Meadowview Notes 2004-2005

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In the year 2004, Meadowview was blessed with above average rainfall, but not excessive amounts as occurred in 2003. The temperatures also were fairly normal during the growing season, leading to relatively "normal" patterns of canker expansion, making it fairly easy to assess blight resistance.

It has been quite wet in the winter and spring of 2005, delaying plowing until March, like 2004. But once again we were able to get the planting done by early April, because we now have sufficient equipment to prepare orchards quickly once the weather breaks. We have sufficient equipment because of the generous support of TACF members. Like last year, thank you!

Inventory. Our current holdings are in Table 1, and changes from 2004 to 2005 are indicated in Table 2. We now have more than 22,000 trees and planted nuts, about 1,000 more than 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.

Table 3 presents the current holdings of 'Graves' and 'Clapper' third backcrosses in the various state chapters. The number of trees, lines and chapters continues to grow. This year, we count 18771 third and fourth backcross trees and planted nuts in the various chapters. This count is up this year by about 5,000 nuts, reflecting the vigorous breeding occurring in more and more chapters. Hopefully, before long, some of the chapters will be able to breed with additional sources of resistance to 'Clapper' and 'Graves.' We have been trying hard to produce backcross F_2s that I feel will serve as good new sources of blight resistance for the chapters.

Harvest. In 2004, we harvested 2211 nuts from controlled pollinations, which is a respectable number overall. Our yield of nuts per bag was much improved compared to 2003, which was lucky because we pollinated 726 fewer bags in 2004 than 2003. The higher yield may have occurred because we used dried pollen for a number of the pollinations, rather than fresh catkins. Although we have never managed in the past to determine in controlled experiments that the yield of chestnut pollinations is higher with dried pollen than fresh catkins, the predominance of the evidence generated in Meadowview and at the state chapters indicates this is the case. This higher yield from dried pollen is most evident if one examines the yield from pollinations at Meadowview over the years in comparison to the yield at state chapters. Most Meadowview pollinations have been with fresh catkins while most state chapter pollinations have been with dried pollen. The state chapter yields have consistently been much higher. We plan in the future to use dried pollen for most of our pollinations here in Meadowivew.

We placed such a low number of bags in 2004 because a hard frost in the first week of May killed most of the flowers at the Price Research Farm. Although our average first frost-free date is around the 15th of May, warm weather in April caused almost all the tree species in the Meadowview region to be leafed out by the first week in May, about two weeks earlier than usual. Hopefully, this won't happen again in the near future.

Flower death from the spring frost also reduced considerably our harvest of second generation nuts from the third backcross (B_3- F_2). This was a disappointment, as many Clapper lines are coming into production, and I had hoped to finish advancing several of these.

Blight resistance screening in B_3-F_2 seedlings. Two-thousand, four was also the first year in which we screened 'Clapper' B_3-F_2 seedlings for blight resistance. The results of that test are

presented in Table 5. I was very pleased to be able to rank a few trees in most families as highly resistant. However, not shown in the table are the results of the supplemental rankings of the most resistant trees performed the spring of 2005. There, I was not as pleased with the results; only one tree retained the high ranking. I am still fairly satisfied as of October, 2005, with the appearance of the one tree that retained a high ranking.

At this point, I do not consider this a setback because the trees were fairly small when inoculated, only two years old, entering their third growing season. Previously in 1993, when we inoculated F_2s and B_1-F_2s, not many of those survived through the next growing season of 1994. In fact, all the grafted 'Nanking' Chinese chestnut trees included in that test as check trees succumbed by the end of 1995, and 'Nanking' is the most blight-resistant cultivar known in Chinese chestnut. After the 2004 test, five of the highest ranking 'Clapper' B_3-F_2 seedlings survived and bore nuts in 2005 (see below)!

In view of these results, we have decided to start using a staged screening for blight resistance in B_3-F_2 seedlings, first testing their blight resistance with a weakly virulent strain of the blight fungus, to weed out the most susceptible trees. The remaining trees will be tested with a strongly virulent strain a few years later, when they have become large enough to survive the test. Small chestnut trees, even highly blight-resistant ones, are especially likely to die after being inoculated because the blight fungus can grow through the center of their small stems. In older, larger trees, the blight fungus has to grow around the stem to kill the tree. Larger trees also are able to withstand the onslaught of blight better than small trees because they are considerably larger relative to the size of a canker, so it is much less of a systemic stress.

In 2005, we have inoculated quite a few additional B_3-F_2 seedlings, using both strong and weak strains of the blight fungus, as the inoculations were made prior to the decision to move to a staged resistance screening. The inoculations were done on trees located both at Meadowview by our crew and at Penn State University by Sara Fitzsimmons, Tim Phelps, Kim Steiner and their crew. The results of these tests should give us a clearer picture of the levels of blight resistance we can expect to see in the future. Patience is indicated!

Effective population size. In 2002 and 2003 (Journal of The American Chestnut Foundation, 16:7-18 and 17:7-14), I presented the results of computer simulations of the inbreeding levels expected in the products of our breeding program, based on assumed pedigrees of B_3-F_4 trees. This year, I was able to calculate the inbreeding effective population size of our breeding stock from the results of those simulations, and include the contributions of our chapter breeding programs to the effective population size.

Effective population size is an important parameter in population and quantitative genetics, as well as conservation genetics. There is a famous rule in conservation genetics known as the 50/500 rule (Franklin, I.R., & Frankham, R. 1998. How large must populations be to retain evolutionary potential. Anim. Conserv. 1:69-71.), which states that, for obligate outcrossers such as chestnut trees, the effective population size must be at least 50 to avoid immediate collapse of a population from inbreeding depression and at least 500 for mutation to offset the slow loss of alleles from genetic drift.

The basic building block of our breeding program is a set of 20 lines from one source of blight resistance. The 20 lines were chosen in order to capture alleles occurring at frequencies of greater than 5% (1/20). One source of resistance is used in order to ensure a fairly high level of homozygosity for blight resistance in later generations. The effective population size depends not only on these 20 lines, but also on how rapidly the real population size is increased from straight B_3 to B_3-F_4. It is approximately the harmonic mean of the size of each generation. The chapters then add additional genetic diversity from their trees and also adaptation to their local conditions.

Table 6 presents the results of these calculations and indicates that our basic building blocks of 20 lines will have an inbreeding effective population size of 72. Adding contributions from 5 chapters builds it to 248, and adding another source of blight resistance for five chapters would double it. Thus our effective population sizes are comfortably above the thresholds of the 50/500 rule. It should be noted that these calculations assume there will be no variation in family sizes. The inevitable variation in family size, especially at B_3-F_3 and B_3-F_4 will reduce the effective population size. But it does appear we will be OK in this regard. [As a side note, the effective population size of the West Salem, WI, stand of American chestnut trees would be about 30 or 35 if all nine founders contributed equally to the bulk of the second generation, perhaps explaining why that population does not appear to be foundering from inbreeding depression].

I would like to thank Lou Silveri, Dave Lazor, Chandis Klinger, Gene Whitmeyer, and Harry Norford for helping out with pollination and inoculation in 2004. They came down on their own and stayed at Emory and Henry College. We also had a group come down under an Elder Hostel program. Sam Fisher, Neil Rich & Chrystle Gates of the Southwest Virginia 4-H Center have been very helpful managing the Elder Hostel program, which would not occur without their initiative. Thank you —this wouldn't get done without your help. If you would be interested in helping pollinate next year, plan on any time in June after the first. (Call 276 944-4631 around June 1). If you would be interested in 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 2005, 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	2082		206
Chinese	814	55	
Chinese x American: F_1	858	22	95
American x (Chinese x American): B_1	386	13	28
American x [American x (Chinese x American)]: B_2	1512	10	81
American x {American x [American x (Chinese x American)]}: B_3	4918	8	75
Am x (Am x {Am x [Am x (Chin x Am)]}):B_4	86	1	1
(Chinese x American) x (Chinese x American): F_2	710	6	6
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F_3	6	1	1
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B_1-F_2	688	3	3
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	365	5	5
[A x (A x {A x [A x (C x A)]})] x [A x (A x {A x [A x (C x A)]})]:B_3-F_2	7295	2	23
Chinese x (Chinese x American): Chinese B_1	191	3	4
Chinese x [American x (Chinese x American)]	41	1	1
Japanese	3	2	2
American x Japanese: F_1	11	2	2
(American x Japanese) x American: B_1	10	2	2
Castanea seguinii	48	1	1
Chinese x Castanea pumila: F_1	9		
Large, Surviving American x American: F_1	272	11	29
(Large, Surviving American x American) x American: B_1	582	6	9
[(Large, Surviving American x American) x American] x American: B_2	126	1	2
Large, Surviving American x Large, Surviving American: I_1	474	14	14
Large, Surviving American: $F_2 = F_1xF_1$, etc, same LS parent	467	5	5
Large, Surviving American Other	59	2	7
Irradiated American x American: F_1	1	1	1
Other	24		
Total	**22038**		

* 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 2004 and 2005 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	-34		-4
Chinese	145	5	
Chinese x American: F_1	241	-1	4
American x (Chinese x American): B_1	-392	-1	-3
American x [American x (Chinese x American)]: B_2	-20	0	-6
American x {American x [American x (Chinese x American)]}: B_3	-357	0	2
Am x (Am x {Am x [Am x (Chin x Am)]}):B_4	0	0	0
(Chinese x American) x (Chinese x American): F_2	0	1	1
[Ch x Am) x (Ch x Am)] x [Ch x Am) x (Ch x Am)]:F_3	0	0	0
[Amer x (Chin x Amer)] x [Amer x (Chin x Amer)]: B_1-F_2	0	0	0
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}:B_2-F_2	22	1	1
[A x (A x {A x [A x (C x A)]})] x [A x (A x {A x [A x (C x A)]})]:B_3-F_2	1459	0	6
Chinese x (Chinese x American): Chinese B_1	49	0	1
Chinese x [American x (Chinese x American)]	0	0	0
Japanese	0	0	0
American x Japanese: F_1	0	0	0
(American x Japanese) x American: B_1	-69	0	0
Castanea seguinii	0	0	0
Chinese x Castanea pumila: F_1	0		
Large, Surviving American x American: F_1	10	2	20
(Large, Surviving American x American) x American: B_1	-49	0	0
[(Large, Surviving American x American) x American] x American: B_2	89	0	0
Large, Surviving American x Large, Surviving American: I_1	89	2	2
Large, Surviving American: $F_2 = F_1 x F_1$, same LS parent	0	0	0
Large, Surviving American: Other	0	0	5
Irradiated American x American: F_1	-2	0	0
Other	0		
Total	**1181**		

* The decreases in B_1, B_2, B_3, and Large, Surviving American B_1 & F_2 trees reflects roguing of trees with inadequate levels of blight resistance. The increases reflect further breeding and collecting.

Table 3. Number of third-backcross chestnut at TACF Chapters in 2005, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock.

Chapter	Number of		
	Nuts or Trees	**Sources of Resistance**	**American Lines**
Maine	1879	2	29
Massachusetts	4345	2	34
Pennsylvania	5371*	2	39
Maryland	418	2	7
Indiana	2970	1	19
Kentucky	802	2	7
Carolinas	1093	2	17
Tennessee	2011	3	19
Alabama	267	1	8
Total	**19156**		

*Numerous B_3-F_2s also have been planted but these are not included in this table.

Table 4. The American Chestnut Foundation Meadowview Farms 2004 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	
Am x Am	American	American	277	267	581		21	60	1
B_1	F_1 Mahogany	American	194	256	472	1	24	39	1
B_2	American	B_1 MusickChinese	234	43	141	0	5	5	1
B_2	B_1 MusickChinese	American	5	19	31	1	3	3	1
B_2	American	B_1 Nanking	106	242	247	5	26	26	11
B_2	B_1 Nanking	American	30	50	108	0	3	4	3
B_2- F_2	B_2 Clapper		865	open pollinated				3	
B_2- F_2	B_2 R1T7	B_2 R1T7	180	60	138	0	5	8	1
B_2- F_3	B_2- F_2 Clapper		191	open pollinated				3	
B_2- F_3	B_2- F_2 Graves		19	open pollinated				1	
B_3	B_2 Gr	American	119	141	447	3	15	30	5
B_4	B_3 Gr	American	1	97	316	0	10	30	1
B_3	American	B_2 Nanking	155	128	162	1	14	21	3
B_3	B2 Nanking	American	0	3	0	1	1	1	1
B_3	American	B_2 R1T7	86	82	141	1	9	17	6
B_3- F_2	B_3 Clapper		1106	open pollinated				17	
B_3- F_2	B_3 Graves	B_3 Graves	26	14	27	0	2	2	1
B_3- F_2	B_3 Graves		555	open pollinated				8	
Chin B_1	F_1 Mahogany	Chin Mahogany	50	98	224	0	10	17	1
F_1	Chinese Kuling	American	13	66	121	3	5	7	1
F_1	Chinese Nanking	American	101	261	499	2	28	51	3
Isa B_2	American	B_1 ScientistsCliffs	431	70	228	1	4	4	2
Isa F_1	American	opDaresBeach	65	63	50	0	6	5	4
Isa I_1	F_1 DaresBeach	B_1 ScientistsCliffs	8	18	21	0	2	2	1
Isa I_1	B_1Ort	B_1 ScientistsCliffs	61	61	114	0	7	13	1
Isa I_1	F_1 Ort	B_1 ScientistsCliffs	69	53	143	0	4	14	1
Total Controlled Pollinations			**2211**	**2092**	**4211**	**19**	**204**	**359**	

*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 5. Blight resistance ratings of 'Clapper' B_3-F_2 trees in 2004.

Code of Mother Tree	Code of Resistant Grandparent	Blight Resistance Rating				
		1	2	3	4	5
CH271	CL285	2	4	11	11	8
CH199	CL112	1	6	14	10	5
CH34	CL198	0	1	2	4	4
CH726	CL130	0	4	17	40	31
CH283	CL98	3	3	12	14	9
CH526	CL287	0	1	12	14	9

Table 6. Effect of adding sets of 20 B_3-F_2 progeny from TACF's chapter breeding program on inbreeding and effective population size, assuming that the base population of B_3-F_1 trees are not inbred.

Number of Chapters	Inbreeding Coefficient at B_3-F_4	Inbreeding Effective Population Size
1	0.02066	72
2	0.01153	130
3	0.00850	176
4	0.00698	214
5	0.00603	248

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
B_3 x American	=	B_4, fourth backcross
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-two 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

Addendum: 2005 Harvest . These notes are coming out late enough to include the results of the 2005 harvest in Meadowview, which are shown below in Table 6.

The most noteworthy event of the 2005 harvest was our first crop of B_3-F_3 nuts from selected Clapper B_3-F_2 trees! We expect this number to increase sharply in future years. We also harvested a large crop of B_3-F_2 nuts; the harvest of nuts from the Clapper source of resistance should go a long way toward completing that generation of crosses.

Our harvest from controlled pollinations was a disappointment: we harvested less than two nuts for every third pollination bag. However, because we managed to place so many bags this year, the total size of the harvest from controlled pollinations was a respectable 1976 nuts.

The low yield may have been associated with cool temperatures in early June after a warm May, which delayed flowering, along with very dry conditions in June. Trees were ready to bloom for several weeks, but didn't, which may have affected pollen viability. This hypothesis is supported by preliminary observation that some pollens shipped to chapters gave very poor yields. This will have to be confirmed by further analysis of the results of chapter pollinations, and further analysis of the results from Meadowview. The analysis is not finished yet because harvest was very late this year, delayed 2 to 3 weeks beyond normal. Another possibility for

2005’s low yield was the hot, dry weather during June; this may have adversely affected pollen germination and growth into the style.

Table 7. The American Chestnut Foundation Meadowview Farms 2005 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	
B_1	F_1 72-211	American	41	100	168	0	15	17	2
B_1	American	F_1 mollissima10	1	38	86	0	3	17	3
B_1	F_1 mollissima10	American	7	70	226	0	7	19	1
B_1	American	F_1 mollissima11	1	43	137	1	3	15	2
B_1	F_1 mollissima11	American	70	90	198	0	11	35	3
B_1	F_1 mollissima12	American	463	253	613	8	25	71	3
B_1	F_1 mollissima13	American	18	9	12	0	1	2	1
B_1	F_1 mollissima7	American	0	23	34	0	2	3	1
B_1-F_2	B_1 MusickChinese	B_1 MusickChinese	120	75	192	0	7	22	2
B_1-F_3	B_1-F_2 Clapper-Graves		3255	open pollinated					10
B_2	B_1 Mahogany	American	46	51	78	0	5	9	3
B_2	B_1 Meiling	American	16	66	124	0	8	27	1
B_2	American	B_1 Nanking	30	305	601	0	35	95	19
B_2	B_1 Nanking	American	127	360	966	2	43	128	12
B_2	American	Japn B_1 PI#104016	14	49	68	0	5	4	1
B_2	Japn B_1 PI#104016	American	16	19	39	0	3	7	1
B_2-F_2	B_2 Clapper		417	open pollinated					3
B_2-F_3	B_2-F_2 Clapper		1990	open pollinated					2
B_3	American	B_2 Graves	9	83	156	0	7	17	4
B_3	B_2 Graves	American	50	175	383	2	14	30	5
B_3	American	B_2 Nanking	14	67	173	2	9	25	7
B_3	American	B_2 R1T7	30	17	40	0	1	2	1
B_3	B_2 R1T7	American	4	49	81	0	4	15	1
B_3-F_2	B_3 Clapper		7399	open pollinated					49
B_3-F_2	B_3 Graves		1451	open pollinated					10
B_3-F_2	B_3 Graves	B_3 Graves	54	27	79	1	3	4	1
B_3-F_3	B_3-F_2 Clapper		118	open pollinated					5
B_4	American	B_3 Clapper	21	81	96	2	8	15	3
B_4	B_3 Graves	American	1	4	2	0	1	0	1
F_2 Chin	Chinese m12xm13	Chinese m12xm13	10	11	18	0	1	1	1
F_1	Chinese Kuling	American	29	85	207	0	3	17	1
F_1	Chinese Mahogany	American	55	131	203	2	15	18	4
F_1	Chinese Nanking	American	27	129	256	2	14	30	1
F_1	Chinese Richwood	American	30	50	98	0	5	12	1
LSA B_1	American	LSA F_1 Amherst	10	51	74	0	8	10	7
LSA B_1	LSA F_1 Amherst	American	10	12	26	0	2	2	1
LSA B_1	LSA F_1 Corrigan	American	0	13	28	0	2	6	1
LSA B_1	American	LSA F_1 NCChamp	0	19	51	0	3	7	4
LSA B_1	LSA F_1 NCChamp	American	9	82	142	0	6	18	3
LSA B_2	American	LSA B_1 Corrigan	6	78	154	0	7	12	1
LSA B_2	LSA B_1 SciCliffs	American	7	24	43	0	2	1	1
LSA F_1	American	LSA Green1	145	95	172	0	9	12	1
LSA F_1	American	LSA opWeekly	56	120	276	0	11	32	1
LSA I_1	LSA B_1 Ort	LSA F_1 Amherst	102	65	250	0	7	15	1
LSA I_1	LSA B_1 Ort	LSA F_1 NCChamp	52	63	110	0	6	18	2
LSA I_1	LSA B_1 Ort	LSA B_1 SciCliffs	20	24	60	0	3	6	1
LSA I_1	LSA B_1 SciCliffs	LSA F_1 NCChamp	51	27	44	0	3	8	1
LSA I_1	LSA F_1 Weekly	LSA B_1 SciCliffs	21	70	97	0	7	15	1
LSA I_2	LSA F_1 DaresBeach	LSA opWeekly	36	37	69	0	1	4	1
LSA I_2	LSA F_1 Weekly	LSA opDaresBeach	40	17	44	0	2	6	1
LSA I_2	LSA opDaresBeach	LSA F_1 Amherst	56	39	93	0	4	9	1
LSA I_2	LSA I_1 GaultSciCliffs	LSA F_1 NCChamp	51	49	88	0	5	16	1
Total Controlled Pollinations			**1976**	**3345**	**7155**	**22**	**346**	**854**	

*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.