Tissue Culture Media Design at Sierra Gold Nurseries

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SGN Research Lab Team

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- Anuradha Kaushal
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UC Davis Walnut Improvement Program

- Chuck Leslie
- Steven Lee

Plant Tissue Culture lab includes around 60 employees, 30 of which specialize only in tissue culture.

Lab Specialists work to create close to 76,000 tissue culture microshoots daily

Acclimatize a minimum of 30,000 TC plants a day, resulting in close to 8 million rootstock TC plants produced per year

Rootstocks Produced via Tissue Culture

Cherries
- Gisela's
- Krymsk® 5 & 6

Apples
- Geneva's
- Bud 10

Pecans
- Riverside

Fruit and Nut
- Krymsk® 86
- SG1®
- MP-29
- Bright's Hybrid® 5

Pears
- OHxF 87 & 97
- Pyro™ 2-33

Walnuts
- Clonal RX1
- Grizzly Clonal Walnut Rootstock™

Pistachios
- Yankee
- Platinum Pistachio Rootstock
- Clonal UCB-1

What obstacles interrupt large-scale plant tissue culture production?

Inconsistent Techniques

- Trainings
- Understanding of event type

Contamination

- Systemic
- Environmental
- Media

Environmental Fluctuations

- Temperature
- Humidity
- CO2

Tissue Culture Performance

- Low multiplication rates
- Poor plant quality
- Genotype

Factors Influencing Tissue Culture

Gelling Agents

- Gellan Gums
- Carrageenan
- Agar

Carbohydrates

- Glucose
- Sucrose
- Sorbitol

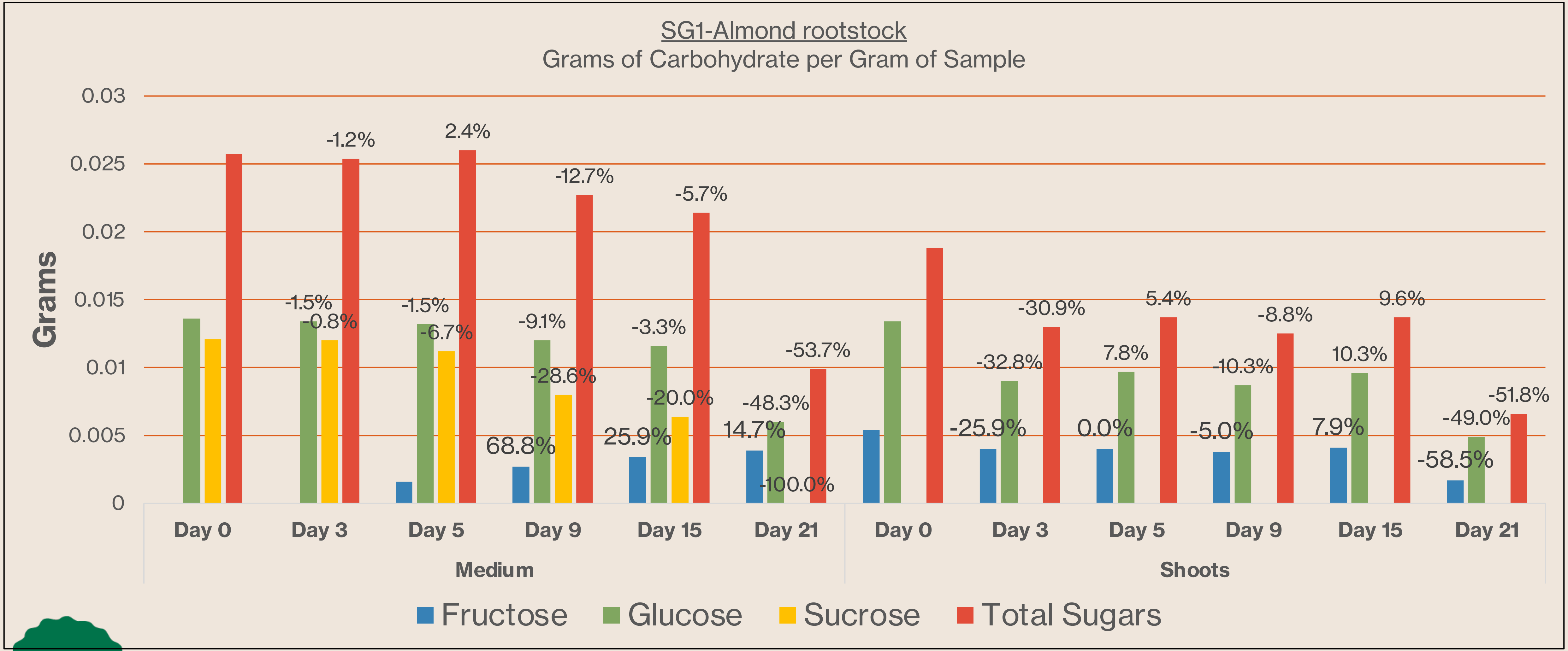
PGRs

- Auxins
- Cytokinins
- Gibberellins

Basal Salts

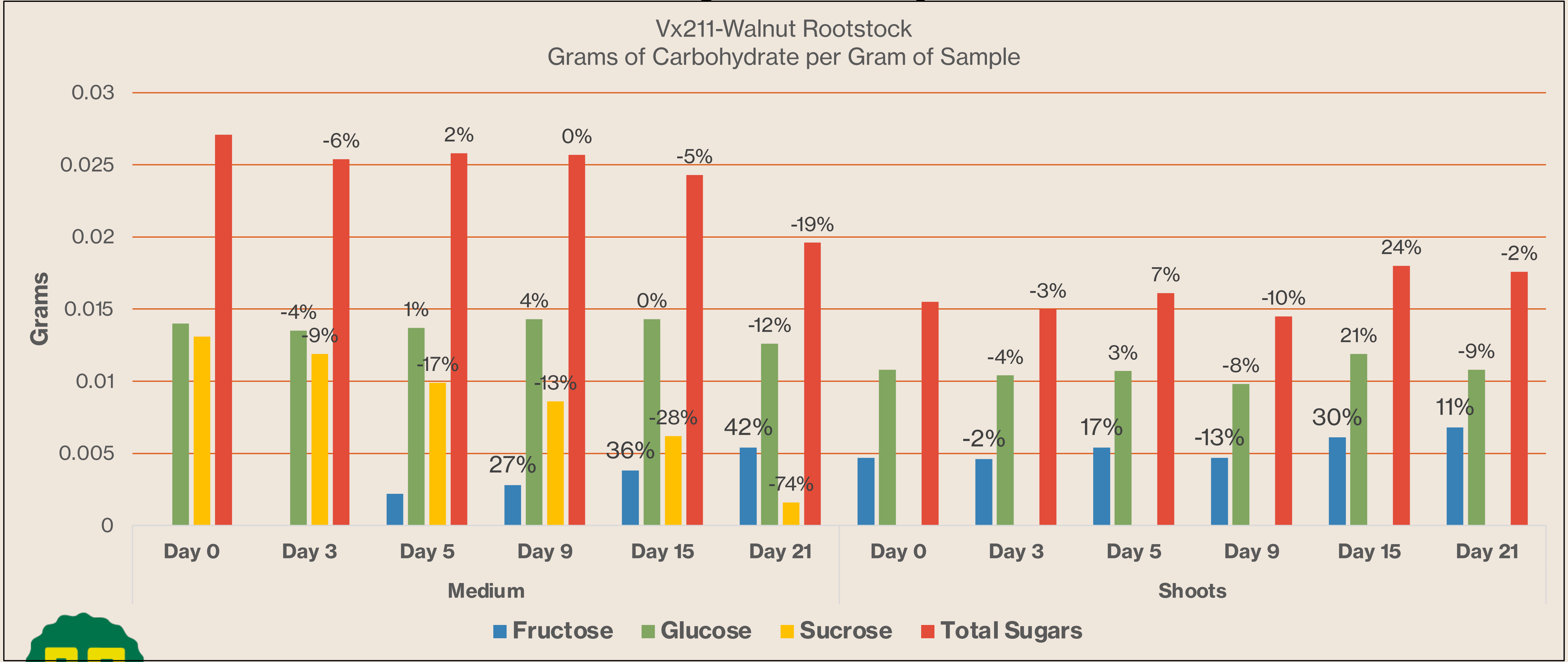
- Total ion concentrations
- Ion ratios

Carbohydrate Uptake



Plants grown on SFN with 15 g/L sucrose + 15/L glucose

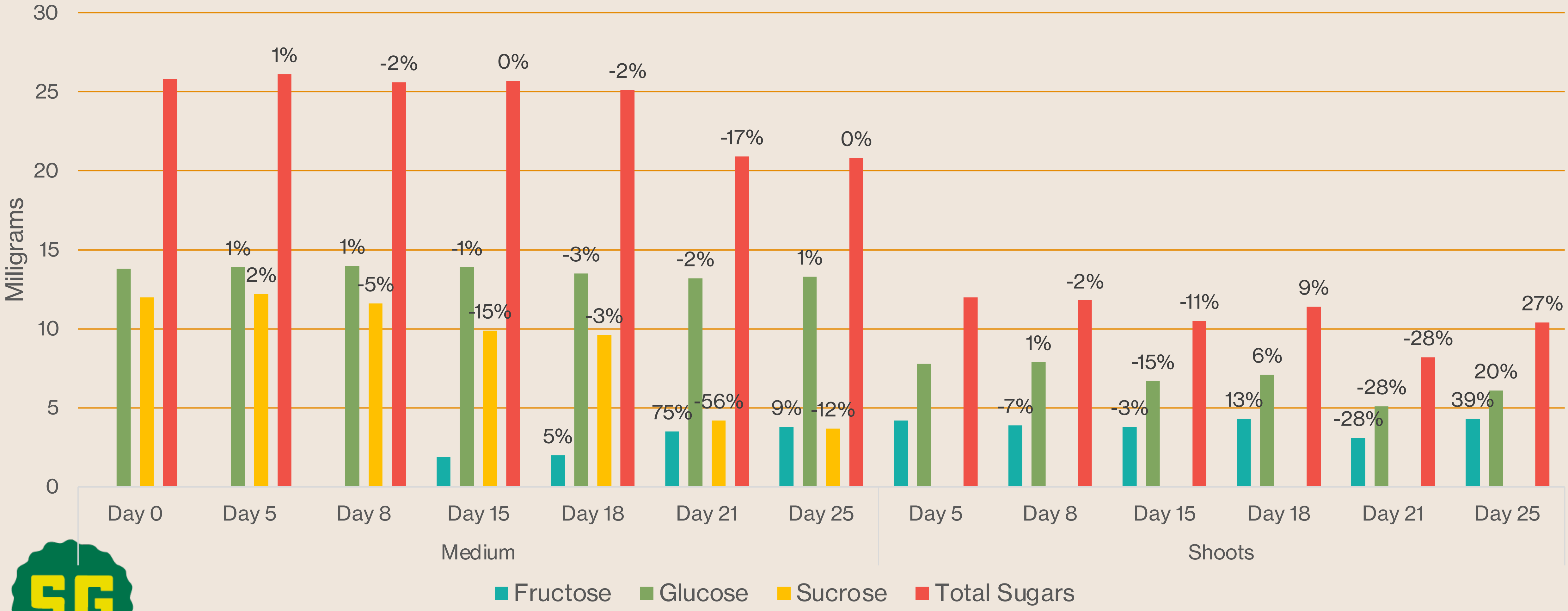
Carbohydrate Uptake



Plants grown on SFN with 15 g/L sucrose + 15/L glucose

Carbohydrate Uptake

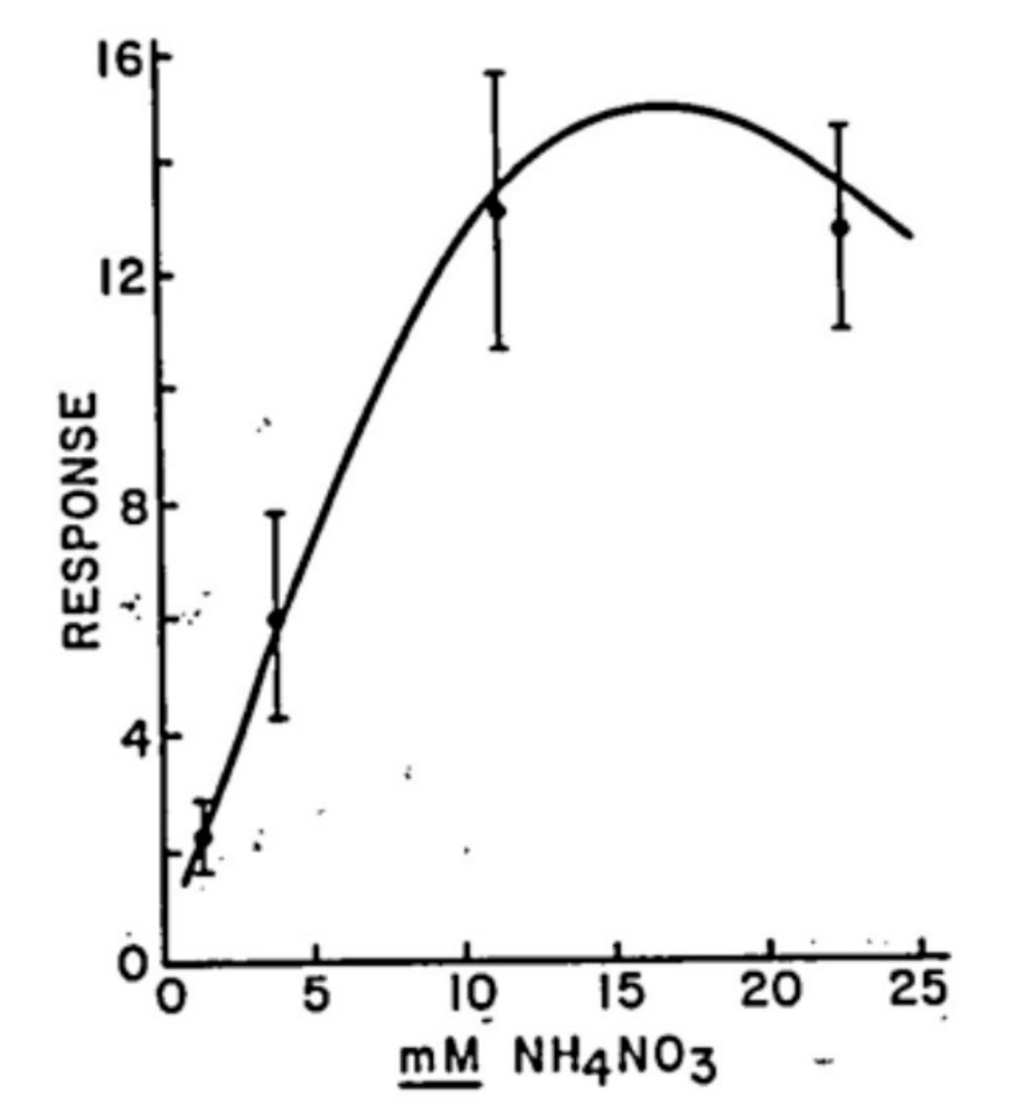
Platinum- Pistachio Rootstock
Miligrams of Carbohydrate per Gram of Sample



Plants grown on SFN with 15 g/L sucrose + 15/L glucose

Improving *Juglans* spp. Micropropagation

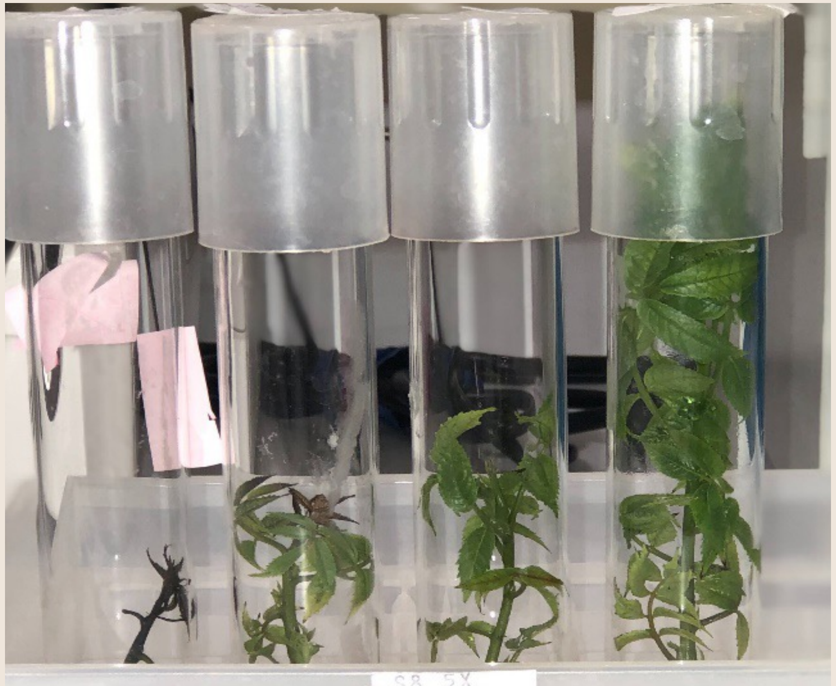
- Walnuts are a high value crop, but micropropagation is often difficult and success low
- DKW basal salts originally designed for paradox hybrid tissue culture
- Newer hybrids and *J. regia* do not perform as well on DKW



Driver and Kuniyuki 1984

Improving *Juglans* spp. Micropropagation

10x DKW

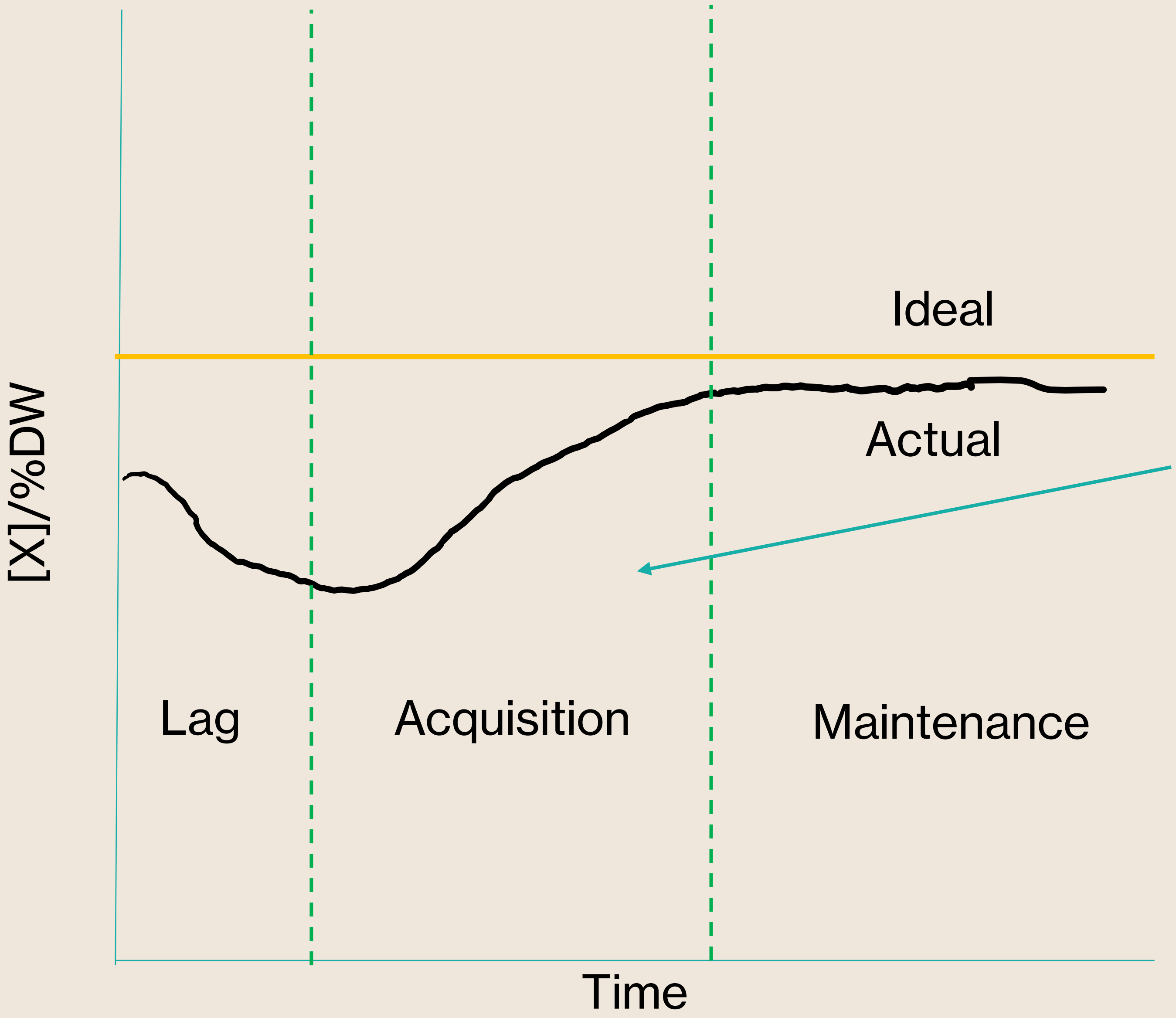


5x DKW

1x DKW

- **Simply changing phytohormones or carbohydrates not enough**
- **Nutrient availability vs accessibility**
- **Tested gelling agents and worked ”backwards” to determine optimum nutrient concentration**

Improving *Juglans* spp. Micropropagation



Duration in this phase influenced by nutrient accessibility. Seek to minimize as much as possible IF true

Improving *Juglans* spp. Micropropagation

Description : SJM-2 D3
Project : Research Lab Nutrient Analysis

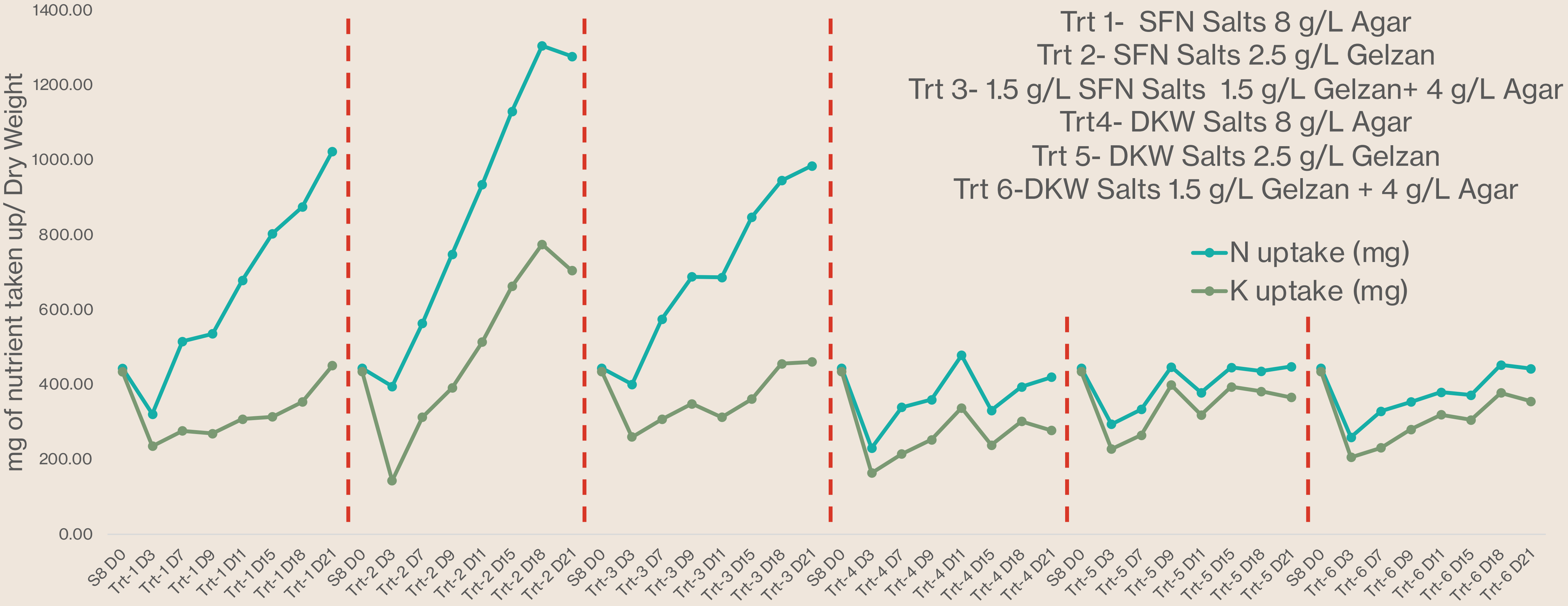
Sample Results - Ag

Constituent	Result	PQL	Units	Note	Sample Preparation Method	Sample Preparation Date/ID	Sample Analysis Method	Sample Analysis Date/ID
Leaf								
Total Nitrogen	7.29	0.05	%		B-2.20	03/18/20:203030	993.13	03/18/20:204335
Phosphorus	1.17	0.0002	%		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Potassium	2.64	0.0058	%		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Calcium	0.576	0.004	%		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Magnesium	0.510	0.004	%		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Zinc	277	0.5	ppm		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Manganese	462	1	ppm		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Iron	151	2	ppm		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Copper	4	1	ppm		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Boron	41.8	0.4	ppm		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364
Sodium	0.030	0.004	%		Dry Ash	03/18/20:203048	6010-Ag	03/19/20:204364

ND=Non-Detected. PQL=Practical Quantitation Limit.

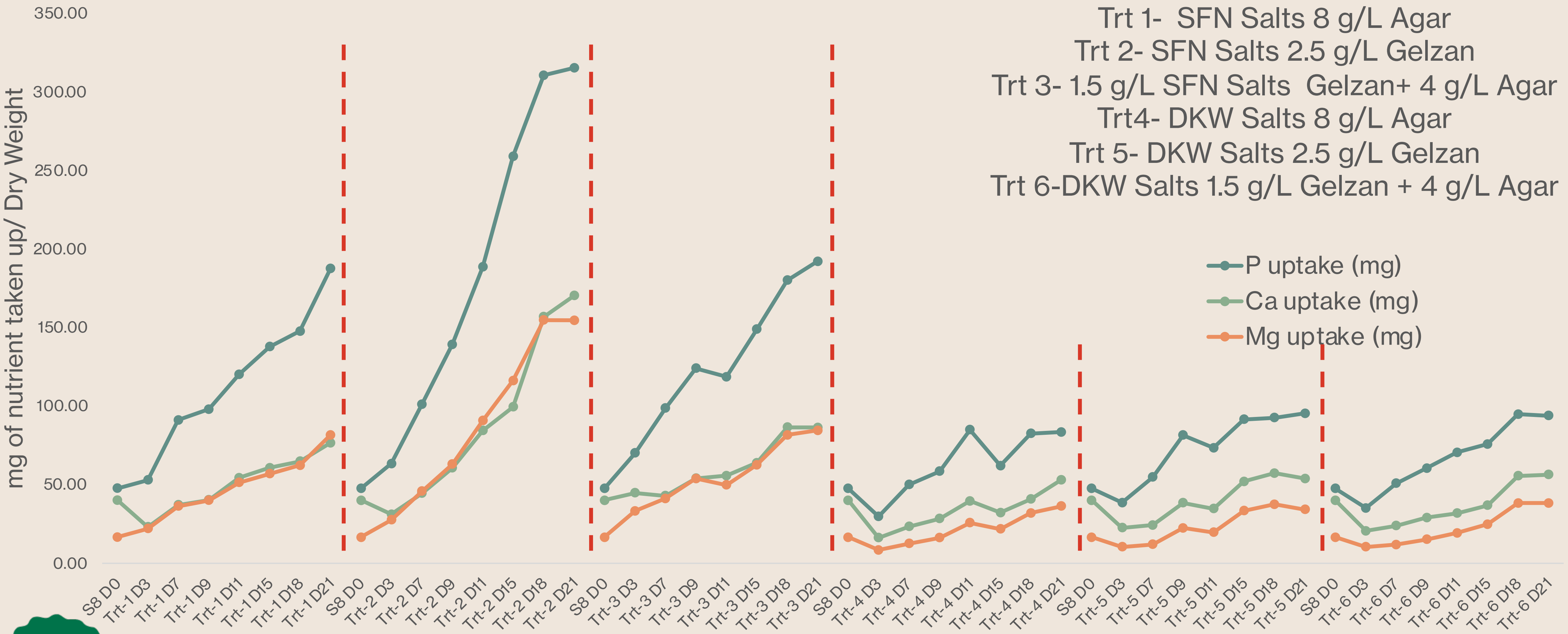
- **Tested different levels of basal salts and gelling agents over the course of a normal cycle period**
- **Rx1 (*J. regia* x *J. micropcarpa*)**
- **Availability vs. Accessibility**

Improving *Juglans* spp. Micropropagation



- Initial dip in nutrient concentration more pronounced in DKW
- Faster and more overall uptake with Gelzan than agar

Improving *Juglans* spp. Micropropagation



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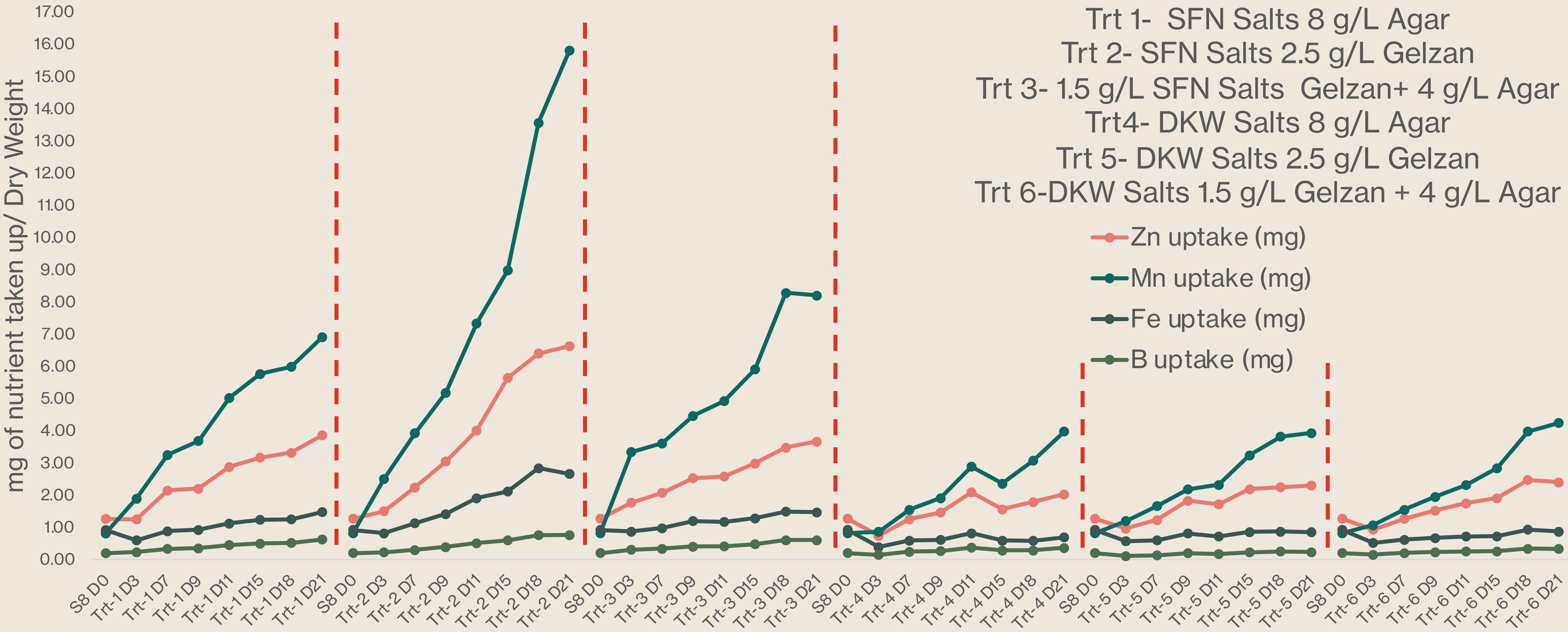

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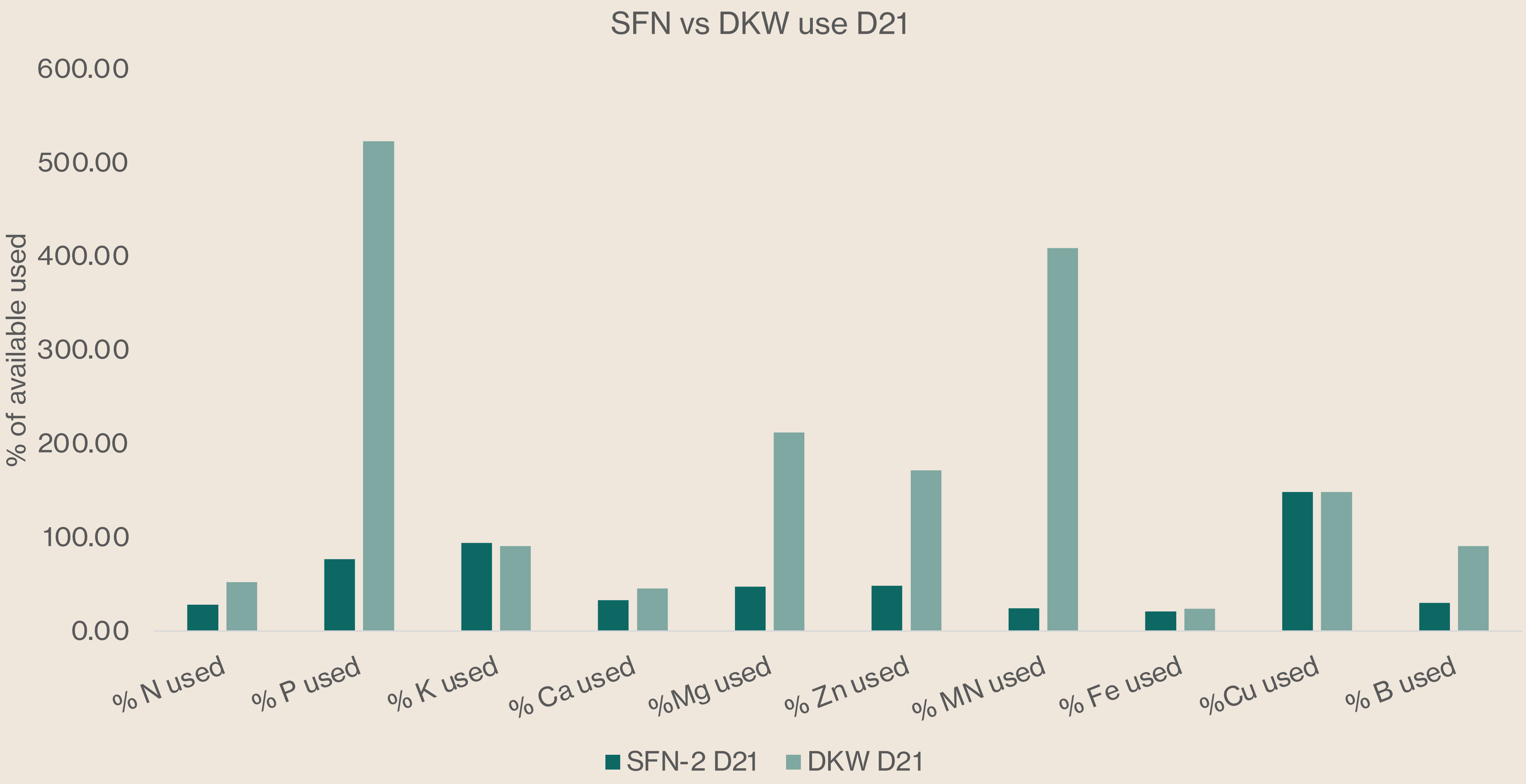


Improving *Juglans* spp. Micropropagation

Compound	SJM	NH4	NO3	K	Ca	Mg	SO4	P	Na	Cl
(NH4)2SO4										
NH4NO3	2100	473.25	1626.75							
NH4H2PO4										
MgNH4PO4+6H2O										
Ca(NO3)2+4H2O	1200		630.16		203.66					
KNO3	1000		613.28	386.72						
Zn(NO3)2+6H2O										
Mg(NO3)2+6H2O	1200		580.37			113.75				
KH2PO4	1400			402.23				318.65		
H3BO3	14.4									
Na2MoO4+2H2O	3.9								0.74	
MgSO4+7H2O	1000					98.61	389.75			
MgCl2 +6H20										
MnSO4+H20	200						113.67			
CuSO4+5H2O	0.25						0.10			
ZnSO4+7H2O	60						20.04			
CaCl2+2H2O										
K2SO4										
FeEDDHA	100									
NiSO4+6H2O	0.005						0.00			
FeSO4+7H2O										
Na2 EDTA										
KI										
CoCl2+6H2O	0.06									0.02
	SUM (mg/L)	473.25	3,450.57	788.95	203.66	212.36	523.56	318.65	0.74	0.02
	SUM (mM/uM)	26.24	55.65	20.18	5.08	8.74	5.45	10.29	0.03	0.00
						mM				
	NO3:NH4 Ratio	2.1								
	Total N (mg/L)	3,923.82								
	Total N (mM)	81.89								
	K:NH4 (mM) Ratio	0.769123								
	K:Mg Ratio	2.309476								
	Ca:Mg Ratio	0.58								
	Ca:K Ratio	0.25								
	NH4:Ca Ratio	5.16								
	NO3:P Ratio	5.409381								

Ion calculator for medium development

Components of new SFN medium

Ion (mM)	SFN	MS	WPM	DKW
NH4	26.2	20.6	5.0	17.7
NO3	55.6	39.4	8.3	34.5
K	20.2	20.0	12.6	19.8
Ca	5.1	3.0	2.1	9.3
Mg	8.7	1.5	0.7	3.0
SO4	5.5	1.7	6.7	12.3
P	10.3	1.2	1.2	1.9
Na	0.0	0.2	0.2	0.3
Cl		6.0	1.0	2.0
Zn	208.7	29.9	29.9	57.1
Mn	1,183.3	100.0	131.9	198.2
Cu	1.0	0.1	1.0	1.0
B	232.9	100.3	100.3	77.6
Mo	16.1	1.0	1.0	1.6
Fe	228.7	100.0	100.0	121.6
Co	0.3	0.1		
Ni	0.0			0.0
I		5.0		
NO3:NH4	2.1	1.9	1.7	1.9
Total N (mM)	81.9	60.0	13.3	52.2
K:NH4 (mM) Ratio	0.8	1.0	2.7	1.1
K:Mg Ratio	2.3	14.0	18.6	6.6
Ca:Mg Ratio	0.6	2.0	2.9	3.1
Ca:K Ratio	0.3	0.1	0.2	0.5
NH4:Ca Ratio	5.2	6.9	2.3	1.9
NO3:P Ratio	5.4	31.5	6.6	17.7

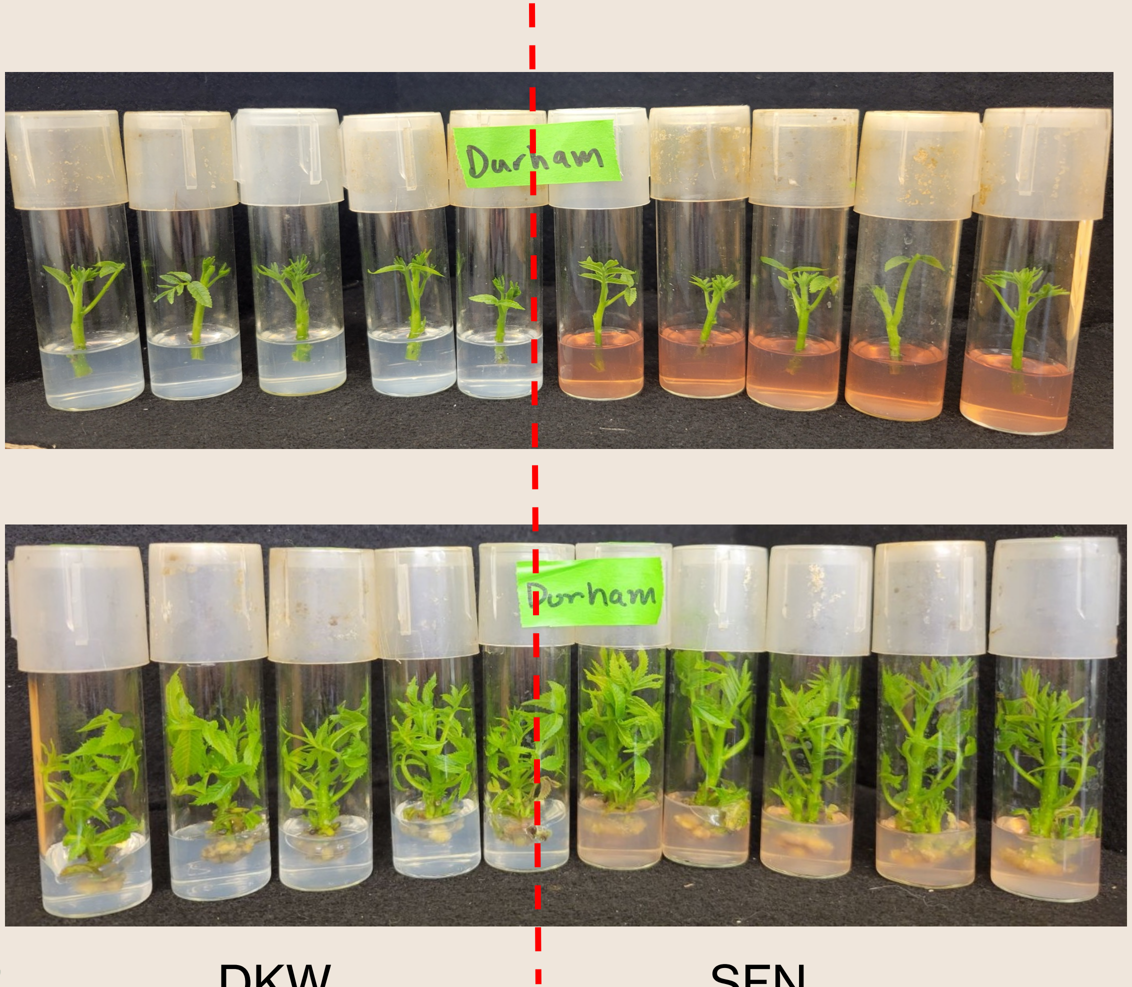
Sure, but is it actually better

- Submitted SFN salts to Chuck Leslie at UC Davis
- Screened 13 diverse *Juglans* genotypes
- Fell into three main groups: English walnut (*Juglans regia*), Texas black walnut (*J. microcarpa*) x English, and Northern California Black (NCB, *J. hindsii*) x English
- 36 microshoots were selected and split between DKW and SFN
- Measured height day 0 and day 21.
- Recorded branching, microshoot color and condition

Rx1 day 21 nutrient sampling

Sure, but is it actually better?

Group 1: English walnuts (*J. regia*)

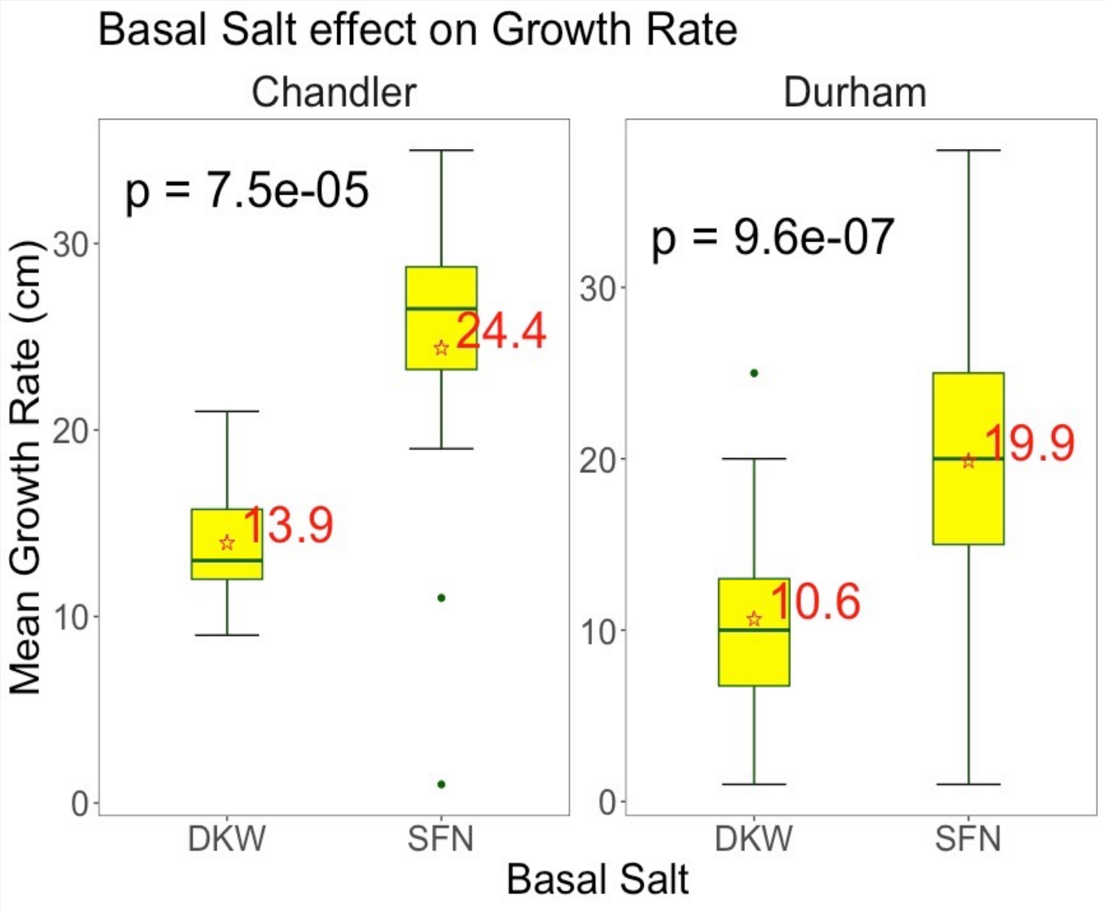


Day 0

Day 21

DKW

SFN



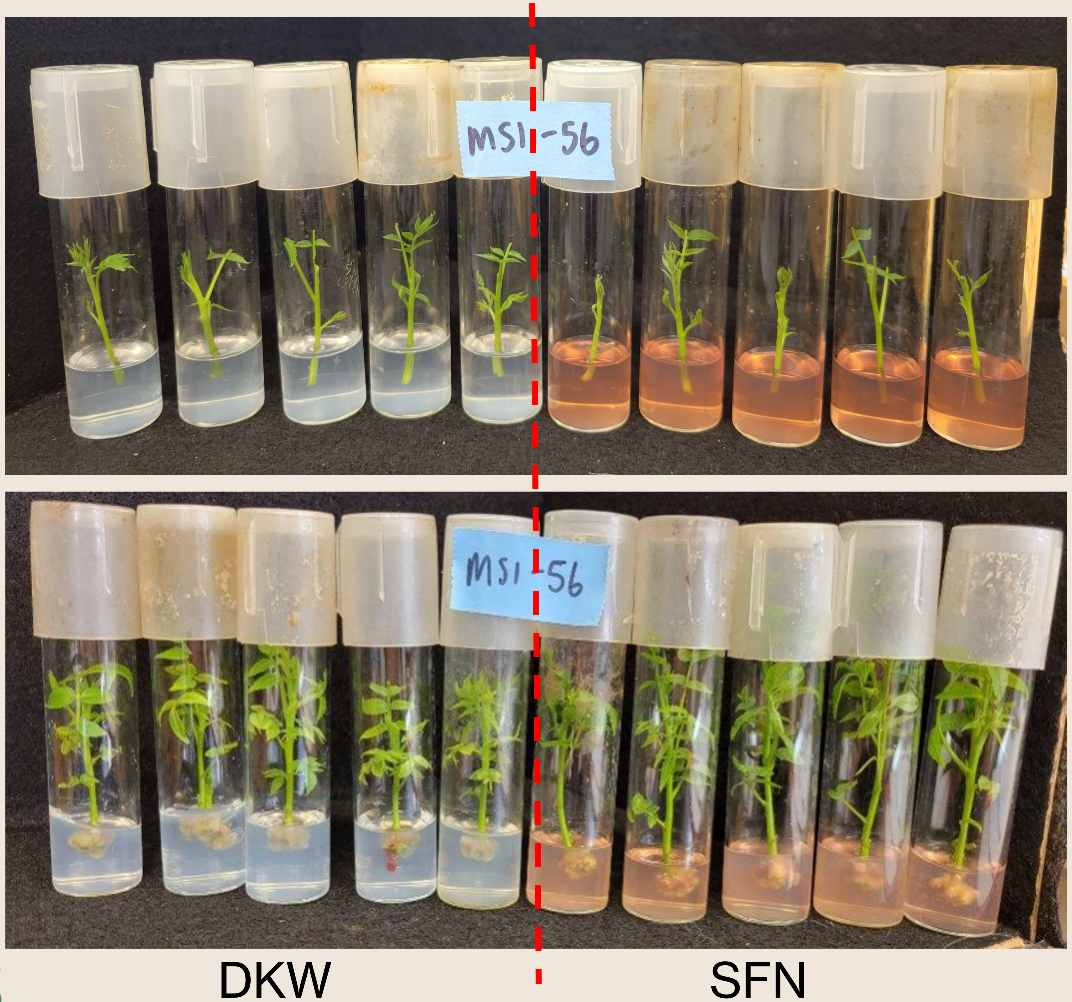
Courtesy of Steven Lee, UCD

Sure, but is it actually better?

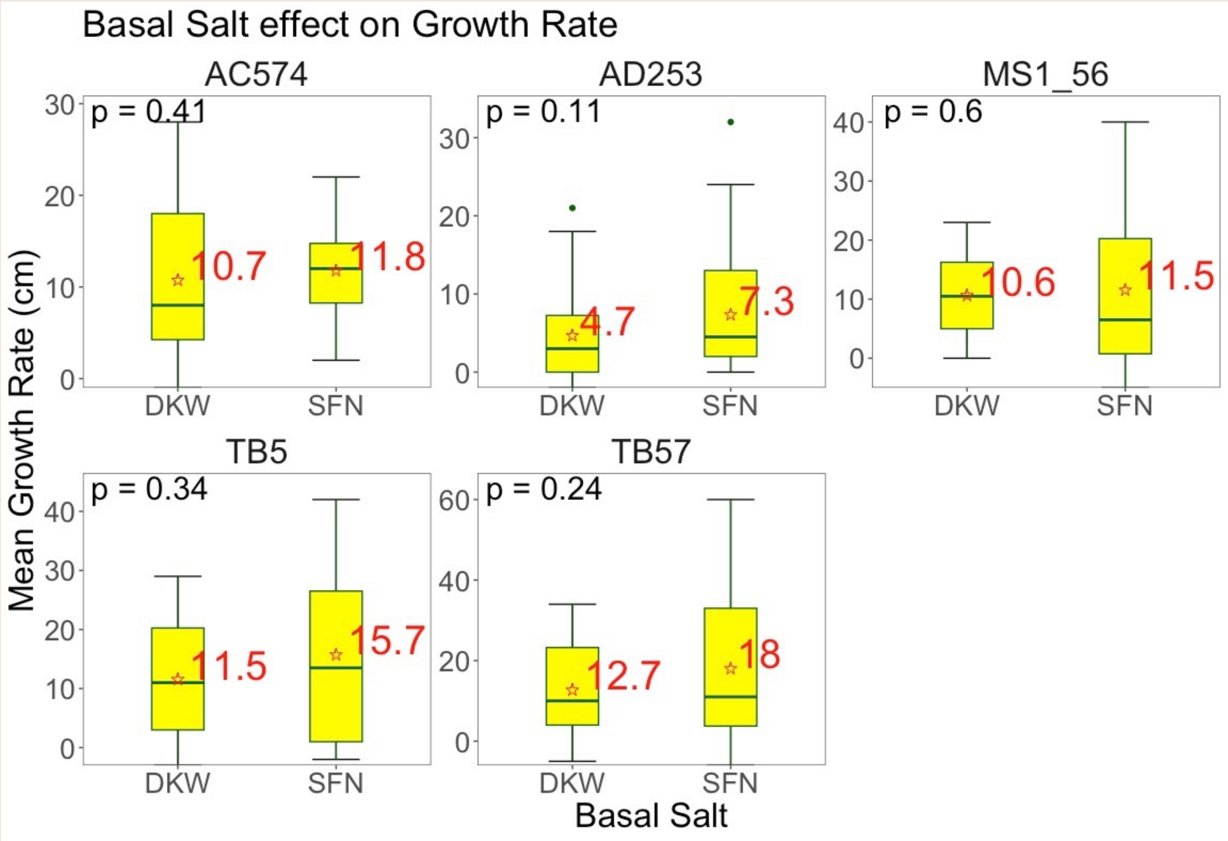
Group 2: Texas black walnut (*J. microcarpa*) x English

Day 0

Day 21



DKW SFN



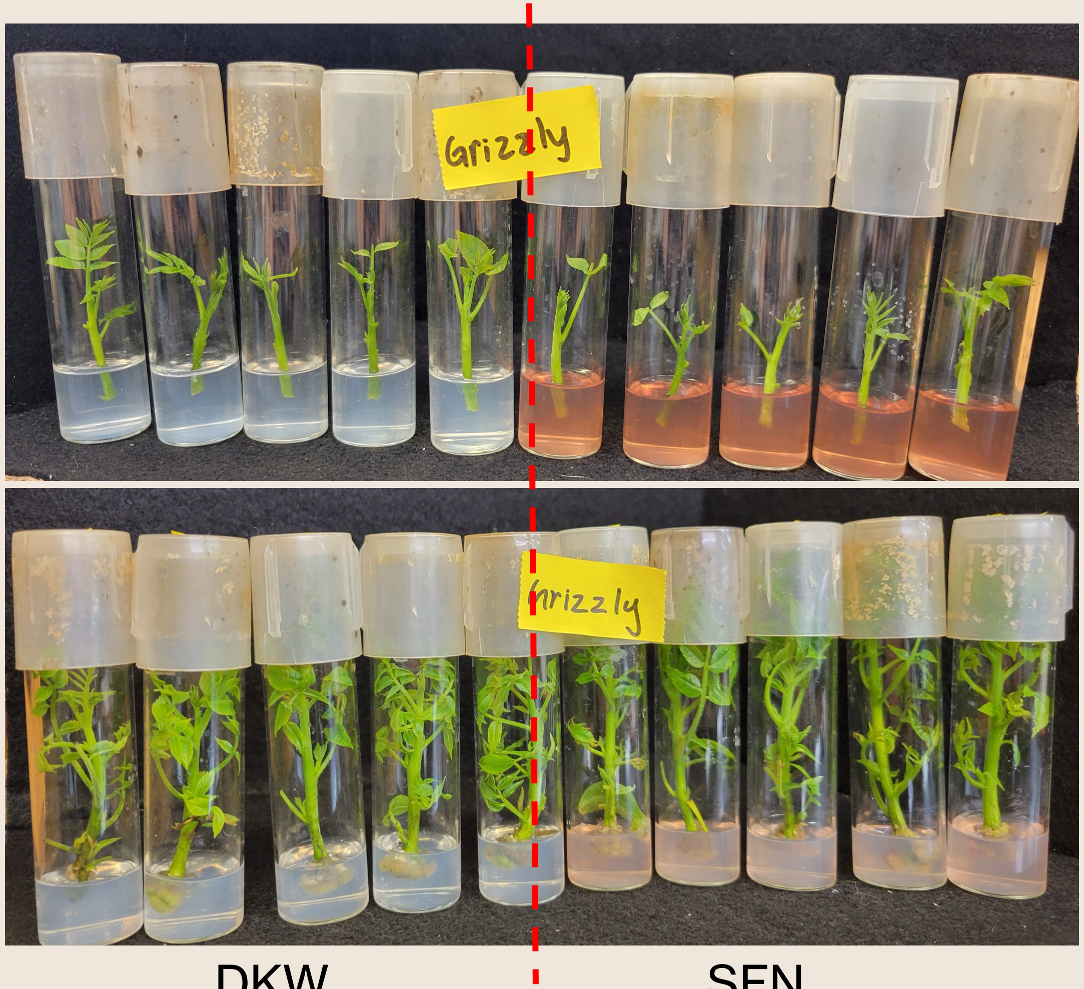
Courtesy of Steven Lee, UCD

Sure, but is it actually better?

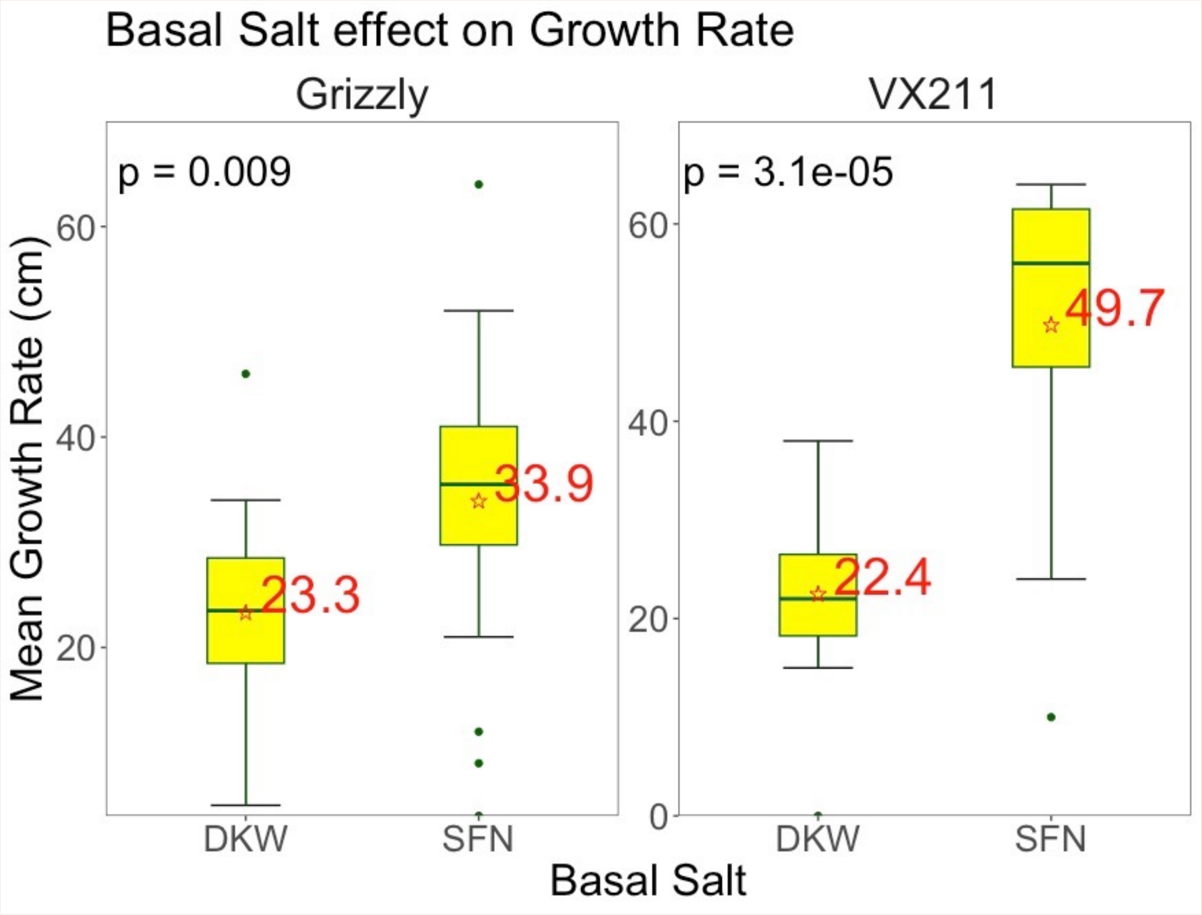
Group 3: Northern California Black (NCB, *J. hindsii*) x English

Day 0

Day 21



DKW SFN



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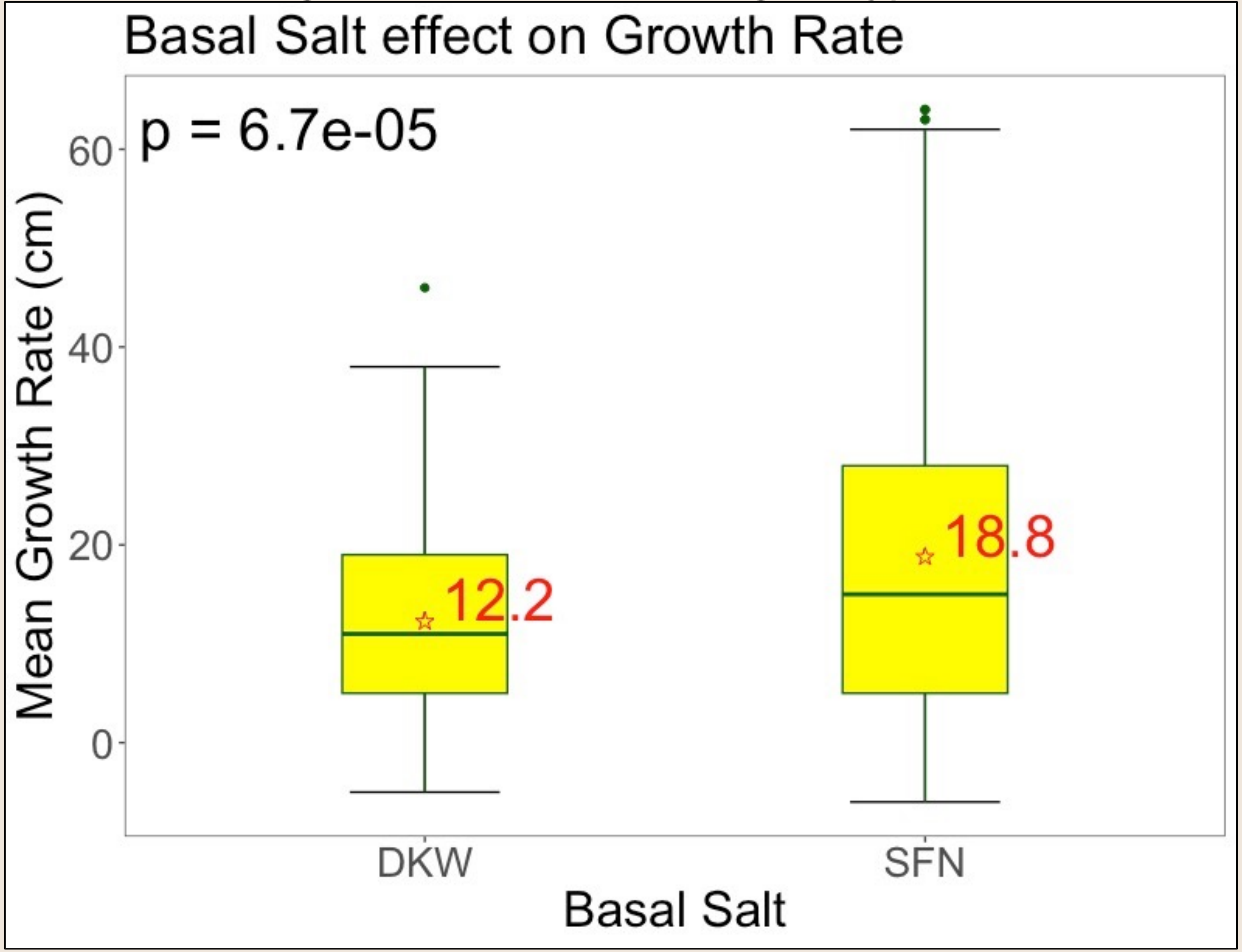
Sure, but is it actually better? YES!

Difference in growth rate across all genotypes



- Culture on SFN medium resulted in greater shoot growth than culture on DKW.
- SFN had greater variability than DKW, but at worst is at least the same as DKW.
- English and NCB x English genotypes exhibited significantly better growth on SFN than on DKW. Growth of the *J. microcarpa* hybrids was similar on both media but again, shoot quality was generally better on SFN

Courtesy of Steven Lee, UCD

Nitrogen Ratios

Viking- Almond Rootstock

Normal elongation Medium:

- Lots of leaf expansion minimal shoot elongations

K:NH4 Ratio	3.1
NO3:P Ratio	3.0

N ratio change:

- Significant shoot elongation

K:NH4 Ratio	2.4
NO3:P Ratio	10.8

TRT	K:NH4 (mM) Ratio	NO3:P (mM) Ratio	Carbohydrate
1	0.6	2.7	Glucose
2	0.6	2.7	Glucose
3	0.6	2.7	Glucose
4	0.6	2.7	Glucose
5	1.2	2.7	Glucose
6	2.4	2.7	Glucose
7	0.6	5.4	Glucose
8	1.2	5.4	Glucose
9	1.2	5.4	Glucose
10	2.4	5.4	Glucose
11	0.6	10.8	Glucose
12	1.2	10.8	Glucose
13	2.4	10.8	Glucose
14	1.2	2.7	Sucrose
15	2.4	2.7	Sucrose
16	2.4	2.7	Sucrose
17	0.6	5.4	Sucrose
18	2.4	5.4	Sucrose
19	0.6	10.8	Sucrose
20	0.6	10.8	Sucrose
21	1.2	10.8	Sucrose
22	0.6	2.7	Sorbitol
23	0.6	2.7	Sorbitol
24	1.2	2.7	Sorbitol
25	1.2	2.7	Sorbitol
26	0.6	5.4	Sorbitol
27	0.6	5.4	Sorbitol
28	2.4	5.4	Sorbitol
29	1.2	10.8	Sorbitol
30	2.4	10.8	Sorbitol

Take Home Message

- Effect of gelling agent on nutrient accessibility
- Carbohydrates are taken up and used in different forms
- Temporal measurement of plant nutrient content led to creation of superior basal salt combination

Questions!?

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