Performance of genetic and genomic improvement in American chestnut

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Carl Zipper, Jared Westbrook, Jason Holliday, Sara Klopf

Virginia Tech and TACF

Background

- Efforts to develop American chestnuts resistant to blight and Phytophthora
  - TACF breeding program
  - Genetically modified chestnuts
- Trees also need be phenotypically (genomically) American AND resilient enough to handle environmental stressors
  - Field test at various locations within range

Overall Research Objectives

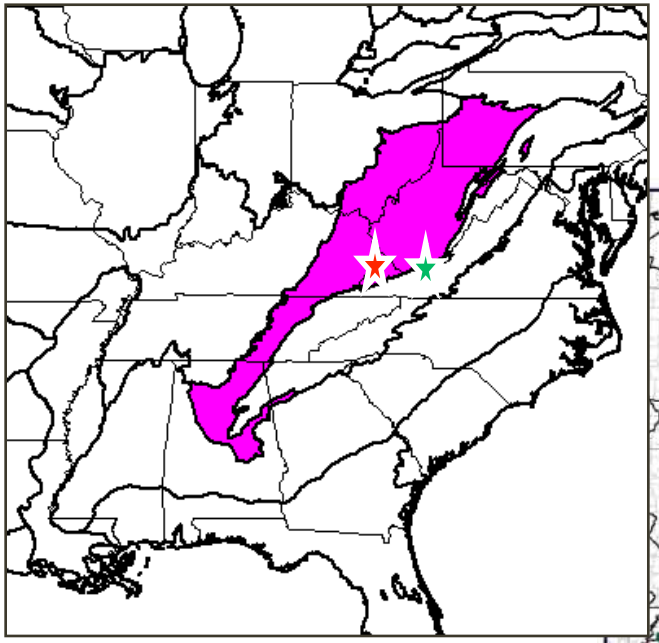
1) Establish mixed plantings of transgenic, hybrid, and native chestnut clones at two field sites

2) Evaluate trees for physiological characteristics, including viability, growth, adaptability, and blight and root rot resistance

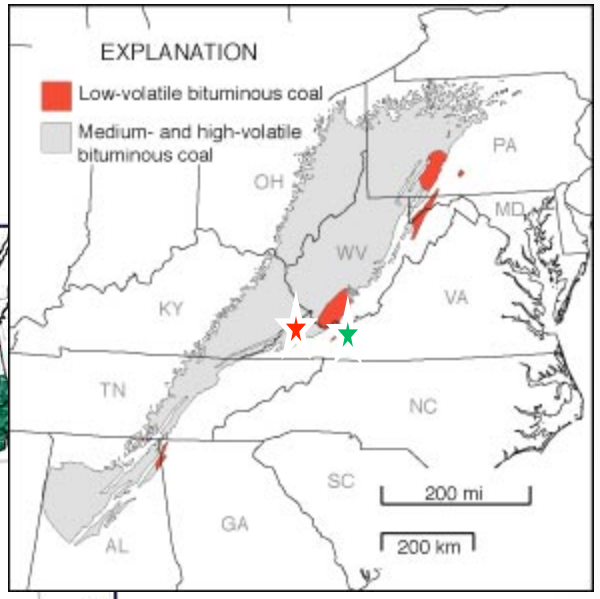
3) Synergize the newly available chestnut genome sequence with TACF backcross populations to develop a genomic selection model based on next generation sequencing

4) Demonstrate and aid communication of FHI approach and technologies

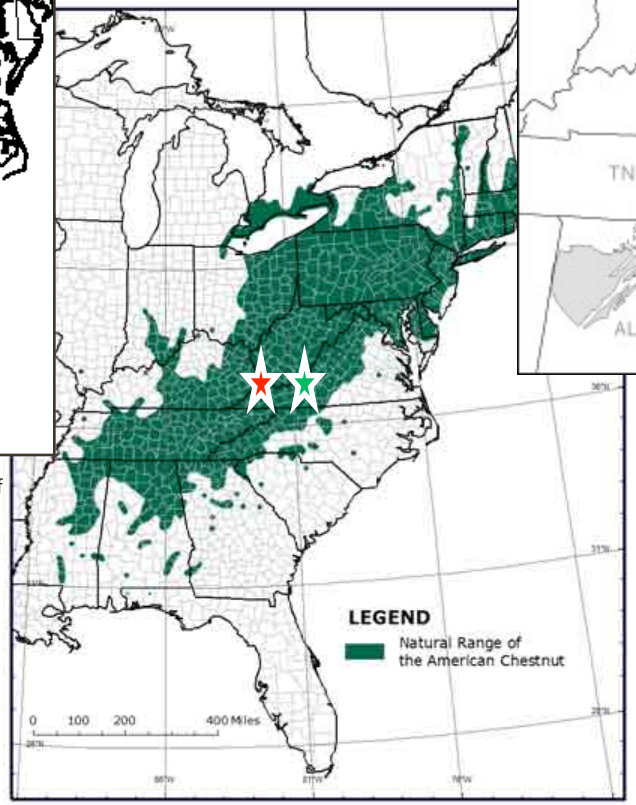
Field tests of GM chestnut



http://www.cas.vanderbilt.edu/bioimages/ecoregions/50402-map.gif



http://pubs.usgs.gov/fs/fs115-99/fig1.jpg



http://www.acf.org/images/about/RangeMap2006sff.jpg

- Powell River Project reclaimed coal mine research site
- Virginia Tech Kentland Farm research site

Rationale for Powell River Project

- Surface Mining Control and Reclamation Act (SMCRA) mandates reclamation but implementation has not been accompanied by widespread replacement of forests disturbed by mining
- Many active and abandoned mine sites comprise prime chestnut habitat (high elevation)
- Lack of competing vegetation

Rationale for Kentland 'Farm'

- Proximity to Virginia Tech allows frequent visit
- Upslope site similar to native chestnut niche
- Easy access for interested parties, but access controlled

Planting methods

- Prior to planting, trees from each construct are assigned to blocks and given Virginia Tech ID #s
- At planting, trees organized in their block groups and randomly assigned to locations within the block
- Trees are each given two 21 g slow-release fertilizer tablets, straw matting was placed to help reduce water loss (June 2013 only), and a tree weed mat (1 m x 1 m) was secured around the base of each tree

Supplemental watering

- Watering begins at planting, continues for ~1.5 months after planting
- Frequency
  - 2 times per week for first few weeks after planting, then as needed
  - Site checks at least biweekly once watering tapers off
- Volume of water
  - Water each tree by time to ensure even watering
  - 0.2-0.3 inches of “rain” per watering in one day

Trees Planted To Date

Research site	Planting Date	# of Trees	Trees planted
Powell River Project	6/13/2013	96	Mix of SE-derived and nut-derived trees from UGA, TACF, and the VA Dept. of Forestry (VDF)
Kentland Farm	11/15/2013	360	Mix of trangenic and non-transgenic trees from UGA and SUNY
Powell River Project	5/13/2014	190	Mix of trangenic and non-transgenic trees from UGA and SUNY

Phenotyping

- Height, basal diameter, crown spread, form, stem count
  - Recorded immediately after planting and at end of growing season
- Assessment of tree health and other variables: numerical scales developed for all characters
  - Includes leaf color, relative leaf density, degree of branch die-back, wounds and/or cankers on stems, etc.
  - Measured right after planting, then monthly during growing season

Phenotyping

- Phenology
  - Bud break at Kentland, next year will visit PRP site 1-2x in the spring to capture a ‘snapshot’ of bud break
    - Do the trees have normal growth and dormancy?
  - Flowering phenology at both sites
  - This year, will record bud set phenology as well

Powell River Project Preliminary Results

Clockwise from top left: laying down straw mats for moisture control, securing weed mats around all trees, providing supplemental water to trees

Powell River Project May 2014 cohort (Photo taken May 2015)

Powell River Project June 2013 Cohort

Survival (%) as of June 2015

Source	Nut/SE-derived	Tree Variety	Mean
TACF	Nut	B3F3, Clapper	33.33%
TACF	Nut	B3F3, Graves	50.00%
UGA	Tissue	B3F3, Clapper	45.45
UGA	Tissue	B3F3, Graves	54.54
UGA	Tissue	76 x 5 OP (VDF)	52.38

- SE-derived trees are surviving slightly better after two full years (51.8% vs. 42.8%)
  - Best survivors are SE-derived Graves trees
  - SE-derived Clapper and Graves families both had higher survival than nut-derived

June 2013 Cohort First Year Growth

Source	Tree Variety	Mean (cm)
TACF	B3F3, Clapper	21.79
TACF	B3F3, Graves	23.93
UGA	B3F3, Clapper	9.22
UGA	B3F3, Graves	31.53
UGA	76 x 5 OP (VDF)	-0.58

- Tissue-derived Graves trees grew the most, followed by nut-derived trees, which on average died back in the previous growing season)

Powell River Project May 2014 cohort

Survival (%) as of June 2015

Gene	Background genetics	Mean	Mean number of trees/ block
CAD	76-5xOP-2B	92.3	6.25
Prox	76-5xOP-2B	100	4.25
NPR1	RxT-22B	100	1.25
TL/TAGL	AW3-46B	33.3	0.75
Cyst1	WB484-3	50	0.5
ETF1	WB484-3	75	1
None	76-5 x OP (ACxCCxJC)	95	27
None	Nagle (AC)	100	5

- Low survival for some constructs due to low replication, too soon to draw conclusions

Powell River Project May 2014 cohort

Growth as of June 2015

Transgenic?	Genotype	Gene	Mean change in height (cm)	Standard deviation
Yes	76-5XOP	CAD	17.9	17.0
Yes	76-5XOP	Prox	18.5	14.9
Yes	AW3-46B	TL/TAGL	1.0	0.0
Yes	RxT-22B	NPR1	21.0	9.1
Yes	WB484-3	Cyst1	15.0	0.0
Yes	WB484-3	ETF1	20.7	15.2
No	76-5XOP	None	5.4	27.2
No	Nagle	None	12.0	20.6

Canker incidence

- One Graves nut-derived and one SE-derived tree also showed symptoms of blight

Canker with stromata on Clapper nut-derived tree (S. Klopf 2015)

Canker Incidence

2013 Cohort

Source	Type	Tree Variety	# trees with cankers	# trees with flagging	% trees with possible blight that are dead/dying
TACF	Nut	B3F3, Clapper	5	1	11.1%
TACF	Nut	B3F3, Graves	3	1	4.2%
UGA	Tissue	B3F3, Clapper	5	2	27.3%
UGA	Tissue	B3F3, Graves	9	1	18.2%
UGA	Tissue	76 x 5 OP (VDF)	3	0	9.5%

Canker Incidence

2014 Cohort

Transgenic?	Gene	Genotype	% trees	Mean canker severity*	Standard deviation
No	None	76-5XOP	62.07	2.56	0.98
No	None	Nagle	60.00	3.33	1.53
Yes	CAD	76-5XOP	4.17	1.00	0.00
Yes	Cyst1	WB484-3	0.00	0.00	0.00
Yes	ETF1	WB484-3	0.00	0.00	0.00

*For trees with cankers present

Flowering at Powell River Project

Date	Tree ID (VT tag #)	Gene	Flowers Removed (#)
5/28/2015	85	76-5xOP-2B	7
5/28/2015	88	76-5xOP-2B	4
5/28/2015	499	Prox	2
5/28/2015	475	CAD	6
5/28/2015	80	76-5xOP-9A	2
5/28/2015	75	76-5xOP-7B	10
5/28/2015	56	76-5xOP-3A	1
5/28/2015	109	76-5xOP-7A	1
5/28/2015	57	76-5xOP-10A	12
5/28/2015	83	76-5xOP-5D	11
5/28/2015	B29	Nagle 1D	10
5/28/2015	105	76-5xOP-3A	9
5/28/2015	94	76-5xOP-12	22
5/28/2015	103	76-5xOP-10B	3
6/3/2015	42	76-5xOP-2B	5
6/3/2015	85	76-5xOP-2B	1
6/3/2015	88	76-5xOP-2B	1
6/3/2015	47	76-5xOP-10B	2

Kentland Farm Fall 2013 cohort (Photo taken June 2014)

Kentland Farm Survival as of June 2015

Source	Gene	Background	Mean survival
SUNY	CC Laccase	Ellis (AC)	1.6
SUNY	Oxalate oxidase	Ellis or WB275	4.3
UGA	B-Gluc	WB484-3	55.6
UGA	CBS1	76-5xOP-2B	55.0
UGA	CBS1	WB484-3	50.0
UGA	GUSi	76-5xOP-2B	14.3
UGA	Lac	WB484-3	39.5
UGA	PRP	WB484-3	26.0
UGA	Thaum	RxT-22B	37.5
UGA	Thaum	WB484-3	11.8
UGA	YFPGUSi	RxT-29B	0
UGA	YFPGUSi	WB484-3	33.3
SUNY	Empty vector control	Ellis	0
SUNY	None	Ellis	0
SUNY	None	Qing (CC)	60.0
UGA	None	AC, multiple families	40.0

Kentland Farm Growth as of June 2015

Source	Gene	Background	Mean height change (cm)	Standard deviation
SUNY	CC Laccase	Travis	-4.5	0.00
SUNY	none	Ellis 1	n.d.	n.d.
SUNY	none	Empty vector	n.d.	n.d.
SUNY	none	Qing	34.67	16.75
SUNY	Oxo	Darling	18.00	8.49
UGA	B-Gluc	WB484-3	24.60	10.81
UGA	CBS1	76-5xOP-2B	37.05	12.50
UGA	CBS1	WB484-3	5.00	0.00
UGA	GUSi	76-5xOP-2B	27.00	0.00
UGA	Lac	WB484-3	7.94	18.81
UGA	PRP	WB484-3	9.42	7.33
UGA	Thaum	RxT-22B	11.56	5.56
UGA	Thaum	WB484-3	16.50	7.07
UGA	YFPGUSi	RxT-29B	n.d.	n.d.
UGA	YFPGUSi	WB484-3	9.50	14.85

Powell River Project May 2014 cohort (Photo taken May 2015)

Kentland Farm Fall 2013 cohort

Early flowering May/June 2014

Flowering at Kentland

Date	# Trees	Gene	Flowers Removed (#)	Nuts Collected (#)
5/19/2014	2	CBS1	9	NA
6/2/2014	4	CBS1	14	NA
6/10/2014	4	CBS1	4	NA
8/13/2014	4	CBS1	0	1
5/4/2015	10	CBS1	249	NA
5/8/2015	12	CBS1	94	NA
5/8/2015	2	Qing	99	NA
5/12/2015	2	CBS1	2	NA
5/12/2015	3	Qing	17	NA
5/14/2015	1	CBS1	1	NA
5/14/2015	2	Qing	10	NA
5/22/2015	1	CBS1	3	NA
5/22/2015	1	None, Nagle	7	NA
6/1/2015	2	None, Nagle	5	NA

- Flowering observed from May 19 to June 10 in 2014, and May 4 to June 1 in 2015
- Flowers removed, double-bagged, and disposed of

Kentland Farm Issues and Solutions

1) Timing of Fall 2013 planting

   Issue: Trees (primarily from SUNY) leafed out during warm period immediately after planting, cold snap right afterward killed many of the trees

   Solution proposed: Delay planting, keep all trees in greenhouse until dormant

   Actions taken: No additional plantings at the Kentland Farm site to date

Kentland Farm Issues and Solutions

2) Heavy rodent damage

Issue: Heavy herbivory/damage from rabbits, voles, moles, etc.

- Possibly due to limited food due to a non-mast year and exacerbated by an exceptionally harsh winter

Solutions proposed: Increased weed control before and after planting, added rodent guards to trees to minimize aboveground damage, more aggressive poison baiting

Actions taken: Aggressive weed control (chemical and mechanical) to make site less appealing over winter, rodent guards installed, poison bait stations placed and kept fresh all winter

Take home message

- In spite of poor soil, the Powell River Project site is amenable to restoration with transgenic chestnut
  - Excellent survival/growth
  - Very little evidence of cankers on transgenic plants

- One particular transgene (CBS) resulted in prodigious flowering
  - Continued monitoring necessary to determine if this is a direct effect of the transgene or perhaps a side-effect of plant health/ vigor coming out of the greenhouse
  - May impact our ability to conduct longer term tests with this construct (difficulty removing all flowers as the trees age)

Plans for 2015/16

- Plant third cohort (transgenic and non-transgenic) on Powell River Project mine site
- Plant second cohort at Kentland Farm?
  - Will depend on tree availability and permitting
- Continue phenotyping existing plantings at Kentland Farm and Powell River Project
- Test root and soil samples from both sites for Phytophthora
  - Testing by Bartlett Tree Research Lab (for free!)
- Blight inoculation at both sites

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U.S. Endowment for Forestry and Communities

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