Meadowview Notes 2006-2007

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Meadowview experienced fairly normal rainfall in the summer of 2006, and adequate rain in the fall, but it was fairly dry in January thru March of 2007, and extremely dry in May and early June. There was enough rainfall in April to delay spring plowing for local farmers, but our plowing in February and March was not hampered by wet weather beyond late February.

Inventory.
Our current holdings are presented in Table 1, and changes from 2006 to 2007 are indicated in Table 2. We now have more than 33,000 trees and planted nuts, an increase of approximately 7,400 over last year (Table 2). The addition of B_3-F_2 trees has been offset by the removal of straight backcross trees as we have made selections and rogued the rejects. We now have screened all of our 'Clapper' B_3 trees for blight resistance and completed rouging of rejects in those orchards. We also are largely finished screening and roguing our 'Graves' B_3 trees. Many of the B_3 trees listed in Table 1 are from sources of blight resistance other than 'Clapper' or 'Graves.' There also is now significant roguing of rejected B_3-F_2 trees, so their numbers may be close to peaking, with new plantings offset by removals. We have some hope that we will finish planting 'Clapper' B_3-F_2 seed in the next two to three years.

Our 14 state chapters have numerous additional 'Clapper' and 'Graves' B_3 trees to those reported in Table 1. The numbers of trees in the chapters is now reported separately in the Spring edition of the Journal.

Harvest.
The most noteworthy event of the 2006 harvest (Table 3) was that it was our largest crop ever, including seed from open pollination. The crop from controlled pollinations was our second largest ever, and we placed the largest number of bags ever this year.

Part of the reason for the large number of bags was that Bob Paris headed up an effort to make a number of crosses among pure Chinese chestnut trees, to see whether we can determine if multiple sources of resistance to chestnut blight exist within the Chinese chestnut population. We also made crosses on a more limited scale with European, Japanese, and large surviving American chestnut. In January of 2007, we made grafts of the Chinese parents in the greenhouse, and in the spring we planted the grafted parents along with the progeny from the crosses in three plots. Based on emergence data, we had to redo some cross combinations in the summer of 2007. We hope that examination of the progeny under blight screening will reveal additional genes for resistance not already in use in TACF's breeding program. Additional sources, if found, could prove valuable to the the breeding program by possibly providing increased levels of resistance. However, if no new sources of resistance are found, we will know that the current breeding efforts are being maximized with all of the available resistance from Chinese chestnut.

The yield from controlled pollinations was average, about one nut per pollination bag, which was a welcome respite from last year, when yield was very poor.

We used a large number of dried pollens this year, but the yield from those pollinations was much worse than some of the chapters report, around 0.7 nuts per bur. This was significantly ($p<0.05$) better than the yield of pollinations with fresh catkins, however, which was about 0.4 nuts per bur. The yield from open pollinations was about 1.0 nuts per bur.

The open-pollination yield from older, larger trees may have been higher (about 1.3 nuts per bur) than the yield from smaller younger trees (about 0.8 nuts per bur). This difference in tree size also may be associated with the better yield seen in some chapter pollinations than in pollinations at Meadowview. After accounting for tree size, there were no apparent differences between the yield from open-pollinated backcross trees and the yield from open-pollinated pure species. This question will need to be revisited when it is not confounded with tree size.

Last year, William White measured the germination of all pollens sent out to the state chapters and compared that to nut yield. As we found in three previous years of this comparison, there was no correlation between the percentage of pollen grains that germinated and nut yield. This past winter, William worked long hours to optimize the pollen germination assay. We found that pollen and sugar concentration were critical factors in germination, with optimal germination occurring at concentrations of about 4 mg of pollen per 1.5 ml of 0.5% (w/v) sucrose solution. More complete instructions are posted on our website at
http://www.acffarms.org/papers/Pollen%20germination%20assay.pdf

With the improved assay, we measured the germination of all pollens sent out this year. Several B_3 pollens germinated at over 70%, which is much higher than reported previously for chestnut. Most B_2 and B_3 pollens germinated at levels comparable to those seen in pure species, although pollens from more pure species will need to be examined to confirm this. It will be interesting to see whether we can establish a correlation with nut yield using germination data from the improved assay.

One point that emerged concerning the pollen assay was that pollens that had been taken to the field appeared to have poorer germination than those that had remained in the refrigerator. It may be helpful to store your pollen in a cooler on ice except when in use. To avoid freeze injury, you may want to ensure that the pollen is insulated from the ice itself by crumpled up paper or a similar item. However, if you remove pollen from the refrigerator, be sure to let it warm before removing the vial cap, to avoid condensation of water on the cold pollen.

Improving chestnut establishment on croplands.
Bob has also begun a multi-year experiment (2006-2010) to address some of the challenges in establishing chestnut on lands previously used for cropping. We suspect that chestnut establishment and growth on land formerly used for cropping may be affected by the type of crop previously grown. As TACF approaches the time when chestnut plantings will be on the increase by land owners, we think it will prove valuable to know how to most efficiently and successfully grow chestnut. We designed this experiment to examine the effects of chestnut growth following corn, tobacco, and grass crops on land that has been tilled for crop production, and on pasture land that has remained relatively undisturbed. We hope that, based on this experiment, we will be able to make recommendations for chestnut establishment on various types of land.

Blight resistance screening in B_3-F_2 seedlings.
The year 2006 was the third in which we screened 'Clapper' B_3-F_2 seedlings for blight resistance and the second for 'Graves' B_3-F_2 seedlings. The results of the 'Clapper' tests are presented in Table 4 and those for the 'Graves' test in Table 5. This year we inoculated many more 'Clapper' and 'Graves' B_3-F_2 seedlings than in 2004 and 2005. However, as discussed in last year's Meadowview Notes, we have moved to staged screening for blight resistance in B_3-F_2 seedling seed orchards, which means this year we only inoculated with a relatively nonaggressive, but virulent, strain of the blight fungus. This will separate out the weakest trees, those in resistance classes 4 and 5. Next year, in 2008, we will inoculate the stronger trees from the 2006 test with an aggressive strain of the blight, to separate out the strongest trees.

Interestingly, this year, for the first time, we saw statistically significant family differences between American backgrounds, for both the 'Graves' and 'Clapper' sources of blight resistance. Further testing will reveal whether the difference has biological significance.

Overall, Fred Hebard has cautious optimism that we will be able to recover highly blight-resistant progeny among the B_3-F_2 seedlings from the 'Clapper' and 'Graves' sources of resistance, but we certainly do not have firm evidence for this yet. Stay tuned!

We would like to thank Lou Silveri, Dave Lazor, Dick Olsen, Steve Berilovits, and Sam Fisher for helping with pollinations and inoculations. Special thanks to Dave Slack for volunteering two days a week all year round for the past two years(!), and to Ignazio Graziosi for interning this summer. Also, we need to acknowledge the role of George Sykes and Danny Honaker in keeping the farms running from day to day. Thanks to all —this wouldn't get done without their help. If you are interested in helping to pollinate next year, plan on any time in June (call 276 944-4631). If you are interested in learning more about the Elder Hostel program, call 617 426-8055 or write 75 Federal St., Boston MA 02110.

We would like to remind all TACF members that you are welcome to visit the farms at any time. We are in a white house on the northeast side of Virginia Route 80, one-third of a mile southeast of Exit 24 on Interstate 81, the Meadowview Exit. We generally are there during normal work hours, but it might be good to call ahead (276 944-4631).

Table 1. Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms in May 2007, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Type of Tree	**Number of**		
	Nuts or Trees	**Sources of Resistance**	**American Lines***
American	2161		222
Chinese	1149	53	
Chinese x American: F_1	511	20	83
American x (Chinese x American): B_1	582	16	40
American x [American x (Chinese x American)]: B_2	1683	11	95
American x {American x [American x (Chinese x American)]}: B_3	1683	9	78
Am x (Am x {Am x [Am x (Ch x Am)]}):B_4	30	3	3
(Ch x Am) x (Ch x Am): F_2	253	5	9
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F_3	6	1	1
[Am x (Ch x Am)] x [Am x (Ch x Am)]: B_1-F_2	471	4	6
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	223	5	7
(Am x {Am x [Am x (Ch x Am)]}) x (Am x {Am x [Am x (Ch x Am)]}):B_3-F_2	18169	2	35
B_3-F_3	217	1	5
Chinese x (Chinese x American): Chinese B_1	191	3	3
Chinese x [American x (Chinese x American)]	41	1	1
Chinese x Chinese	2255		
Chinese x Japanese	109		
Chinese x European	140		
Chinese x Large, Surviving American	28		
European	1	1	1
European F_1	2	1	1
Japanese	13	3	3
Japanese F_1	11	2	2
Japanese B_1	10	2	2
Japanese B_2	133	2	2
Japanese x European	157		
Japanese x Large, Surviving American	42		
Castanea seguinii	48	3	3
Large Surviving American F_1	548	15	32
Large Surviving American B_1	531	8	27
Large Surviving American B_2	94	2	6
Large Surviving American I_1	1411	19	21
Large Surviving American F_2	374	5	10
Large Surviving American other	146	10	13
Other	33		
Total	**33456**		

* The number of lines varied depending on the source of resistance. We will have to make additional crosses in some lines to achieve the desired number of 75 progeny per generation within a line. In keeping with past practice, the number of lines for each source of resistance are added separately; thus, progeny from two sources of resistance that share an American parents would be counted as two lines rather than one line (this only occurs rarely).

Table 2. Changes between 2006 and 2007 in the number of chestnut trees and planted nuts of different types at TACF Meadowview Research Farms, including changes in the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Type of Tree	**Increase or Decrease* in Number of**		
	Nuts or Trees	**Sources of Resistance**	**American Lines**
American	-1		-13
Chinese	457	2	
Chinese x American: F_1	-12	-2	-7
American x (Chinese x American): B_1	157	1	7
American x [American x (Chinese x American)]: B_2	124	1	4
American x {American x [American x (Chinese x American)]}: B_3	-2135	0	1
Am x (Am x {Am x [Am x (Ch x Am)]}):B_4	21	2	2
(Ch x Am) x (Ch x Am): F_2	-457	-1	3
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F_3	0	0	0
[Am x (Ch x Am)] x [Am x (Ch x Am)]: B_1-F_2	-298	0	-2
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	-118	0	-2
(Am x {Am x [Am x (Ch x Am)]}) x (Am x {Am x [Am x (Ch x Am)]}):B_3-F_2	5793	0	6
B_3-F_3	96	0	3
Chinese x (Chinese x American): Chinese B_1	0	-1	-1
Chinese x [American x (Chinese x American)]	0	0	0
Chinese x Chinese	2255		
Chinese x Japanese	109		
Chinese x European	140		
Chinese x Large, Surviving American	28		
European	1		
European F_1	-1	0	0
Japanese	10	1	1
Japanese F_1	0	0	0
Japanese B_1	0	0	0
Japanese B_2	110	1	1
Japanese x European	157		
Japanese x Large, Surviving American	42		
Castanea seguinii	0	2	2
Large Surviving American F_1	220	2	3
Large Surviving American B_1	128	1	16
Large Surviving American B_2	0	0	3
Large Surviving American I_1	722	9	3
Large Surviving American F_2	-74	-1	2
Large Surviving American other	-29	1	3
Other	-2		
Total	**7443**		

* The decreases in Chinese, F_1, B_3, and Large, Surviving American trees reflects roguing of trees with inadequate levels of blight resistance. The increases reflect further breeding and collecting.

Table 3. The American Chestnut Foundation Meadowview Farms 2006 nut harvest from controlled pollinations and selected open pollinations.

Nut Type	Female Parent	Pollen Parent	Pollinated			Unpollinated Checks			Number of American Chestnut Lines*
			nuts	bags	burs	nuts	bags	burs	
AxA	American	American	225	83	131	0	9	16	2
B_1	European F_1	American	95						1
B_1	mollissima11 F_1	American	102	171	332	1	12	28	3
B_1	mollissima12 F_1	American	286	284	812	0	23	90	2
B_1-F_3	Clapper;Graves B_1-F_2		2657		1446	open pollinated			10
B_2	72-211 B_1	American	6	107	174	0	11	17	2
B_2	American	Nanking B_1	152	211	449	6	25	61	19
B_2	Nanking B_1	American	301	706	2071	0	87	235	18
B_2	PI#104016 Japn B_1	American	193	126	405	1	13	33	3
B_2-F_2	Clapper B_2		182		161	open pollinated			3
B_2-F_3	Clapper B_2-F_2		5872		4342	open pollinated			5
B_2-F_3	Graves B_2-F_2		729		698	open pollinated			3
B_3	American	Meiling B_2	5	44	268	0	8	14	1
B_3	American	Nanking B_2	68	108	277	3	13	30	7
B_3	Meiling B_2	American	10	15	48	0	2	8	1
B_3-F_2	Clapper B_3		9365		11215	open pollinated			47
B_3-F_2	Graves B_3		7609		10092	open pollinated			27
B_3-F_2	Graves B_3	Graves B_3	194	324	1037	0	32	145	3
B_3-F_3	Clapper B_3-F_2		97		134	open pollinated			7
B_4	American	R11T14 B_3	9	20	30	0	5	8	2
B_4	Douglas B_3	American	8	22	38	0	2	3	1
B_4	R11T14 B_3	American	6	122	196	0	10	15	1
F_1	Kuling Chinese	American	7	77	132	0	8	17	1
F_1	Nanking Chinese	American	212	364	610	0	40	40	1
F_1	Richwood Chinese	American	11	19	34	0	2	4	1
F_1	opMacBoyd Chinese	American	15	32	55	0	2	3	1
LSA F_1	American	DaresBeach LSA F_2	141	58	198	0	5	11	2
LSA B_1	Amherst LSA F_1	American	9	15	30	0	2	6	1
LSA B_1	DaresBeach LSA F_2	American	1	39	68	0	4	8	1
LSA B_1	NCChamp LSA F_1	American	20	171	257	0	14	25	2
LSA B_2	NCF179 LSA B_1	American	4	28	52	0	2	4	1
LSA F_1	American	CareyMac2 LSA op	144	108	241	0	61	22	6
LSA F_1	American	WayahBig LSA op	54	76	213	0	10	37	4
LSA F_1	CareyMac2 LSA op	American	6	14	47	0	1	3	1
LSA F_1	GaultSciCliffs LSA I_1	American	15	19	42	0	1	1	1
LSA F_1	WayahBig LSA op	American	7	21	53	0	1	1	1
LSA I_1	Ort LSA B_1	Amherst LSA F_1	172	71	219				2
LSA I_1	Ort LSA B_1	Gault LSA F_2	60	75	229	0	8	40	1
LSA I_1	Ort LSA F_1	Weekly LSA F_1	129	100	179	0	3	6	1
LSA I_1	SciCliffs LSA B_1	Amherst LSA F_1	164	122	297	0	13	34	2
LSA I_1	SciCliffs LSA B_1	Weekly LSA F_1	200	76	292	0	8	27	1
LSA I_1	SciCliffs LSA F_2	Weekly LSA F_1	14	17	31	0	1	1	1
CxC	Mahogany Chinese	Nanking Chinese	319	289	494	2	30	39	5
CxC	Nanking Chinese	Mahogany Chinese	43	209	416	0	22	47	2
	Parent 1	**Parent 2**							
CxC	Eighteen Chinese	Mahogany Chinese	773	344	546	0	31	46	
CxC	Eighteen Chinese	Meiling Chinese	227	431	657	2	31	48	
CxC	Eighteen Chinese	Nanking Chinese	558	373	593	1	28	39	
LSAxJ	Five LSA American	Japanese	45	87	197	0	8	11	
LSAxC	Five LSA American	Nanking Chinese	27	66	122	0	9	15	
ExJ	European	Japanese	147	25	61	0	4	8	
ExC	European	Chinese	143	29	62	0	2	5	
JxC	Japanese	Chinese	119	84	143	0	6	17	

Total Controlled Pollinations	**5446**	**5782**	**12838**	**16**	**609**	**1271**

*The number of American lines for this table is restricted to the number of American chestnut trees that were direct parents, not grand parents, of progeny.

Table 4. Number of 'Clapper' B_3-F_2 seedlings ranked in various blight resistance classes in 2006.

Susceptible Great Grandparent	LS Mean Resistance Rating**		Standard Deviation of Resistance Rating	Number of Progeny Tested	Blight Resistance Class*		
					3	4	5
QBF3CL	4.0	BC	0.7	295	75	146	74
LFR4T14	4.0	C	0.7	308	98	103	107
QBA1CL	4.1	BC	0.7	65	16	31	18
LFR4T10	4.1	BC	0.7	132	39	40	53
Bu3C1C	4.2	BC	0.6	72	13	38	21
LFR4T9	4.2	BC	0.7	628	142	226	260
LFR4T12	4.3	BC	0.6	23	2	10	11
LFR4T1	4.3	B	0.7	142	29	49	64
QBF2CL	4.4	ABC	0.6	96	17	34	45
HBF2C	4.6	A	0.5	83	5	17	61
QBF3CL	4.0	BC	0.7	295	75	146	74

* Trees were only inoculated with a weak, but virulent strain of the blight fungus in early June. A rating of 3 indicates that the cankers were small, about 1-cm long, 5 months after inoculation. A rating of 4 indicates the cankers were slightly larger, 2-4 cm long, and a rating of 5 indicates the cankers were over 5 cm long.
** Means followed by the same number are not significantly different at $p<.0.05$ by a Tukey-Kramer HSD test.

Table 5. Number of 'Graves' B_3-F_2 seedlings ranked in various blight resistance classes in 2006.

Susceptible Great Grandparent	LS Mean Resistance Rating**		Standard Deviation of Resistance Rathing	Number of Progeny Tested	Blight Resistance Class*		
					3	4	5
HBF1G	3.1	C	0.6	10	8	2	0
QBF3G	3.7	B	0.7	137	57	51	29
HBW1G	3.8	ABC	0.8	31	12	10	9
Bu3C3C	3.9	AB	0.8	263	89	88	86
PaulGalloway	4.0	A	0.7	415	125	170	120

* & ** See footnotes to Table 4.

A Quick Guide to Chestnut Breeding Terminology

Parents		Offspring
American x Chinese	=	F_1, "F-one"
F_1 x F_1	=	F_2, F-two
F_2 x F_2	=	F_3, F-three
F_1 x American	=	B_1, first backcross, or B-one
B_1 x American	=	B_2, second backcross, or B-two
B_2 x American	=	B_3, third backcross, or B-three
B_3 x American	=	B_4, fourth backcross, or B-four
B_1 x B_1	=	B_1-F_2, B-one F-two
B_1-F_2 x B_1-F_2	=	B_1-F_3, B-one F-three
B_2 x B_2	=	B_2-F_2, B-two F-two
B_2-F_2 x B_2-F_2	=	B_2-F_3, B-two F-three
B_3 x B_3	=	B_3-F_2, B-three F-two
B_3-F_2 x B_3-F_2	=	B_3-F_3, B-three F-three