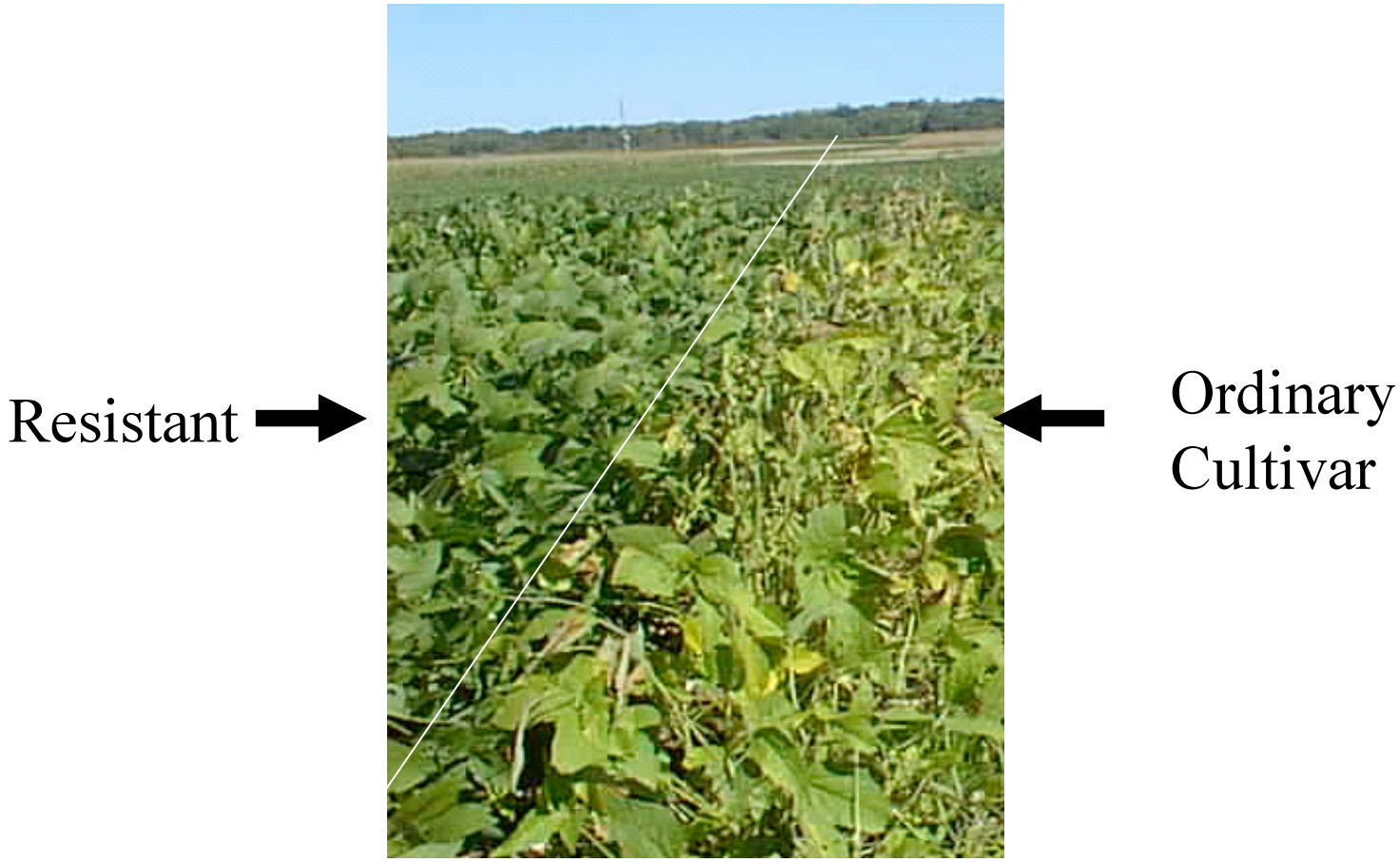
SCN COORDINATION REPORT BREEDERS MEETING 2010



Jim Orf, University of Minnesota

1. We have used PI 88788, Peking, Pi209332, PI437654 and Cloud as sources of resistance
2. We do not use hill plots in the field. We score rows by taking a sample at the beginning of the season (Pi) and at the end (Pf) and an egg count at the end of the season. In the greenhouse we use the female index (FI)
3. We use markers for LG G and A2 for MAS
4. We use 2 and 4 row plots (30 inch) for yield as well 4 and 8 or 10 row plots (10 inch); for aphids we use greenhouse and field screens compared to known checks; phytophthora is screened in the greenhouse; SDS is mainly greenhouse but also some field and BSR is greenhouse.
5. no new publications
6. no recent germplasm releases, recent variety releases--MN0606CN and MN1413CN

Stella Kantartzi <kantart@siu.edu>James Klein III jklein@siu.edu SIUC

1. Resistant sources are from PI88788 and PI437654.
2. Greenhouse with pots for early selection. Lines are scored when entered into the SCN, North, or South Regional Test.
3. None
4. Working on cultivars with multiple disease resistance SCN, SDS, RKN, frogeye leaf spot, and charcoal rot.
5. None
6. Releasing LS03-4294 as a variety.

William Schapaugh wts@ksu.edu ;

Cooperator: Tim Todd, nematologist, KSU

1. We continue to focus on combining PI88788 or Peking resistance with STS resistance in conventional lines in MGâ €™s 3-5, but use â€œHartwigâ€ sources for some populations.
2. All lines are screened for resistance to race 3 in a greenhouse assay with secondary emphasis given to races 14 and 1.
4. In 2007 through 2009, soil samples were collected from SCN-infested soybean fields across the state to characterize the diversity of soybean cyst nematode populations in Kansas. Approximately sixty samples from locations in central and eastern Kansas have been collected. The Heterodera glycines (HG) type test is being performed on each SCN population collected to characterize the virulence of each population, with nearly fifty type tests completed to date.

William Schapaugh wts@ksu.edu ; Cooperator: Tim Todd, nematologist, KSU

4. (cont) Results indicate that virulence on differentials PI88788 and PI54316 is common, with female indices >10% observed for at least one SCN population from 38% of counties sampled. In contrast, no populations with female indices greater than 3% were observed for PI 437654. Further characterization of the HG type will continue in 2010

5. Schapaugh Jr., W.T., T. Todd, J. Reese, J. Diaz-Montano, J. Meng, and C.M. Smith. 2010. Registration of K1639-2 soybean germplasm resistant to soybean cyst nematode and soybean aphid. J. of Plant Registrations 4:1-3.

Silvia Cianzio scianzio@iastate.edu Iowa State University

1. PI 507354; PI 90763; PI 567516C; PI 437655; PI 438489B
2. **Field plots.** Advanced experimental lines are entered into the ISU SCN-resistant Soybean Variety Trials. Varieties are planted at 3 locations, 4 replications, 4 row plots – 20 feet long. A soil sample is taken from each plot at planting and again at harvest. The number of SCN eggs/100cc of soil is determined. The HG Type is also determined for each field location. Yield and other agronomic traits are also determined for each plot.

Greenhouse. Naturally SCN infested soil with a known HG Type is used for all greenhouse testing. Screening begins on seed derived from single plants pulled from maturity separation populations. 5 seeds from each plant are planted in a coffee cup containing infested soil. Plants are grown for 30 days on a greenhouse bench, roots are inspected for SCN females. Samples estimated with less than or equal to 10% the number of females, compared to susceptible checks, are selected to advance to 1st year yield trials.

Silvia Cianzio scianzio@iastate.edu Iowa State University

Selections from the 1st year yield trials are screened in waterbaths in cone-tainers; 3 seeds from each line are planted in cone-tainers containing infested soil; 2 replications are used. After emergence, cone-tainers are thinned to 1 plant. After 30 days, females on the outside of the root are counted on each plant. Lines with less than or equal to 10% the number of females, in comparison to the susceptible checks, are selected to advance to the SCN Regional Test and the ISU SCN-resistant Soybean Variety Trials. Additional SCN screening takes place in the SCN Regional Test.

3. We do not use marker-assisted selection.

4. Lines with possibly other disease resistances, in addition to SCN, i.e. Phytophthora, SDS, IDC, BSR, are screened for the corresponding disease resistance at the time the line is entered in the SCN Regional Tests.

Silvia Cianzio scianzio@iastate.edu Iowa State University

5. No research publications.
6. **Germplasm releases**

AR1 (IA2039BC) has SCN resistance (PI 88788 source)
A95-684043 – Jacques J285 x [Archer x (Cordell x Asgrow A2234)]
AR4SCN, AR5SCN, AR6SCN, AR7SCN, AR8SCN – PI 88788 x Columbia

Cultivar releases

IAR1008BC (SCN/Phyto) (PI 88788 and *Rps1k*)
IAR2101 SCN (PI 88788, and PI 507354)
IAR3001 Phyto/SCN (PI90763, PI 88788, and *Rps8*)

Brian Diers, University of Illinois

1. We are using advanced experimental lines as resistant parents in our breeding program. These lines trace their resistance to the resistance sources PI 88788, PI 437654, and PI 468916 (SCN partially resistant Glycine soja line). We used the cultivar LD00-3309 (PI 88788 resistance) and the germplasm line LD00-2817 (PI 437654 resistance) extensively as SCN resistant parents in the breeding program.
2. The SCN resistance testing is being done using a water bath system in a greenhouse.
3. We are conducting marker-assisted selection for SCN resistance for only the resistance QTL that we mapped from G. soja on soybean chromosomes 15 (linkage group E) and 18 (linkage group G). We would like to do marker-assisted selection for *rhg1*, but have not been able to do this because of patent issues.
4. We recently completed research showing that by combining the chromosome 15 and 18 SCN resistance QTL from G. soja PI 468916 with resistance genes from PI 88788 or PI 437654, we can achieve greater resistance than using genes from only one resistance source. We are currently emphasizing the development of high yielding experimental lines that combine the G. soja resistance QTL with resistance genes from other sources.

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Brian Diers, University of Illinois

4. (cont) We completed a field study to compare the impact of *rhg1* from PI 437654 and *rhg1*-b from PI 88788 on SCN reproduction, yield and other agronomic traits. There was no clear indication of which allele was most effective in our field environments and rhg1-b from PI 88788 gave a positive impact on yield even in environments containing nematode populations that can overcome this source of resistance.
5. 5. Diers, B.W., T. Cary, D. Thomas, A. Colgrove, and T. Niblack. 2010. Registration of LD00-2817P soybean germplasm line with resistance to soybean cyst nematode from PI 437654. Crop Sci. In press.
6. 6. We have released several SCN resistant cultivars for private branding. We released LD00-2817 as a germplasm line (see the registration article above). This line has SCN resistance from PI 437654.
7. We are using traditional breeding and marker-assisted selection to breed for high yielding SCN resistant cultivars. We can no longer detect evidence of a yield drag associated with SCN resistance from PI 88788. We are still struggling to combine PI 437654 resistance with high yield in maturity group II and III backgrounds.

Jiang, Guo-Liang <Guo-Liang.Jiang@sdstate.edu>SDSU

1. SCN is a major problem for soybean production in South Dakota. The soybean breeding program at South Dakota State University uses PI88788 and Peking as the primary sources of resistance to SCN.
2. We rely on our plant pathology department for initial greenhouse screening and regional SCN trials for information on field performance and resistance of lines to specific HG types. Our pathology group currently is not HG typing soil samples. We rely on regional data to identify which HG types are present in our South Dakota growing areas.
3. We are currently in the process of incorporating SCN resistance into conventional soybean lines with high yield and other economically important traits.

Jiang, Guo-Liang <Guo-Liang.Jiang@sdstate.edu>SDSU

4. In the past, our breeding program worked closely with molecular biologists using SSR markers to help facilitate transfer of SCN resistance. We intend to continue research on identification and application of SSR markers for SCN resistance in soybean breeding and research. We will also focus on development of elite germplasm with SCN resistance integrated with high yields, good quality and/or other resistances.
6. SDSU released SD1161RR/SCN in 2007 with SCN resistance from PI88788. SDSU is no longer working on RR1. We are working on a limited basis with RR2 including entry submitted for RR2Y trait introgression with some resistance to SCN (data from regional SCN trials data).

Vince Pantalone
University of Tennessee

1. We collaborate with Dr. Prakash Arelli, USDA-ARS, Jackson, TN on a project to develop new soybean lines with durable resistance to SCN (See Dr. Arelli's report for further details). In that regard, we anticipate drafting a request during 2010 to release the high yielding line JTN-5203. JTN-5203 is a MG V conventional soybean line with resistance to multiple races of SCN. Averaged over five environments of the 2009 Tennessee State Variety Test, JTN-5203 ranked second highest for seed yield, producing over 4,500 kg ha-1, which was not significantly different than the top yielding entry. In that test, JTN-5203 exhibited strong tolerance to a heavy and uniform infestation of SDS: it scored an SDS DX value 0.7 (where DX scores among 36 entries ranged from 0.0 to 55.0, with a DX mean of 20.3).
2. In other work, we will be conducting field trials of high yielding late MG IV and early MG V lines which have resistance of multiple races of SCN inherited from the parental lines 'Anand' and 'Fowler'. Greenhouse studies conducted this winter will confirm HG type resistance. Two additional years of field yield trials will be necessary before considering the possibility of release.

TN Conclusions

3. Most of our glyphosate resistant populations include SCN resistance as a planned purpose from the cross of parental lines.
7. In basic research, salicylic acid (SA) is a critical signal for activation of plant defense responses both at the site of infection and systemically in distal tissues. We are working with a functional genomicist to characterize SA-related genes that may play critical roles in SCN resistance in our advanced breeding (see poster at this workshop by Jingyu Lin).

Arelli, Prakash Prakash.Arelli@ARS.USDA.GOV,
USDA Jackson, TN

1. Based on the genetic and molecular marker diversity studies, a set of PI lines diverse from Peking, PI88788 and PI437654 have been identified for breeding soybeans for durable resistance to nematodes. These PIs include; PI507354, PI467312, PI567516C, PI438489B, PI437655, PI567328, PI89772, PI22897 and PI494182.
2. Field evaluations to identify SCN resistance lines have not been successful. We have been using greenhouse evaluations using either water -bath system with controlled temperature and lighting or 3” clay pots with under bench heating and lighting (controlled). Most recently, we have been using 3” clay pots for screening. Cultured nematode populations for most prevalent HG Types (Races 2,3,5 and 14) maintained and increased in the greenhouse have been valuable for identifying resistant progenies. We published these methodologies in refereed journals.

Arelli, Prakash Prakash.Arelli@ARS.USDA.GOV, USDA

Jackson, TN

3. Since 2002 we have been confirming resistance to SCN using known SSR markers for marker assisted selection which has been very effective in my breeding program. Primarily we use Satt 162, Satt 632, Satt 309 and Satt 574 markers. Advance generations are used for MAS. DNA is harvested from field or greenhouse grown progenies by pressing leaves onto Whatman FTA Cards. It is PCR based. Another set of SSR markers Satt 592, Satt 331 and Sat_274 are highly useful in MAS for resistance derived from PI567516C to LY1 a synthetic nematode population which overcomes resistance of Hartwig.
4. In the field, we routinely eye ball and score for fungal diseases such as Frogeye leaf spot, Stem canker, SDS and evaluations for Charcoal rot is done in collaboration.

5. (1). Prakash Arelli, Lawrence Young and Vergel Concibido. 2009. Inheritance of resistance in soybean PI 567516C to LY1 nematode population infecting cv. Hartwig. Euphytica 165: 1-4.

   (2). Prakash Arelli and Dechun Wang. 2008. Inheritance of Cyst nematode resistance in a new genetic source, Glycine max PI494182. J. Crop Sci. Biotech. 11: 83-90.

6. Soybean JTN-5503, JTN-5303 (high yielding, MG V with resistance to multiple nematode populations). First germplasm lines developed and released using MAS for nematode resistance.

JTN-5109, JTN-5209 and JTN-5203 are being released soon. These are again nematode resistant and MG V soybean lines.

7. Our primary objective is to diversify nematode resistance in soybeans and identify new sources of resistance in Max and Soja. We are currently screening several hundreds of PI lines from Soybean Germplasm Collection (courtesy of soybean curator) for identifying additional resistant sources.

Thomas E. Carter, Jr.
Thomas.Carter@ARS.USDA.GOV, USDA-ARS,
Raleigh

1. Using the cross Cook (MG7) x Anand (MG5), we developed breeding lines via SSD which were subsequently selected visually for agronomic characteristics.
2. The best 100 lines or so were screened for SCN resistance by Steve Koenning (NCSU) in the greenhouse in pots. Approximately 8 resistant lines were identified and 2 performed well agronomically. The first, group 7 breeding line N02-7084, appears to have the same resistance as Anand and has topped the NC State Variety Trials (NCSCVT) for conventional materials over the past 3 years in it's maturity group. It is also being used as a check in the USDA Southern Uniform tests (USDA-UT).

Thomas E. Carter, Jr.
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6. The group 5 breeding line, N02-7002 is resistant to race 2 but not all SCN races. It is performing well in the NCSVT and USDA-UT). N02-7084 will be released in the spring 2010 and will be one of the first group 7 cultivars with race 2 resistance. N02-7002 is undergoing further testing.
4. Prakash Arelli is performing confirming SCN marker analysis on these lines.
7. We have a small ongoing SCN resistance program.

Transgenics with the RLK and WxENIL hybrids

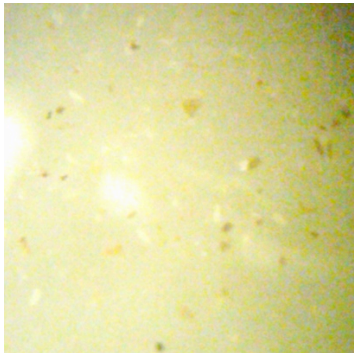
David Lightfoot - SIUC

1. Forrest (Peking), Hartwig (PI437654), Pyramid (PI88788), X5 transgenic with RLK (X5::RLK),
2. Waterbath with 2,000 eggs per tube and infested soil in pots.
3. *Rhg1* (LG G, Chr18) with SIUC-TMD001 (intron of RLK at RHG1), SIUC-Sca005 (core promoter of RLK at RHG1) and 3 SNPs within gene to separate 9 alleles for 5 alloproteins. *Rhg4* (LGA2; Chr8) with SIUC-SagB100B10a and b; the a marker is dominant because it is in the deletion of the *I* seed coat color gene. That deletion is larger in S than R cultivars. *Rhg3* (LG D2, Chr17), BARC-Satt543, SIUC-SCAR-OZ19, SIUC-Sat H30M22. This locus is free from lien by patents other than SIUCs US Patent # 6,300,541.
4. Recovered SDS resistance in X5::RLK and its progeny in crosses to Williams x Essex NIL and ExF2. Therefore, *rhg1* is pleiotropic to *Rfs2*. Root growth was restricted by the RLK when not infested which may explain the yield drag of Peking allele of *rhg1.* Will field test for yield effects in 2010.

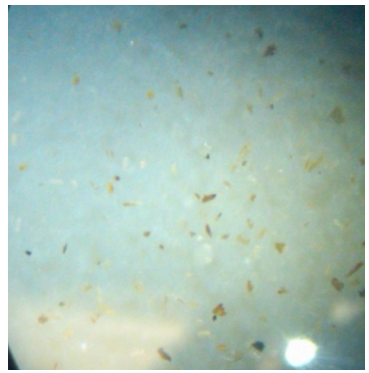
RLK transgene SCN reduction by *rhg1/Rfs2*

Cultivar	x5RLK	x5
SCN IP	60±11	100±13
RLK Gene	+	-

No chlorosis



Nonspecific chlorosis



RLK transgene leaf scorch reduction by *rhg1/Rfs2 14 dai*

Cultivar	x5	x5RLK	EF23	EF85
Fusarium	+	+	+	+ 10^4 cfu
Gene	-	+	+	-

DS	2.0	0	1	3
-				

RLK transgene leaf scorch reduction by *rhg1/Rfs2 – 21 dai*

Cultivar	EF85	EF23	x5	x5RLK 10^4 cfu
Fusarium	+	+	+	+
Gene	-	+	-	+

DS	3.0	1.5	3.0	1.0
	-			

David Lightfoot - SIUC

5. Kazi S, Shultz J, Afzal J, Hashmi R, Jasim M, Bond J, Arelli PR, Lightfoot DA.2010. Iso-lines and inbred-lines confirmed loci that underlie resistance from cultivar 'Hartwig' to three soybean cyst nematode populations. Theor Appl Genet. 120:633-640

Afzal AJ, A Natarajan, N Saini, M J Iqbal, MA Geisler, H El Shemy, R Mungur, L Willmitzer and DA Lightfoot. 2009. The nematode resistance allele at the rhg1 locus alters the proteome and metabolome of soybean roots. Plant Physiology 151: 1264–1280.

Karangula UB, M.A. Kassem, L. Gupta, H.A. El-Shemy and D.A. Lightfoot. 2009. Locus interactions underlie seed yield in soybeans resistant to Heterodera glycines. Curr. Issues Mol. Biol. 11 (Suppl. 1): i73-84

Transgenics
Root Stunting by *rhg1*

- **+** **+** **-**

6.4 **3.2** **4.6** **6.3** **(g)**

-

David Lightfoot - SIUC

Afzal AJ, Srour A, Saini N, Lightfoot DA, 2008. The multigeneic *Rhg1* Locus: A model for the effects on root development, nematode resistance and recombination suppression. Nature Preceedings hdl:10101/npre. 2008.2726.1

6. None recently

7. Selection on the abundance of isoflavone synthase protein and maltose may identify Peking derived *rhg1* alleles. Needs to be tested in wider germplasm.

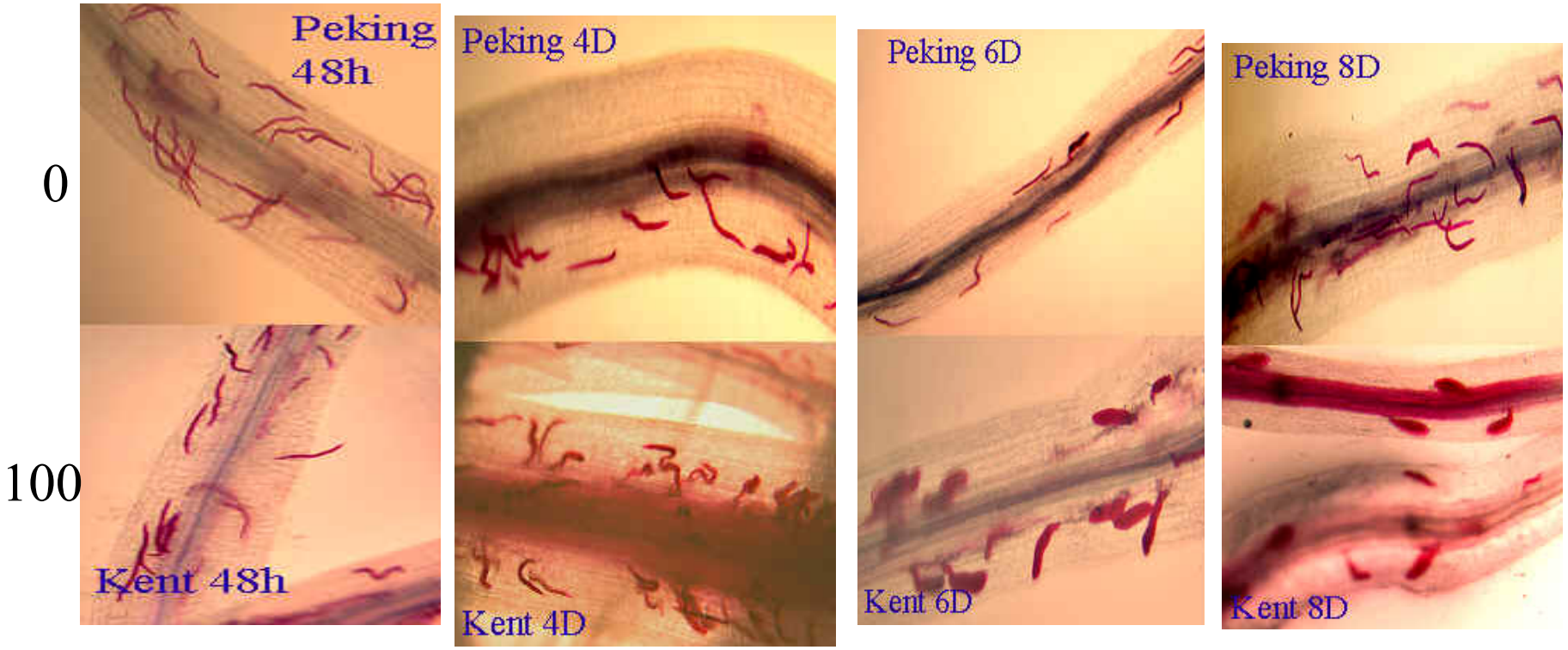
David Lightfoot - SIUC
Marked Germplasm

		Yield MG	SDS Habit	Yield	SDS R SDS	Leaf DX	Genes	Root R	SCN R
F	**94**	**4.5**	**Det**	**3.33**	**3.27**	**1.4**	**6**	**Yes**	**None**
	13	**4.5**	**Det**	**3.15**	**3.09**	**0.1**	**6**	**Yes**	**3**
x	33	4.7	Det	2.98	2.85	6.2	5	Yes	3,14
	34	4.5	Det	3.09	2.95	18.9	5	Yes	2,3,14
H	77	4.5	Det	3.33	2.65	62.6	0	No	None
ExF	**23**	**5.1**	**Det**	**3.63**	**3.48**	**1.1**	**6**	**Yes**	**3**
	44	5.1	Det	3.56	3.51	1.2	6	Yes	None
PxD	85	5.1	Det	3.62	3.05	24.5	0	No	None
	98	**4.0**	**Semi**	**nd**	**3.47**	**1.1**	**4**	**No**	**3,14**

Conclusions

1. **Twenty different sources of resistance are being used.**
2. **Evaluations are mainly greenhouse assay based, waterbath or pots, with field confirmations**
3. **Markers are only used by a minority of programs (4 of 10) largely due to patenting issues on A2 and G.**
4. **SCN associated diseases include FLS, SDS, Charcoal Rot.**
5. **There were 8 peer reviewed publications.**
6. **There were 10 germplasm and 11 cultivar releases with resistances to races 2, 3, 5, and 14.**
7. **New methods being tested included new PIs, biomarkers of resistance (SA, maltose) and separation of seed yield drag from PI437654 alleles.**

SCN Root Invasion
What does co-dominant mean?
IP=40-50



Invasion of soybean after inoculation by SCN race 3. Peking is resistant to SCN race 3, while Kent is susceptible (from B. Mathews)

http://bldg6.arsusda.gov/benlab