G. longicalyx project introgressing reniform nematode resistance into cotton

Support

Cotton Incorporated
Texas A&M University
USDA-ARS (Cotton Pathology Research Unit)

Scientists and assignments

Hybrid development and breeding.....Al Bell

Cytogenetics & molecular markers.....David Stelly
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Resistance phenotyping.....Forest Robinson

Objective: Introgress resistance from *G. longicalyx* to produce package of germplasm + markers giving industry incentive and means to develop cultivars that yield well and reduce populations in infested fields

Strategy: Cross *G. hirsutum* with *G. longicalyx* hybrids and select for resistance in progeny from repeated backcrosses onto *G. hirsutum* up to BC_6 or BC_7, monitoring progeny cytogenetically and morphologically, then obtain homozygous resistant progeny that yield and lack deleterious recessive exotic genes from hybrids. Develop markers during introgression beginning with AFLP progressing to SSR or other transportable markers.

Benchmarks and Pitfalls: Introgression into *G. hirsutum* chromosomes, irreversible gene block (e.g. introgression into A genome inversions), low marker density or homology near resistance gene(s), differential expression of multiple resistance genes upon recombination within alien segment.

Triple species hybrids (A.A. Bell, 1980's)
used by USDA-ARS to introgress
reniform nematode resistance
from *G. longicalyx* into upland cotton

1. **HLA hybrid (Bell's strategy: substitute F for A)**

   (*G. hirsutum* x *G. longicalyx*)^2 x *G. armourianum*

   Genomes: (AD x F) ⟶ (FAD)^2 x D ⟶ (FADD)

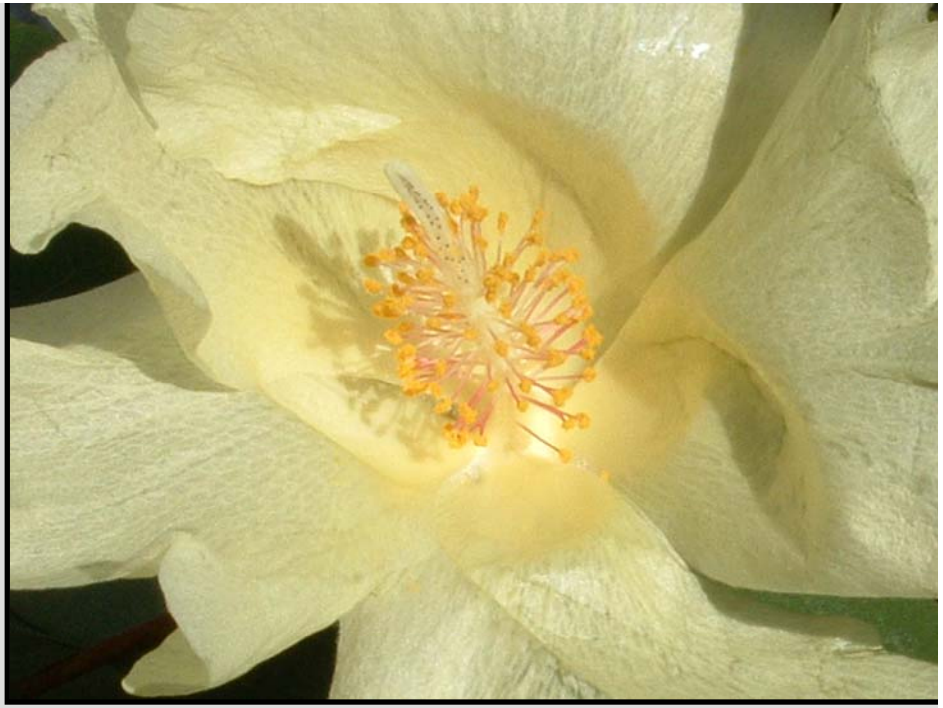
2. **HHL hybrid (Bell's strategy: substitute F for D)**

   (*G. hirsutum* x *G. herbaceum*)^2 x *G. longicalyx*

   Genomes: (AD x A) ⟶ (AAD)^2 x F ⟶ (AADF)

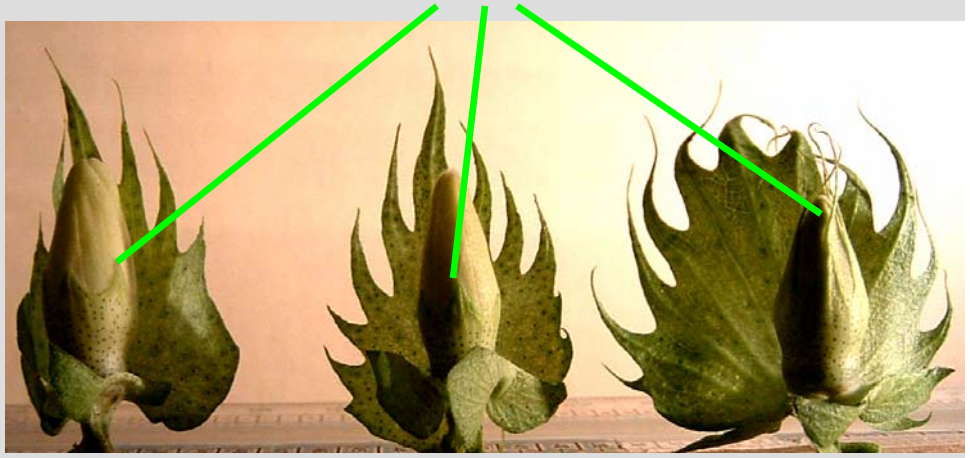
[Note: *G. hirustum* (AADD) 'TM-1' (Deltapine 14) × *G. longicalyx* (FF) hexaploid created by Dr. Meta Brown.]

HHL hybrid – Red petal spots from *G. herbaceum*



HLA hybrid – Pink staminal filaments from *G. armourianum*

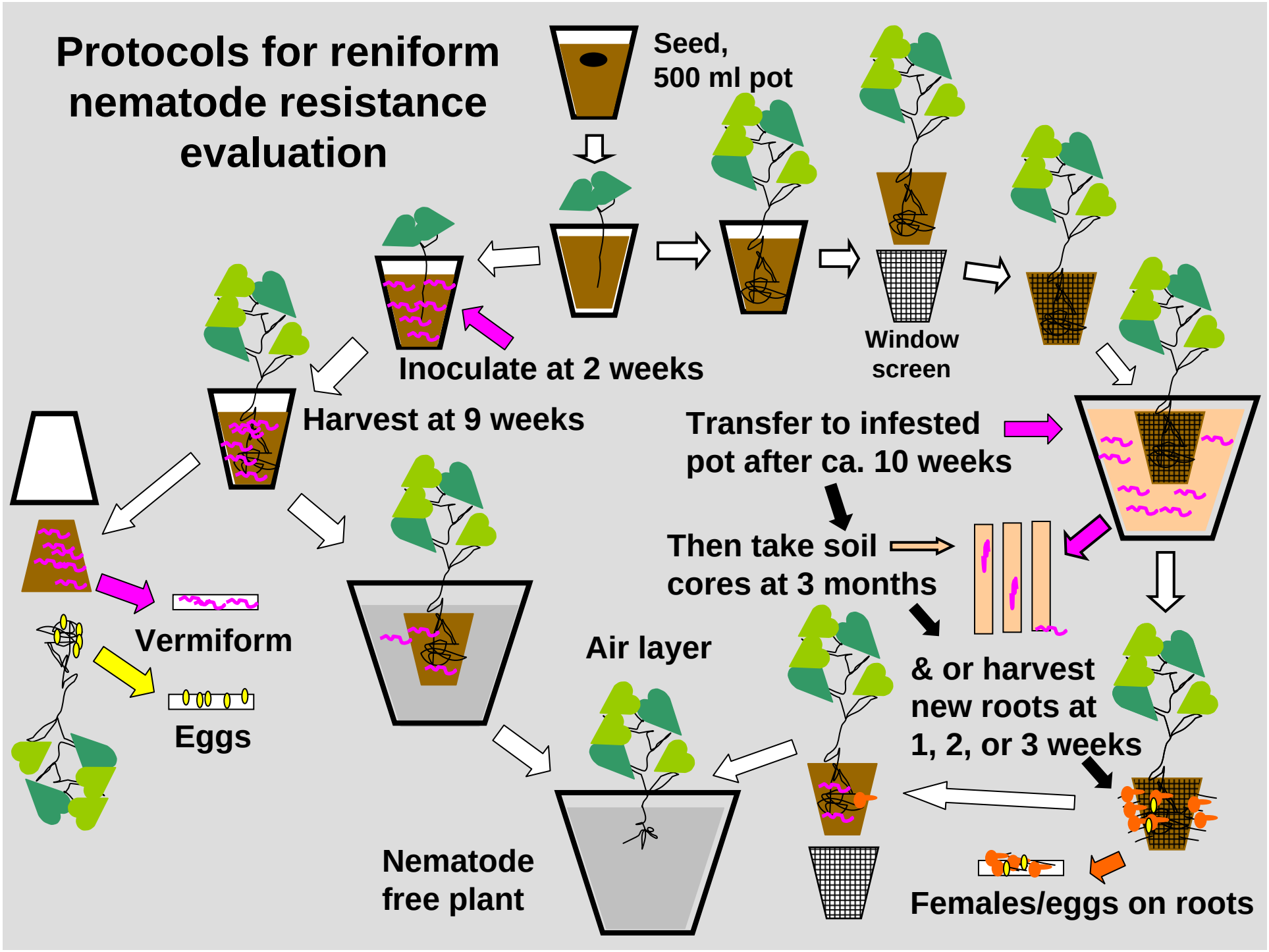
Stem pubescence



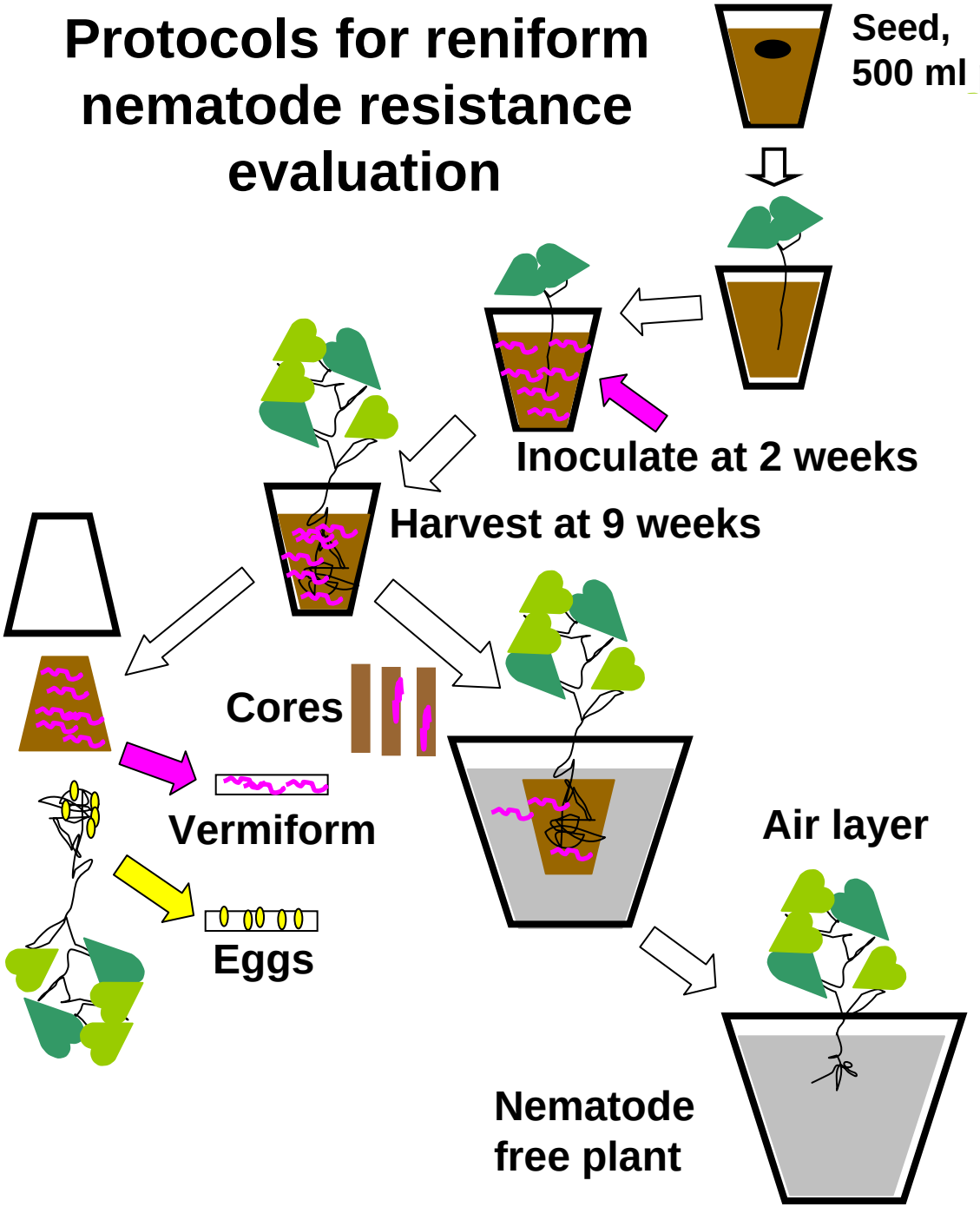
G. hirsutum

BC1 F1

Al Bell with hybrid (Bell on right)


Protocols for reniform nematode resistance evaluation
Seed, 500 ml pot
Window screen
Inoculate at 2 weeks
Harvest at 9 weeks
Transfer to infested pot after ca. 10 weeks
Then take soil cores at 3 months
& or harvest new roots at 1, 2, or 3 weeks
Females/eggs on roots
Vermiform
Eggs
Air layer
Nematode free plant

Protocols for reniform nematode resistance evaluation



Progeny status 17 months ago (January 2004)

BC_1 Set A - 1992
(ca. 90 plants, nematode resistance not selected and lost at the BC_2

BC_1 F_1 2000-2001
Of 89 plants from resistant mothers, 41 were immune or highly resistant (28<1% ck & 41<2% ck)

BC_2 - 2002
Of 58 plants from DP&L 458 mothers, 12 were immune or highly resistant (10<1% ck & 12<2% ck) ; pollen from immune or highly resistant BC_1 plants.

BC_1 Set B -1999
(69 plants, 3% highly fertile, 10 immune, 9 resistant)

BC_2 Set B 2000-2001
Of 63 plants from resistant mothers, 5 were immune or highly resistant (3<1% ck & 5<2% ck); male parents RKN-res.

BC_3 Set B 2002
Of 53 plants from D&PL 458 mothers, 19 were immune or highly resistant (12 <1% ck & 19<2% ck); pollen from immune BC_1 plants.

BC_4 Set B 2003-2004
Seed in mature bolls of NemX and DP&L 458; pollen from immune BC_3 plants; BC_4's not yet tested.

BC_1 Set C - 2002
(604 from HLA X NemX, 104 were male fertile; of these 34 (33%) were immune or highly resistant.

BC_2 Set C, 2003
Immune BC_1 used to pollinate NemX and DP&L 458; bolls almost mature)

BC_1 Set D -2002
(21 from HHL X NemX)
4 were self fertile; of these, 1 (25%) was immune.

BC-2 Set D, 2003
Immune BC_1 used to pollinate NemX and DP&L 458; bolls almost mature)

Total plants phenotyped for resistance = 470, Jan 2004

Families in Set B (mixed HLA & HHL)

	# 2	# 77	# 83	# 84	# 85	# 103	# 122	# 132
BC2	3	11	7	37	12	29	5	2
BC3	2	17	7	6	23	3	3	5
BC4	4	18	6 (10)	32	4 (40)	4 (10)	9	4
BC5	(10)	4 (20)	(20)	4 (10)	(20)	(10)	(20)	(10)
BC6		(30)		7 (20)				

Status – June 9, 2005
Number of resistant plants identified. (Plants currently being tested in parentheses)

Families in Set C (HLA set)

	# 4	# 18	# 21	# 26	# 34	# 35	# 45	# 61	# 75	# 77	# 81	# 91	# 99	# 103
BC2	3	4	6	2	2	2	4	3	2	6	5	2	2	3
BC3	4	4	0	3	3	1	5	4	4	2	5	5	1	4
BC4														
BC5														
BC6														

Families in Set D (HHL set)

	# 3	# 5	# 7	# 11	# 14	# 17
BC2	1	3	3	6	5	5
BC3	0	4	1	4	2	5
BC4						
BC5						

Other generations in Families in Set B

	# 2	# 77	# 83	# 84	# 85	# 103	# 122	# 132
BC1S1				34		13		
BC2S1						(16)		
BC3S1		19 (108)		57 (204)				
BC4TC		(70)						

Example of resistance assay data set - % of susceptible control

Code	Family	Parent	Generation	Plant number 1	2	3	4	5	6	7	8	9	10
95	BC1 77	BC3 1-6 self	**BC3S1**	4	4	63	51	50	3	3	2	42	93
			BC3S1	37	37	4	4	1	84	60	1	1	45
			BC3S1	6	5	61	54	1	1	109	6	121	3
			BC3S1	3	114	9	2	3	91	66	6	3	96
			BC3S1	120	69	2	46	123					
96	BC1 84	BC3 3-15 self	**BC3S1**	71	28	1	17	0	0	0	21	0	0
			BC3S1	2	3	38	0	35	1	10	1	0	22
			BC3S1	1	7	2	55	0	0	3	14	1	0
			BC3S1	0	1	0	1	0	0	1	1	0	67
			BC3S1	1	9	0	1	1					
97	BC1 84	BC3 3-24 self	**BC3S1**	1	1	0	0	94	0	16	5	1	1
			BC3S1	3	2	1	2	2	0	20	1	3	2
			BC3S1	1	1	0	1	0	0	1	1	1	0
98	BC1 2	BC3 1-9 BC*	**BC4**	4	0	92	69	5	57	87	136	1	78
99	BC1 77	BC4 47-10 BC	**BC5**	77	115	108	8	21	3	89	123	2	1
100	BC1 77	BC3 4-10 BC	**BC4**	2	224	9	6	115	78	1	86	99	54
101	BC1 83	BC3 52-7 BC	**BC4**	4	2	118	1	72	39	0	3	2	134
102	BC1 83	BC2 5-2 BC	**BC2**	3	77	90	45	149	66	91	2	6	85
103	BC1 85	BC3 11-10 BC	**BC4**	5	1	1	66	76	73	105	80	29	3
104	BC1 103	BC3 33-3 BC	**BC4**	128	61	88	3	129	3	54	2	73	4
105	BC1 103	BC2 15-21 BC	**BC2**	144	73	5	0	88	78	2	4	1	113
106	BC1 122	BC3 19-5 BC	**BC4**	2	1	37	3	5	3	5	3	3	2
107	BC1 132	BC3 56-8 BC	**BC4**	127	5	1	38	4	43	79	123	6	0
108	**DP-16**		**Sus. Ck.**	157	108	116	116	119	54	105	113	119	98
109	**GB-713**		**Res. Ck.**	2	6	3	1	2	14	8	2	5	1

5% or less

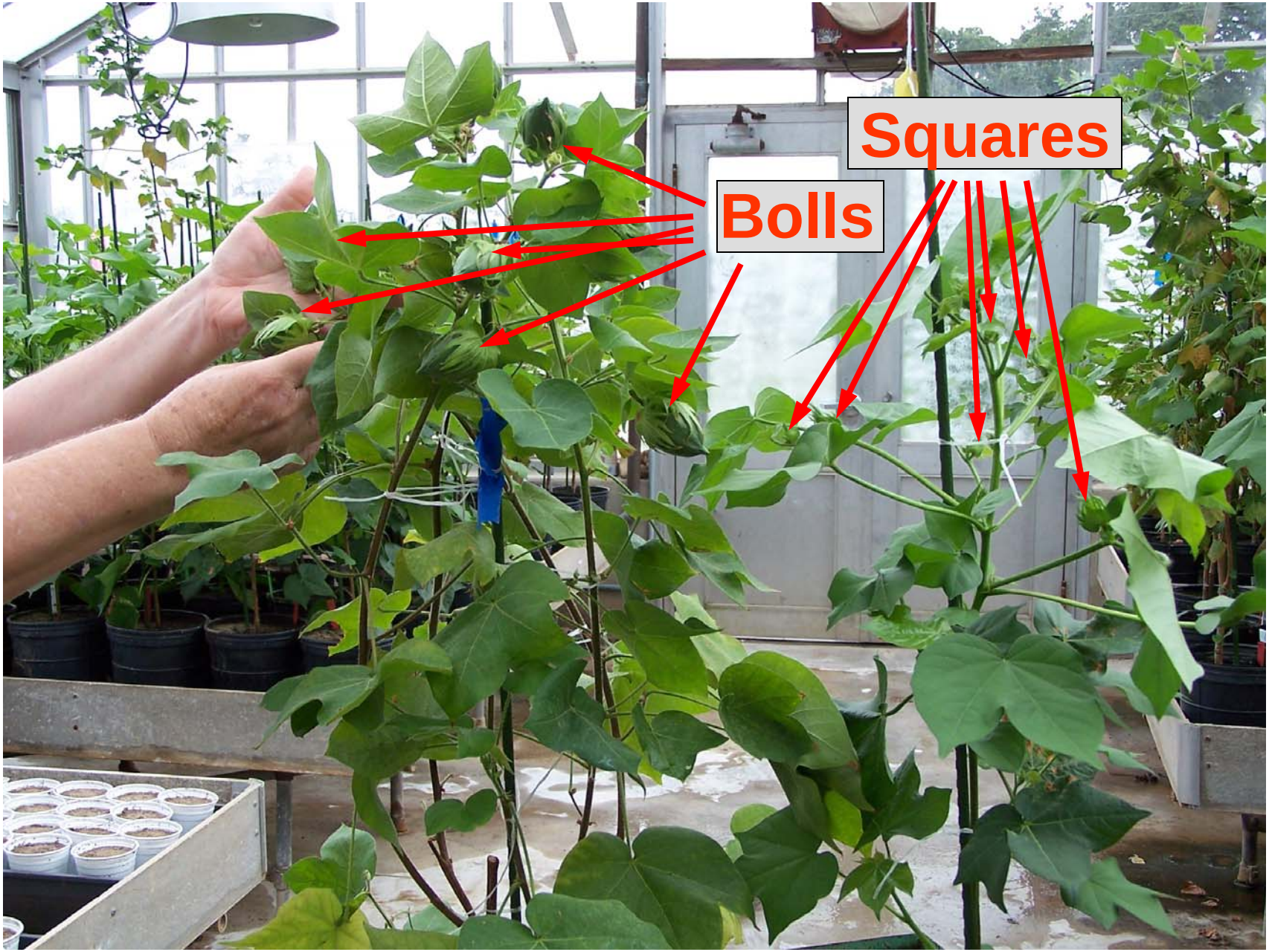
> 5%, < 1/3

1/3 to 1/2

> 50%

Resistant BC_4
plant with bolls

Young resistant BC_6
plant with squares



Resistant BC_4 plant with bolls

Young resistant BC_6 plant with squares