  - Elevate expression, alter alleles transgenically.
- Possible to use in other plants?

Now that we know what Rhg1 encodes:

Can we enhance *Rhg1* by classical breeding? or GMOs?

Why has *Rhg1* resistance been so durable over decades?

What is the basis of emerging SCN 'race-specificity' differences between different *Rhg1* sources?

Why does Peking-source *Rhg1* need *Rhg4* for full function, while the widely used PI88788-source does not need *Rhg4*?

Some Current Efforts:

Biochemistry:

Race-Specificity:

Functional Testing of New Allele Combinations:

Some Current Efforts:

Biochemistry:
Is the putative amino acid transporter really an amino acid transporter, and/or a transporter of other compounds?

Is the putative α-SNAP really an α-SNAP?

What compounds are made with/without strong expression of the 2610 protein?

Race-Specificity:

Functional Testing of New Allele Combinations:

Some Current Efforts:

Biochemistry:

Race-Specificity:
What are the gene sequences in other *Rhg1* sources?
(whole genome sequencing of Hg-Typing soybean lines)

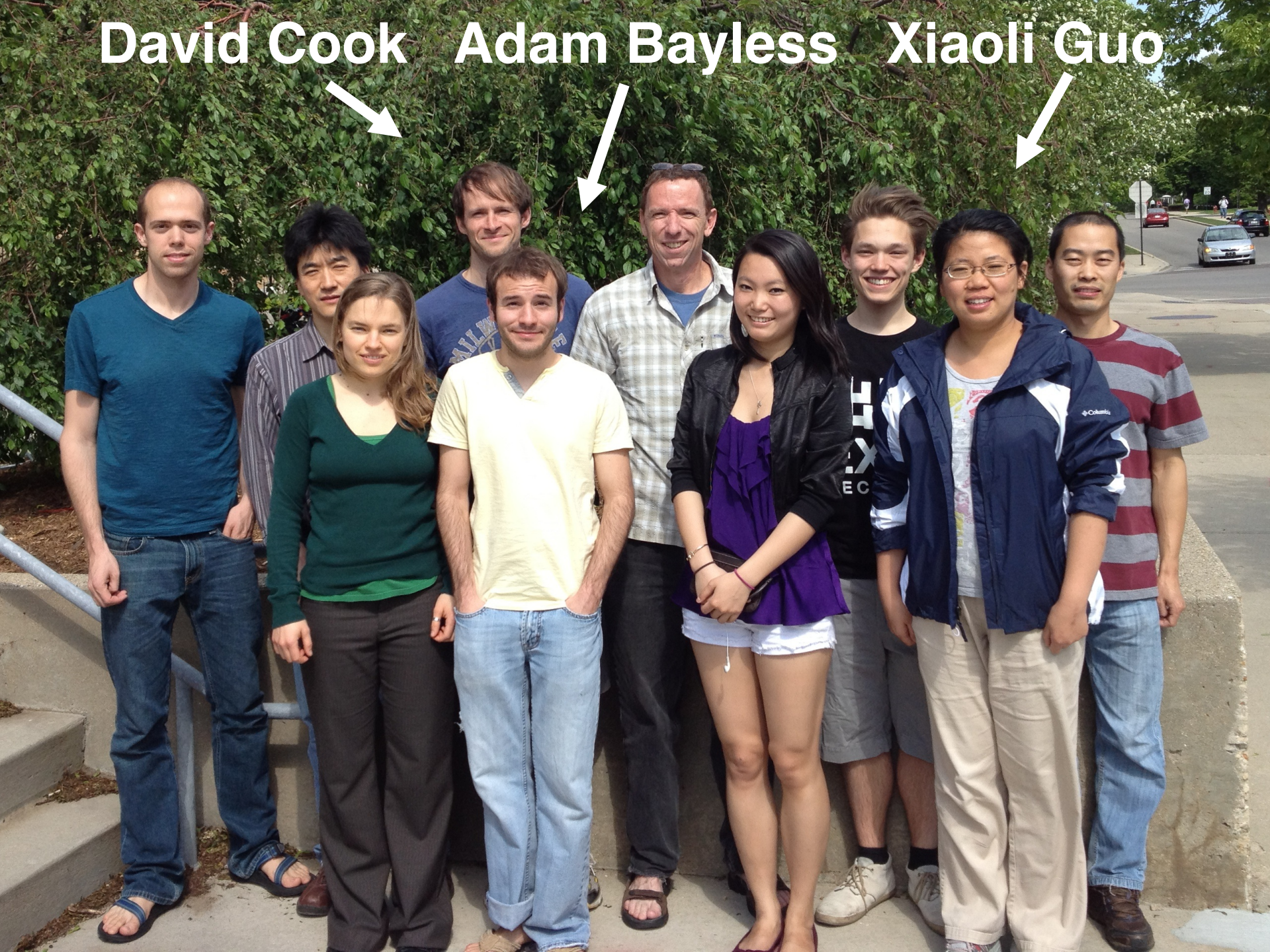
Functional Testing of New Allele Combinations:

Some Current Efforts:

Biochemistry:

Race-Specificity:

Functional Testing of New Allele Combinations:
Efficacy and Race-Specificity – alter level or ratio of expression of the three genes, and/or combine different *Glyma18g02590* alleles.


David Cook
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Thank You!