

A man wearing a blue long-sleeved shirt, blue jeans, and a blue cap stands in a field of chestnut trees. The trees are lush green and appear to be in a field or orchard. The background shows a hazy sky and some distant structures.

Survival and Growth of Chestnut Backcross Seeds and Seedlings on Surface Mines After 8 Years

Jeff Skousen et al.
West Virginia University 06.04.2015

Chestnut

**“Redwood
of the East”**



**1 in 4 Trees
in the
Eastern US Forest
were Chestnuts**



**Strong,
Resistant to
Degradation**

Old Stump

Cradle to Grave....



Prolific Nut Producer

Importance of livestock feed



Disaster Strikes!

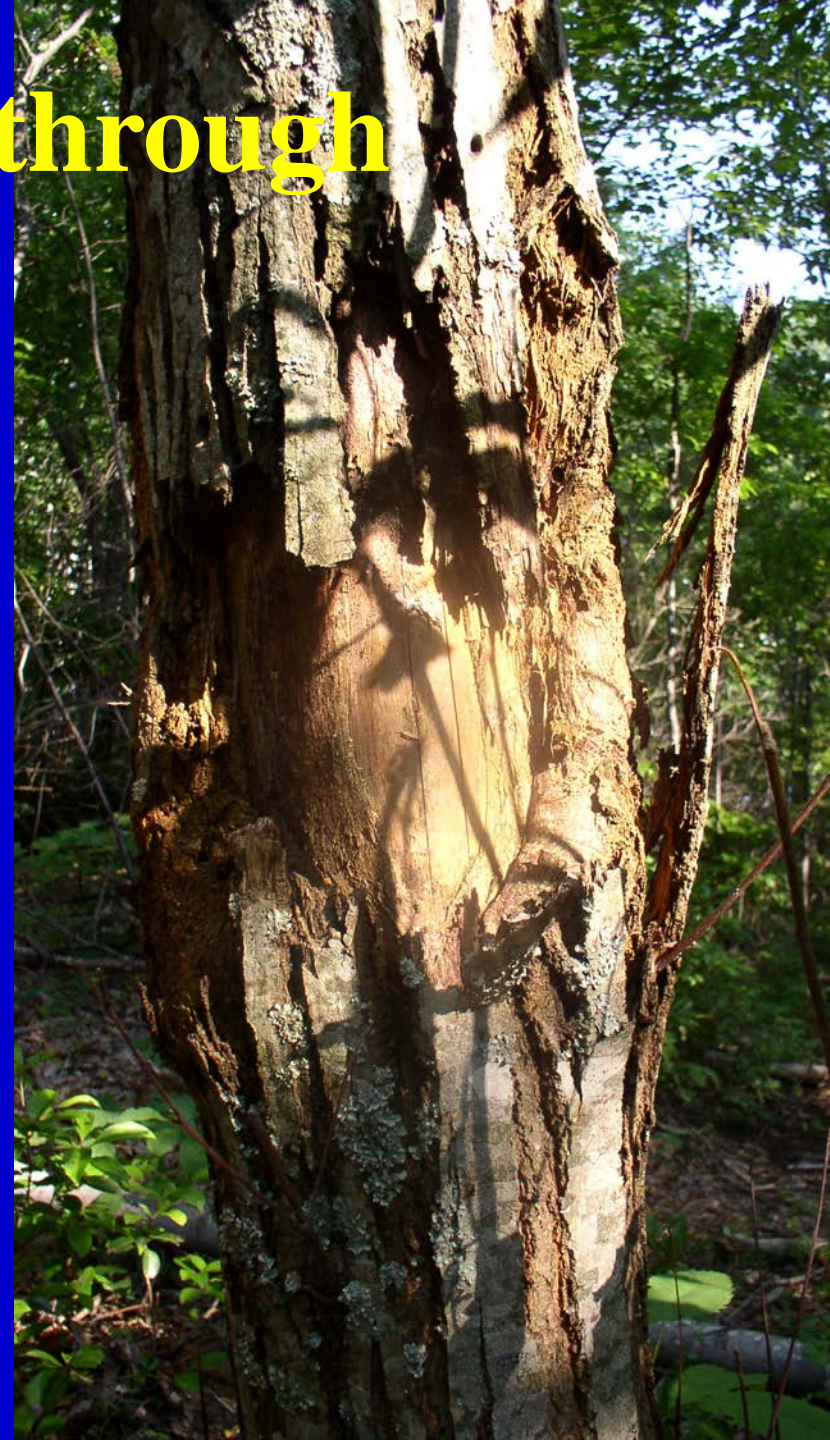
Blight – Discovered 1904



1950 - Entire Range Affected



Blight fungus infects through wounds in the bark.



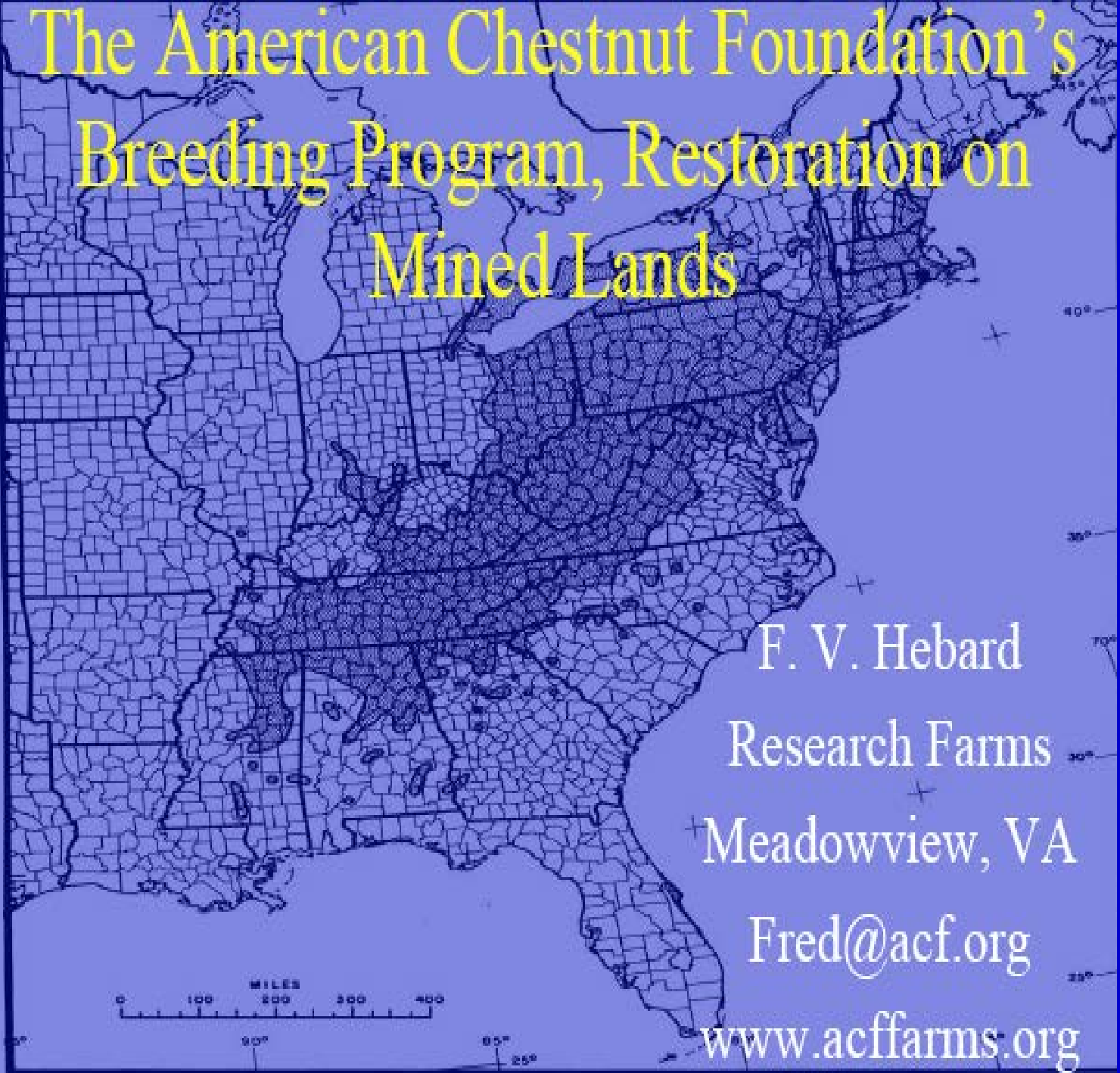
The American Chestnut Foundation

There's Hope!



2007 8 9

The American Chestnut Foundation's Breeding Program, Restoration on Mined Lands



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Backcrossing

Expected
Proportion
Chinese

1/2

C x A

F₁ x A

1/4

B₁ x A

1/8

B₂ x A

1/16

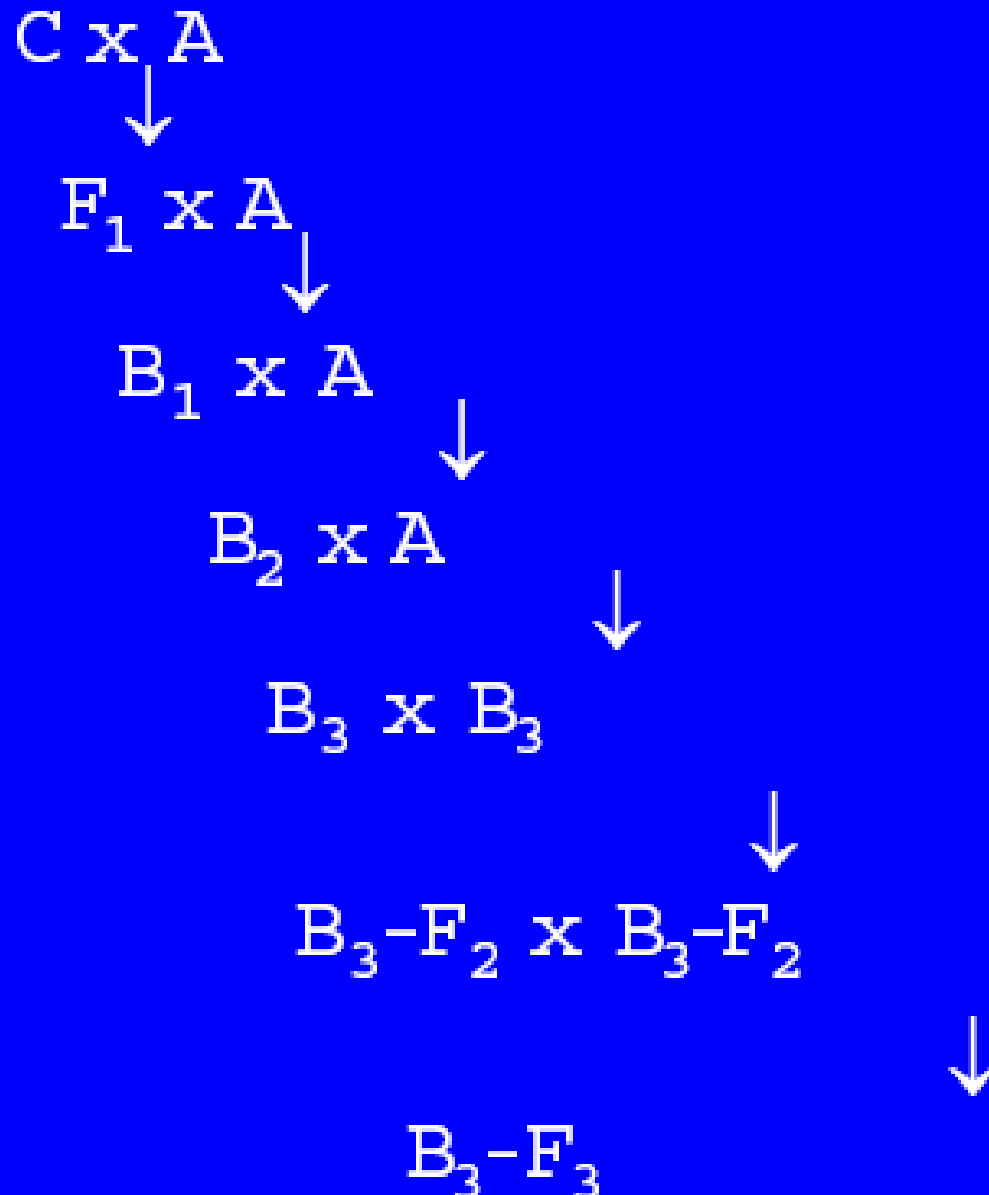
B₃ x B₃

1/16

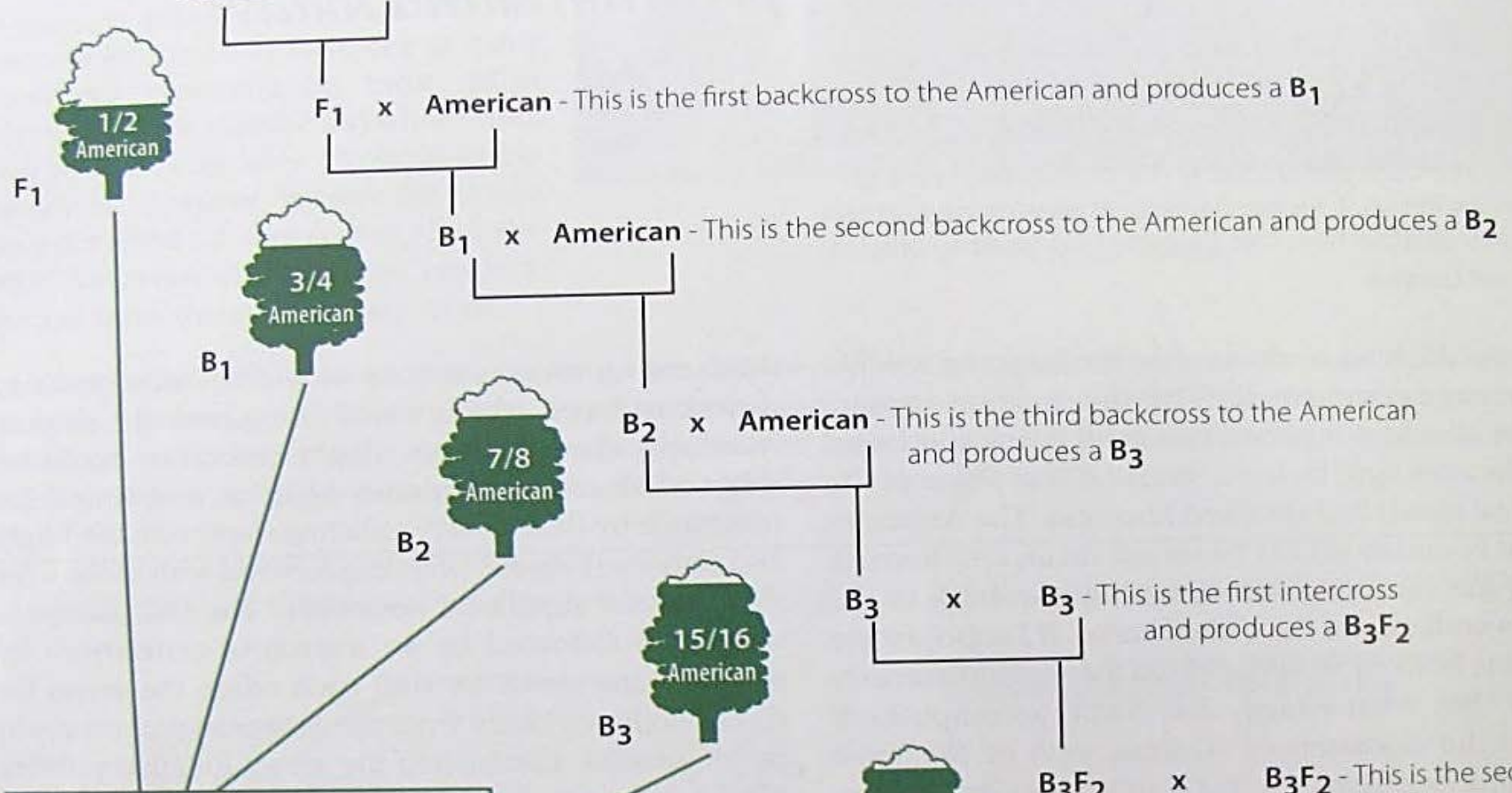
B₃-F₂ x B₃-F₂

1/16

B₃-F₃



Chinese x American - This cross produces an F_1



Each generation is inoculated with blight fungus and only those trees with the highest resistance are used to breed future generations. The trees are also visually inspected, and only trees with the fewest Chinese characteristics are selected.

B_3F_3
This is the final intercross and is expected to show a high level of blight resistance in forest test plantings

Three areas of research:

**Crosses between American and Chinese
Hypovirulence on Blight (virus on blight)
Phytophthora – black root rot**

**Blight-resistant
Chinese to
American B₃-F₂.**

**Nuts from this
generation will be
planted back into
the forest and onto
mined land.**





Chestnuts at my farm

05/15/2016



05/15/2016



05/15/2016



09/23/2012



OPERATION SPRINGBOARD



Rebirth of the American Chestnut

**Appalachian Regional
Reforestation Initiative (ARRI)**

TACF and Several Universities

Chestnut Tree Planting in Kentucky



Chestnut planting of seedlings in West Virginia



07/21/2011

Study Sites and Objectives

1. Glory Study – Seed – 2008

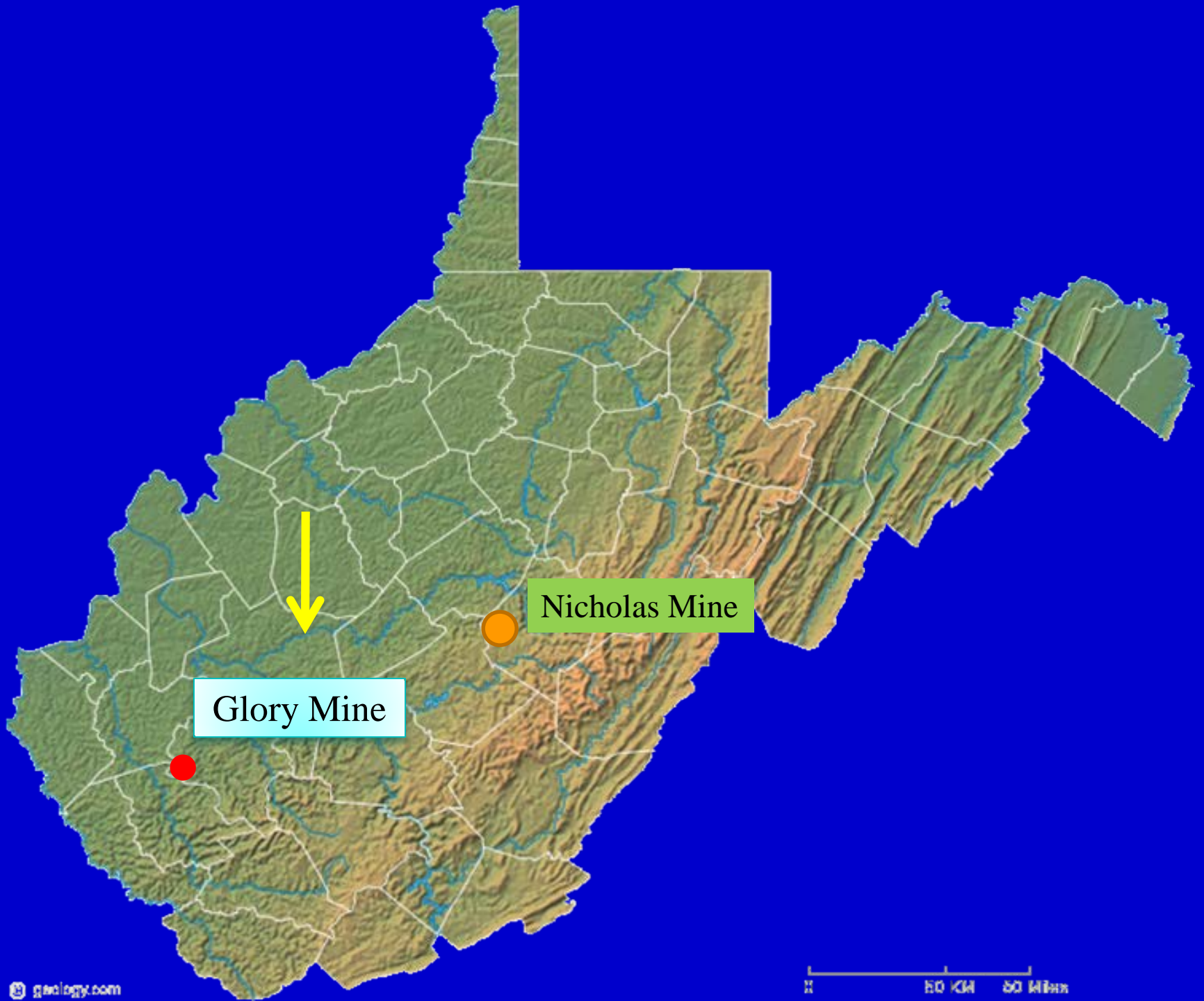
8 yrs

2. Nicholas Study – Seedlings – 2009

7 yrs

Survival and Growth of 5 Stock Types

(Amer, Chin, B_1F_3 , B_2F_3 , B_3F_2)



Glory Mine

Nicholas Mine



Glory Mine

Mixed, loosely-graded
Brown-Gray mine soil

2008 5 1

NO SHELTER TREES

REP 1			REP 3	
B ₂ F ₃	B ₃ F ₂		AMERICAN	CHINESE
AMERICAN	CHINESE		CHINESE	B ₂ F ₃
B ₁ F ₃	B ₂ F ₃		B ₃ F ₂	AMERICAN
B ₃ F ₂	B ₁ F ₃		B ₂ F ₃	B ₃ F ₂
CHINESE	AMERICAN		B ₁ F ₃	B ₁ F ₃
REP 2			REP 4	
B ₂ F ₃	B ₂ F ₃		B ₁ F ₃	AMERICAN
CHINESE	AMERICAN		AMERICAN	B ₁ F ₃
AMERICAN	B ₁ F ₃		CHINESE	B ₂ F ₃
B ₁ F ₃	CHINESE		B ₂ F ₃	B ₃ F ₂
B ₃ F ₂	B ₃ F ₂		B ₃ F ₂	CHINESE

**Randomized
Split-Plot
Design
with
four
replications**

SHELTER TREES

REP 1			REP 3	
CHINESE	AMERICAN		B ₂ F ₃	AMERICAN
AMERICAN	B ₁ F ₃		B ₁ F ₃	B ₂ F ₃
B ₂ F ₃	CHINESE		B ₃ F ₂	B ₃ F ₂
B ₁ F ₃	B ₃ F ₂		AMERICAN	B ₁ F ₃
B ₃ F ₂	B ₂ F ₃		CHINESE	CHINESE
REP 2			REP 4	
B ₂ F ₃	AMERICAN		B ₁ F ₃	CHINESE
B ₃ F ₂	B ₂ F ₃		B ₃ F ₂	B ₃ F ₂
B ₁ F ₃	CHINESE		AMERICAN	AMERICAN
AMERICAN	B ₁ F ₃		CHINESE	B ₁ F ₃
CHINESE	B ₂ F ₃		B ₂ F ₃	B ₂ F ₃

Rep 1

B_2F_3	B_3F_2
AMERICAN	CHINESE
B_1F_3	B_2F_3
B_3F_2	B_1F_3
CHINESE	AMERICAN

No peat

peat

5 seeds of each stock type were seeded in each side with either a Peat or No Peat Treatment

Backcrossing

Chinese American

Expected
Proportion
Chinese

1/2

C x A

F₁ x A

1/4

B₁ x A

1/8

B₂ x A

1/16

B₃ x B₃

1/16

B₃-F₂ x B₃-F₂

1/16

B₃-F₃

So we have
seeds of:

**Chinese
American**

B₁F₃

B₂F₃

B₃F₂



Chestnut planting of seeds in West Virginia





2008 5 1

Planting Procedure

2008 5 1

Peat

2008 5 1

Seed in Peat

2008 5 1

Cover Seed



2008 5 1

Graduate Students didn't do all the work!



2008 5 2

Skousen Planted!



2008 5 2



Sheltered Trees

2008 5 14

Unsheltered Trees

2008 5 14

Seed ID

Block 1 Peat
American

BLIP
AMER

2008 5 14

7 Weeks later



2008 6 24



Statistical Analyses

Data on Survival and Height

Analysis of Variance for each year
peat and stock types – main effects
peat*stock type interactions

2008 6 24



Soil Sampling

5 samples from each block

2008 5 1

Soil Chemical Properties

between trees with and without shelters

Block	pH	P	K	Ca	Mg	CEC	BS
		mg kg ⁻¹	-----	cmol ⁺ kg ⁻¹	-----		%
Shelters	5.8	30	0.2	3.4a	1.9	10	55a
No Shelters	5.5	33	0.2	2.1b	2.0	10	44b



3 years old

09/13/2011



09/13/2011

3 years old



09/14/2011

3 years old



09/13/2011



09/13/2011

8 years old



06.23.2015

8 years old



06.23.2015

8 years old Helpers



06.23.2015

Chestnut seed Survival with and without tree shelters across all five stock types at Glory.

Treat	Survival				
	2008	2009	2010	2011	2015
	----- % -----				
Shelters	81 a	74 a	61 a	59 a	50 a
No Shelters	64 b	48 b	53 b	45 b	38 b
Ave	72	61	57	52	44

Means within columns with different letters are significantly different at p<0.05.

Chestnut seed Height with and without tree shelters across all five stock types at Glory.

Treat	Height				
	2008	2009	2010	2011	2015
	----- cm -----				
Shelters	10 a	37 a	51 a	53 a	42 a
No Shelters	6 b	27 b	37 b	44 b	25 b
Ave	8	32	44	49	34

Means within columns with different letters are significantly different at p<0.05.

Chestnut seed Height with and without peat treatment across all stock types at Glory.

Treat	Height			
	2008	2009	2010	2011
	----- cm -----			
Peat	8	29	41	47
No Peat	9	35	48	51
Ave	8	32	44	49

No significant differences!

Chestnut seed Survival for five stock types with shelters and across peat treatments.

Type	Survival (Tree Shelters)				
	2008	2009	2010	2011	2015
	----- % -----				
Amer	75 b	68 b	48 c	48 c	45 b
Chin	93 a	93 a	93 a	85 a	90 a
B ₁ F ₃	83 b	70 b	68 b	65 b	40 b
B ₂ F ₃	78 b	63 b	48 c	45 c	34 b
B ₃ F ₂	75 b	68 b	53 c	48 c	40 b
Ave	81	74	61	59	50

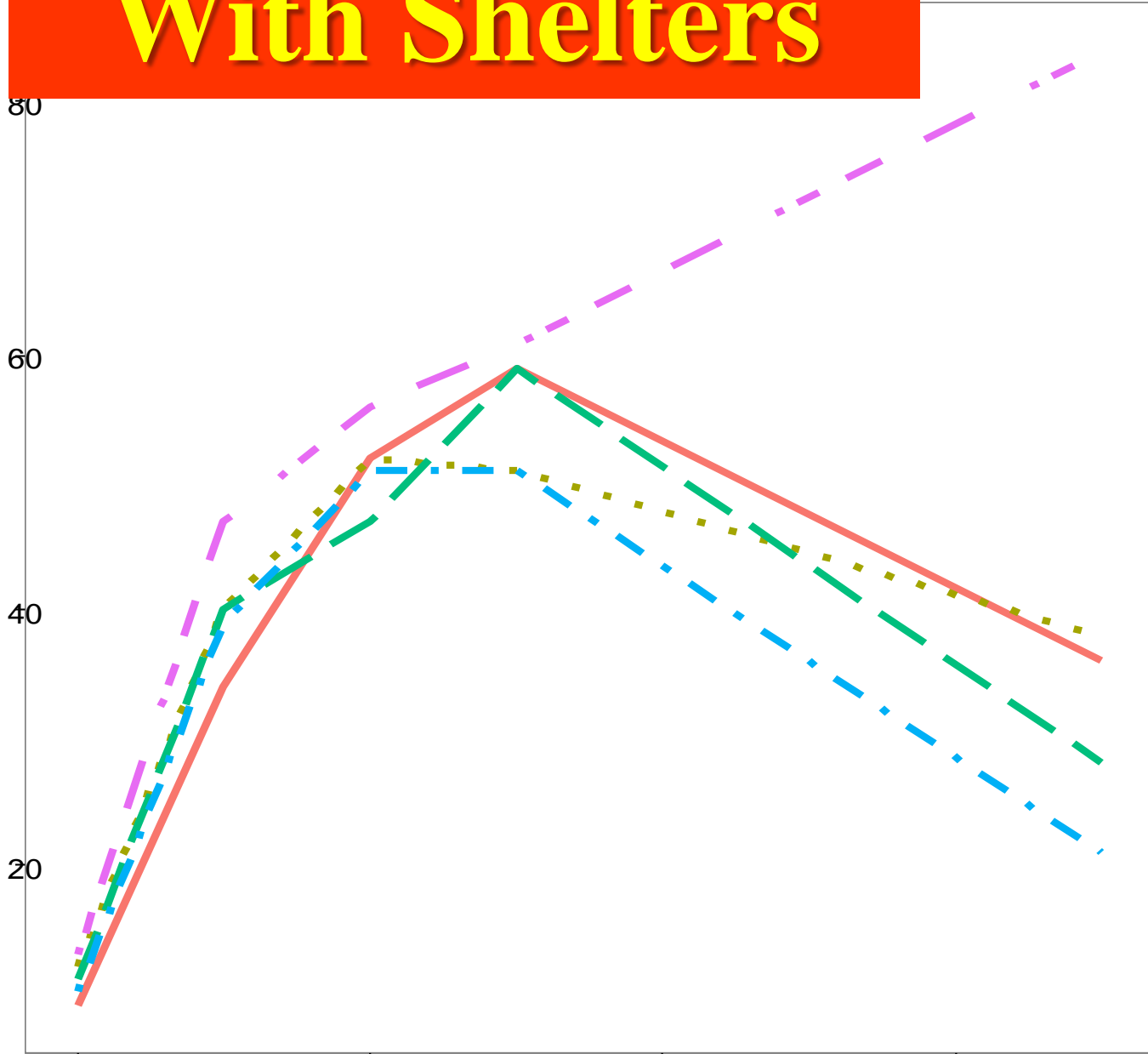
Chestnut seed Height for five stock types with shelters and across peat treatments.

Type	Height (Tree Shelters)				
	2008	2009	2010	2011	2015
	----- cm -----				
Amer	9	31	51	57	36 b
Chin	13	44	56	49	84 a
B ₁ F ₃	11	39	52	51	38 b
B ₂ F ₃	9	37	47	59	28 b
B ₃ F ₂	10	37	51	51	21 b
Ave	10	37	51	54	41

With Shelters

With Shelters

Height (cm)



Treatment

- American
- B1F3
- B2F3
- B3F2
- Chinese

2008

2010

2012

2014

Year

Without Shelters



Conclusions

**Mixed brown/gray sandstone
– pH 5.5 to 5.8**

2008 6 24

Conclusions

After 8th year,

Seeds with tree shelters –
50% survival and 42 cm height

Seeds without shelters –
38% survival and 25 cm height

Conclusions

After 8 years, seeds of

American – 45% and 36 cm

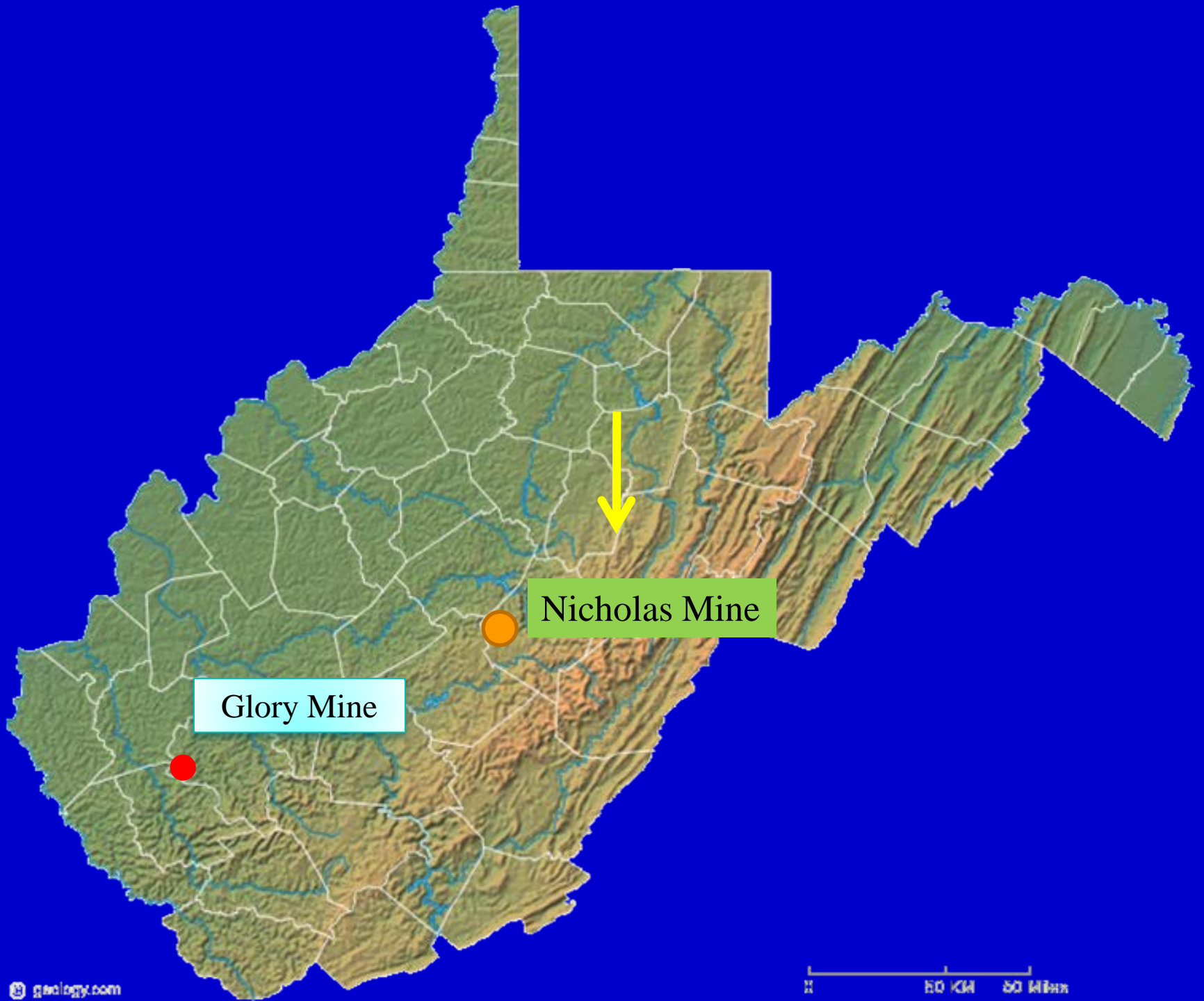
Chinese – 90% and 84 cm

B₁F₃ – 40% and 38 cm

B₂F₃ – 34% and 28 cm

B₃F₂ – 40% and 21 cm

09/13/2011



Glory Mine

Nicholas Mine

Nicholas Study in 2009 – Seedlings and Seeds



End dumped brown sandstone



Heavily graded gray sandstone



Seeds and Seedlings



Hilly, Brown Sandstone

Smooth, Gray Sandstone

BLOCK 1

B ₂ F ₃ - Seed	B ₃ F ₂ - Seedling
AMER - Seed	CHIN - Seedling
B ₁ F ₃ - Seedling	B ₂ F ₃ - Seedling
B ₃ F ₂ - Seed	B ₁ F ₃ - Seed
CHIN - Seed	AMER - Seedling

AMER - Seed	CHIN - Seed
CHIN - Seedling	B ₂ F ₃ - Seed
B ₃ F ₂ - Seedling	AMER - Seedling
B ₂ F ₃ - Seedling	B ₃ F ₂ - Seed
B ₁ F ₃ - Seed	B ₁ F ₃ - Seedling

BLOCK 2

B ₂ F ₃ - Seed	B ₂ F ₃ - Seedling
CHIN - Seedling	AMER - Seedling
AMER - Seed	B ₁ F ₃ - Seedling
B ₁ F ₃ - Seed	CHIN - Seed
B ₃ F ₂ - Seedling	B ₃ F ₂ - Seed

B ₁ F ₃ - Seed	AMER - Seed
AMER - Seedling	B ₁ F ₃ - Seedling
CHIN - Seed	B ₂ F ₃ - Seed
B ₂ F ₃ - Seedling	B ₃ F ₂ - Seed
B ₃ F ₂ - Seedling	CHIN - Seedling

BLOCK 3

CHIN - Seed	AMER - Seedling
AMER - Seed	B ₁ F ₃ - Seedling
B ₂ F ₃ - Seed	CHIN - Seedling
B ₁ F ₃ - Seed	B ₃ F ₂ - Seedling
B ₃ F ₂ - Seedling	B ₂ F ₃ - Seed

B ₂ F ₃ - Seed	AMER - Seedling
B ₁ F ₃ - Seed	B ₂ F ₃ - Seedling
B ₃ F ₂ - Seedling	B ₃ F ₂ - Seed
AMER - Seed	B ₁ F ₃ - Seedling
CHIN - Seedling	CHIN - Seed

BLOCK 4

B ₂ F ₃ - Seedling	AMER - Seedling
B ₃ F ₂ - Seedling	B ₂ F ₃ - Seed
B ₁ F ₃ - Seed	CHIN - Seed
AMER - Seedling	B ₁ F ₃ - Seedling
CHIN - Seedling	B ₃ F ₂ - Seed

B ₁ F ₃ - Seed	CHIN - Seed
B ₃ F ₂ - Seed	B ₃ F ₂ - Seedling
AMER - Seed	AMER - Seedling
CHIN - Seedling	B ₁ F ₃ - Seedling
B ₂ F ₃ - Seed	B ₂ F ₃ - Seedling

BLOCK 5

B ₁ F ₃ - Seedling	CHIN - Seed
B ₂ F ₃ - Seed	B ₁ F ₃ - Seed
AMER - Seed	B ₃ F ₂ - Seed
B ₃ F ₂ - Seedling	B ₂ F ₃ - Seedling
CHIN - Seedling	AMER - Seedling

AMER - Seedling	B ₂ F ₃ - Seed
CHIN - Seed	AMER - Seed
B ₃ F ₂ - Seedling	B ₃ F ₂ - Seed
B ₁ F ₃ - Seed	CHIN - Seedling
B ₂ F ₃ - Seedling	B ₁ F ₃ - Seedling

**Randomized
Split-Plot
Design
with
five
replications**

BLOCK 1

B₂F₃ - Seed	B₃F₂ - Seedling
AMER - Seed	CHIN - Seedling
B₁F₃ - Seedling	B₂F₃ - Seedling
B₃F₂ - Seed	B₁F₃ - Seed
CHIN - Seed	AMER - Seedling

Backcrossing

Chinese American

Expected
Proportion
Chinese

So we have

Seed and Seedlings

Chinese
Amer

$B_1 F_3$

$B_2 F_3$

$B_3 F_2$

1/2

C x A

F₁ x A

B₁ x A

B₂ x A

B₃ x B₃

B₃-F₂ x B₃-F₂

B₃-F₃

1/4

1/8

1/16

1/16

1/16



Planting Procedure - Brown







Seeds



Planting Procedure – Gray



Much more difficult





Seeds

Brown Sandstone Plot



Gray Sandstone Plot



A photograph of a field with young trees and a person in the background. The text is overlaid on the image. The background shows a person standing in a field of young trees, possibly a nursery or a reforestation site. The ground is rocky and uneven. In the distance, there are more trees and a utility pole. The sky is overcast.

Statistical Analyses

Data on Survival and Height

**Analysis of Variance for each year
plant type, stock types – main effects
interactions**

Soil Sampling

15 samples from each site



Nicholas Study - Soil Chemical Properties

Substrate	pH	P	K	Ca	Mg	CEC	BS
		mg kg ⁻¹	-----	cmol ⁺ kg ⁻¹	-----		%
Brown	4.5 b	6 b	0.33	2.9 b	3.6 b	13 b	28 b
Gray	6.6 a	56 a	0.40	9.5 a	6.2 a	8 a	100 a



**SIX SEEDS
out of 250!**

Seeding was a failure this time!



Seedlings:

B₃F₂

2 years old

07/21/2011



B₃F₂
2 years old

07/21/2011

American
2 years old

07/21/2011



**Chinese
2 years old**



06.04.2015

7 years old



06



7 years old

06.04.2015



7 years old

06.04.2015



7 years old

06.04.2015



**Chinese
2 years old
On Gray**

07/21/2011

A man wearing a blue long-sleeved shirt, blue jeans, black boots, and a blue cap stands in a rocky, open field. He is holding a small, young tree with green leaves. The background shows a line of trees under a blue sky with white clouds.

**7 years old
On Gray**

06.04.2015



7 years old

06.04.2015

Chestnut seedling Survival for five stock types

Type	Substrate			
	Brown			
	2009	2010	2011	2015
	----- % -----			
Amer	100 a	100 a	77 ab	68 a
Chin	100 a	96 a	84 a	68 a
B ₁ F ₃	100 a	72 b	88 a	56 ab
B ₂ F ₃	100 a	70 b	70 b	35 b
B ₃ F ₂	89 b	68 b	68 b	30 b
Ave	98	81	77	51

Means within columns with different letters are significantly different at p<0.05.

Chestnut seedling Survival for five stock types

Type	Substrate			
	Gray			
	2009	2010	2011	2015
	----- % -----			
Amer	100 a	72 b	80 b	56 b
Chin	100 a	100 a	100 a	96 a
B ₁ F ₃	80 b	72 b	72 b	75 ab
B ₂ F ₃	87 b	66 b	68 b	43 b
B ₃ F ₂	100 a	44 c	44 c	48 b
Ave	93	61	74	64

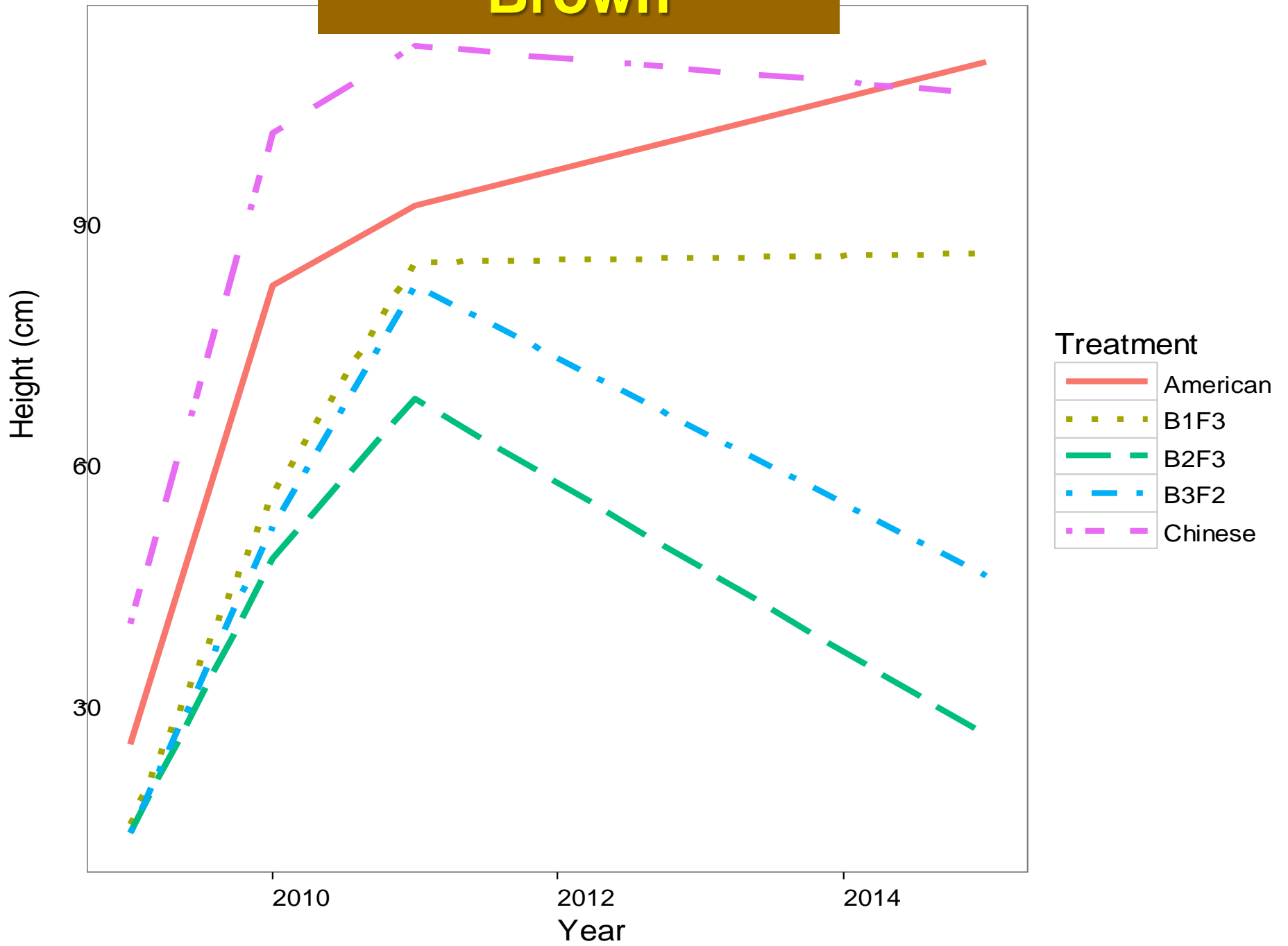
Means within columns with different letters are significantly different at p<0.05.

Chestnut seedling Height for five stock types

	Substrate			
Type	Brown			
	2009	2010	2011	2015
	----- cm -----			
Amer	62 b*	80 b	92 b	110 a
Chin	100 a	101 a	112 a	106 a
B ₁ F ₃	36 c	56 c	77 c	86 b
B ₂ F ₃	33 c	48 c	68 c	26 c
B ₃ F ₂	26 c	52 c	82 bc	46 c
Ave	51	68	86	75

Brown

Brown

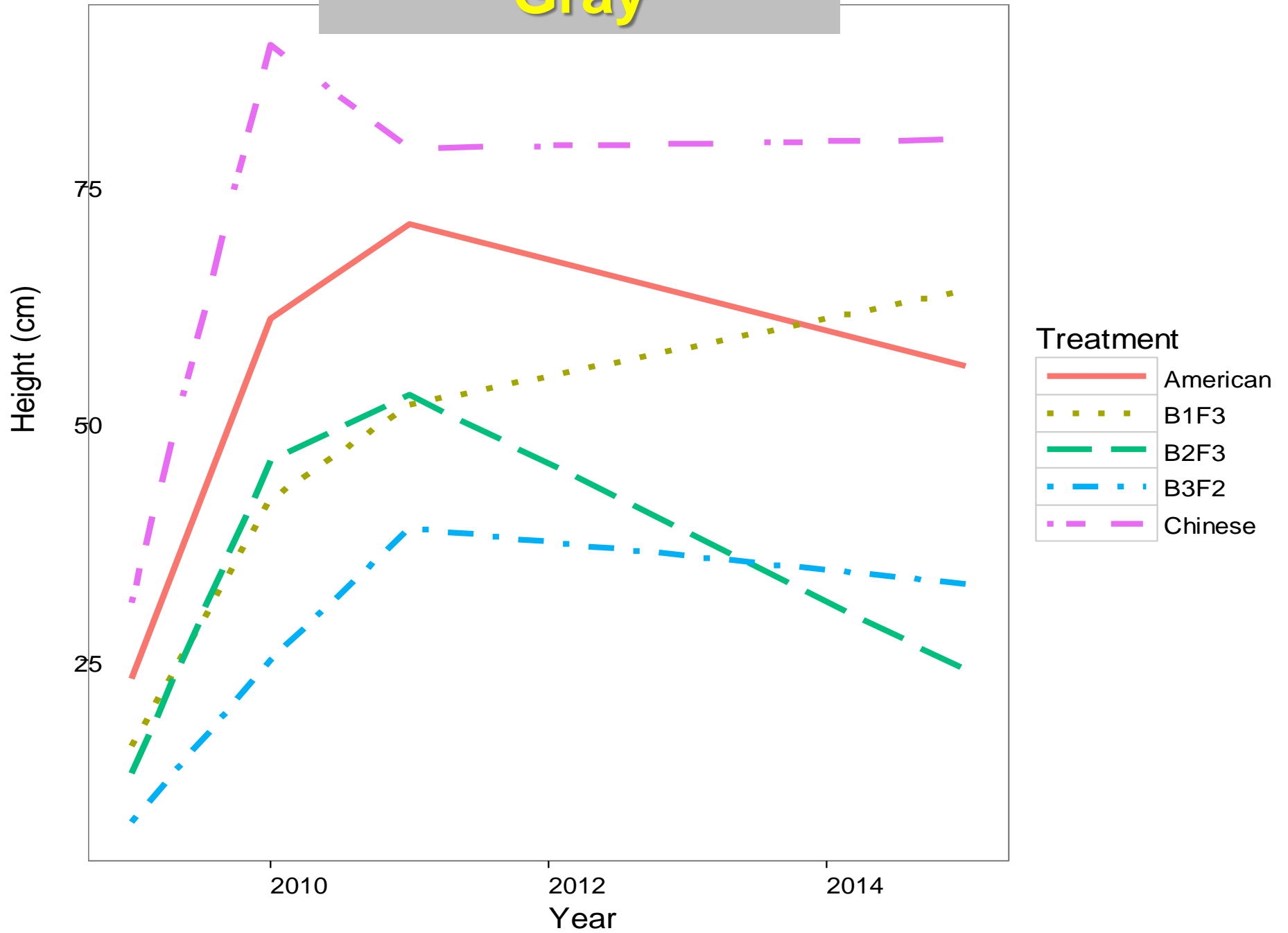


Chestnut seedling Height for five stock types

	Substrate			
Type	Gray			
	2009	2010	2011	2015
	----- cm -----			
Amer	59 b	61 b	71 a	56 b
Chin	84 a	90 a	79 a	80 a
B ₁ F ₃	37 c	42 c	55 b	64 b
B ₂ F ₃	31 c	24 d	52 b	24 c
B ₃ F ₂	22 c	24 d	39 c	33 c
Ave	47	48	59	51

Gray

ray



Conclusions

Brown

pH = 4.5

P = 6 mg/kg

BS = 28%

Gray

pH = 6.6

P = 56 mg/kg

BS = 100%

Conclusions

Only 6 of 250 seeds germinated!

After 7 years,
Ave Survival of Seedlings
51% on Brown
64% on Gray

Conclusions

After 7 years,

Brown

Gray

American	68%, 110 cm	56%, 56 cm
Chinese	68%, 106 cm	96%, 80 cm
B ₁ F ₃	56%, 86 cm	75%, 64 cm
B ₂ F ₃	35%, 26 cm	43%, 24 cm
B ₃ F ₂	30%, 46 cm	48%, 33 cm

**Chestnut
Survival
is similar
to other
hardwood
trees on
mined
land!**



**Chestnut
Growth,
on these sites,
was not as
good as other
hardwood trees
on mined land.**



7 years old

Red Oak

Tulip Poplar



7 years old

Seed Seedling



**These were the largest chestnuts
Most were much smaller.**

06.23.2015

06.04.2015