Salt Toxicity in Grapes

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Scope of Soil Salinity

- **4.5 million acres worldwide**
- **10,000's of acres in CA**
  (Ag Sci and Tech, 1996)
- **29-43% surface soils in Paso Robles area**
- **(Mark Battany)**

Modified from Presentation by Kevin Fort

A History of Salt and War

<u>The Salting of Carthage (146 BCE)</u>

- ‘Salting the Earth’ has been an idiom for societal destruction for millennia.
- Although widely disproven at this point, the salting of Carthage remains as a legend comparing the elimination of the city of Carthage during the Third Punic Wars (146 BCE).

<u>Palestrina (1299 CE)</u>

- Following the Carthaginian example, Pope Boniface VIII ordered the plowing and salting of Palestrina in 1299 CE.

Current role of Salt

Uses

Salts are used widely in our society

- Icing control
- Cleaning
- Food

Definition

Acid + Base = Neutral Salt

- Easily soluble in water
- Separate easily and bind readily

Issues

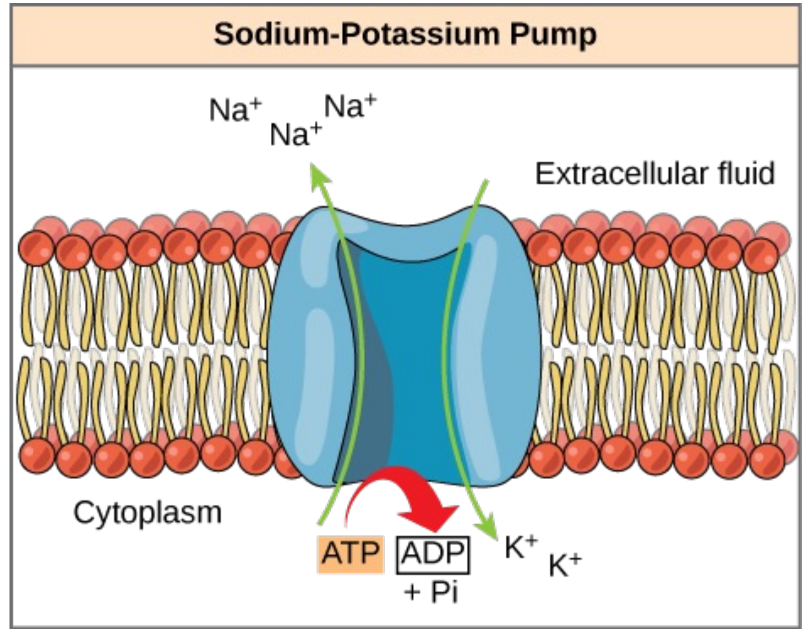
Over use of some salts in fertilization or cleaning can become an issue

- Most commonly, NaCl (sodium chloride) is the salt which causes agricultural issues
- Recycling of winery wastewater for vineyard irrigation = excessive chloride levels in vineyard soils.

Sodium and Potassium

Na^+ and K^+

1. **Similar radius**
   - Na^+ = 227 pm
   - K^+ = 280 pm
2. **Similar charge**



Malignant hyperthermia: A runaway thermogenic futile cycle at the sodium channel level. *Advances in Bioscience and Biotechnology*, *05*, 197–200.

Sodium toxicity in Grapevine

Chloride toxicity in grapevine

Ismail, A. (2013). *Grapes for the Desert: Salt Stress Signaling in Vitis*. Thesis (fig. 8)

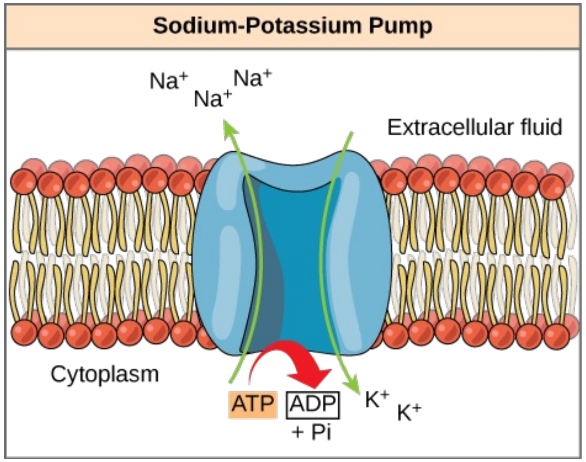
Potassium deficiency symptoms: Photo by Mardi L. Longbottom; AWRI (Australia)

Sodium and Potassium

Grapevine *HKT 1;1*

- The *High-affinity Potassium Transporter* protein in grapevines selects only for potassium.
- But it can be fooled by Sodium
  - (Henderson et al. 2014)

- Cl^- becomes toxic before Na^+ in grapevines and a few other perennial crops



Malignant hyperthermia: A runaway thermogenic futile cycle at the sodium channel level. *Advances in Bioscience and Biotechnology*, *05*, 197–200.

What's the problem?

- Sodium = "Imposter" for entry into plant

- Similar for chlorine and nitrate

Sodium toxicity in Grapevine

Chloride toxicity in grapevine

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Potassium deficiency symptoms: Photo by Mardi L. Longbottom; AWRI (Australia)

What about Chlorine?

Cl^- and NO_3^-

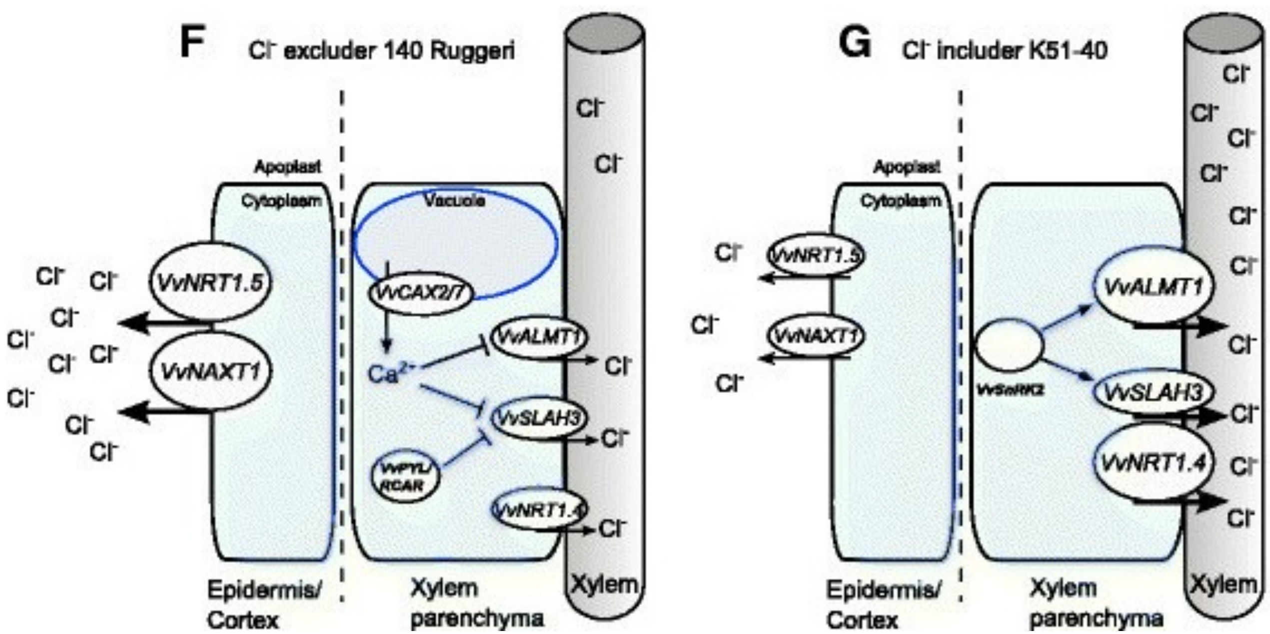
- Similar radius
  - Cl^- = 175 pm
  - NO_3^- = 179 pm

- Similar charge and similar problems as K^+ and Na^+

A difference in action

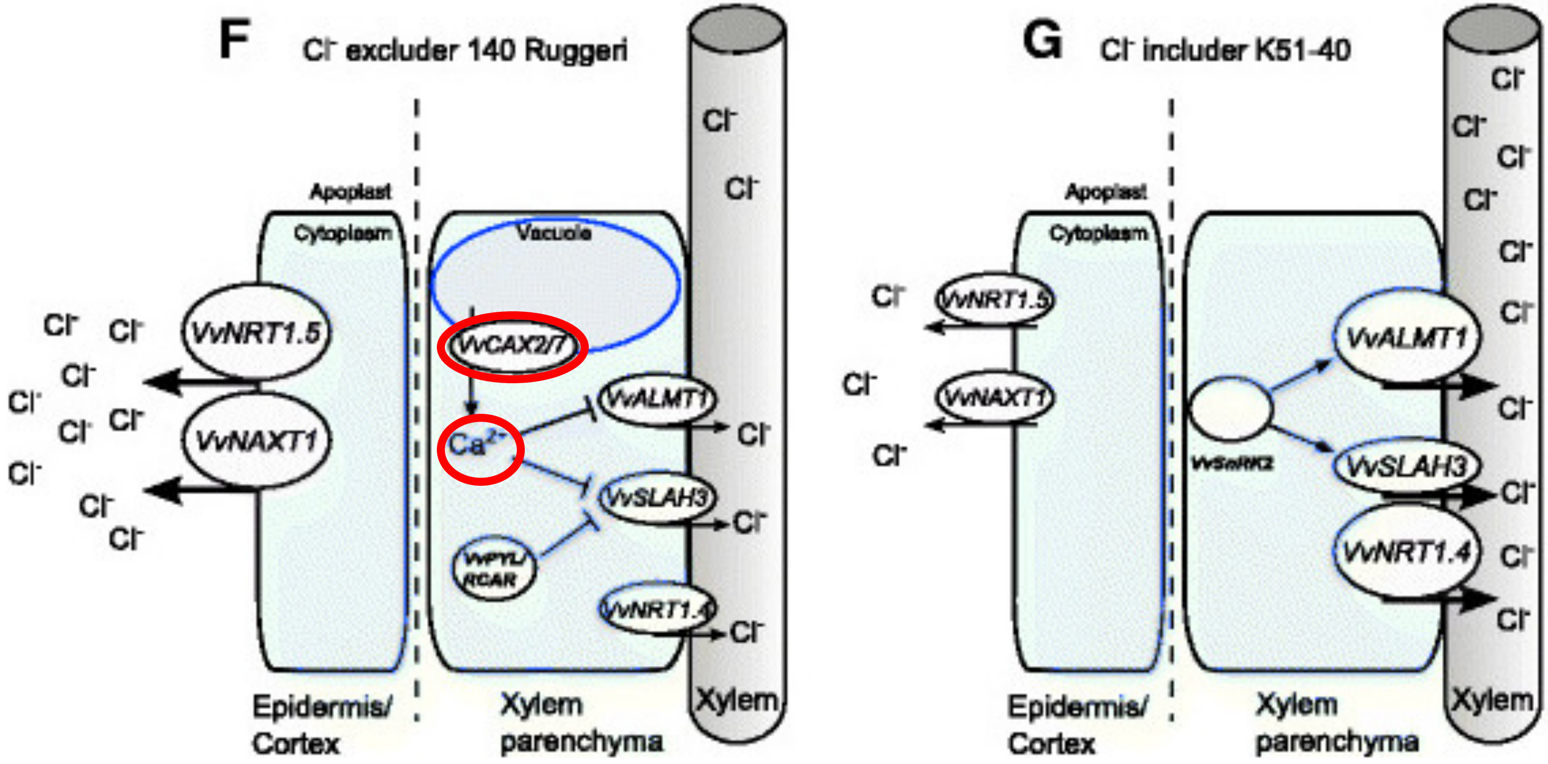
- Salt tolerance in grapevine is. associated with chloride exclusion from shoots.

- Differences between varieties partially arise in the limiting of Cl^- passage between root symplast and xylem apoplast.



Henderson et al. BMC Plant Biology 2014

The *(partial)* model for complex control of salt tolerance



Henderson et al. BMC Plant Biology 2014

What does NaCl do to photosynthesis?

	Applied [NaCl]							
[NaCl]	E $mol * m^{-2} * s^{-1}$		A_{net} $mol * m^{-2} * s^{-1}$		CO_2 $mol * mol^{-1}$		g_s $mol * m^{-2} * s^{-1}$	
0 mM	0.002 ± 0.0004	a	9.10 ± 0.9	a	220.45 ± 14.8	a	0.13 ± 0.03	a
25 mM	0.002 ± 0.0003	a	9.45 ± 0.9	a	216.71 ± 12.4	a	0.12 ± 0.02	a
75 mM	0.001 ± 0.0001	b	6.41 ± 0.7	b	177.54 ± 11.2	a	0.06 ± 0.01	b
100 mM	0.0009 ± 0.0001	b	6.35 ± 0.6	b	169.37 ± 11.3	a	0.05 ± 0.01	b
p value		< 0.001*		< 0.001*		0.573		< 0.001*

Table 2.5. Photosynthesis based on LiCOR measurements

With little [NaCl] applied there are no differences in PS from unsalted vines.

However, at [NaCl] ≥ 75 mMol (≈ 7.5 dS * m^{-1}), stomatal conductance, transpiration, and carbon assimilation rates can drop by half.

This result makes sense when you consider that 40 mMol NaCl (≈ 4.0 dS * m^{-1}) is the widely accepted threshold for defining a soil as 'sodic'

Salt and Drought

Similarities

- Initial stages of NaCl toxicity ≈ Drought induced stress
  - Drop in SWP, stomatal conductance, & transpiration rates

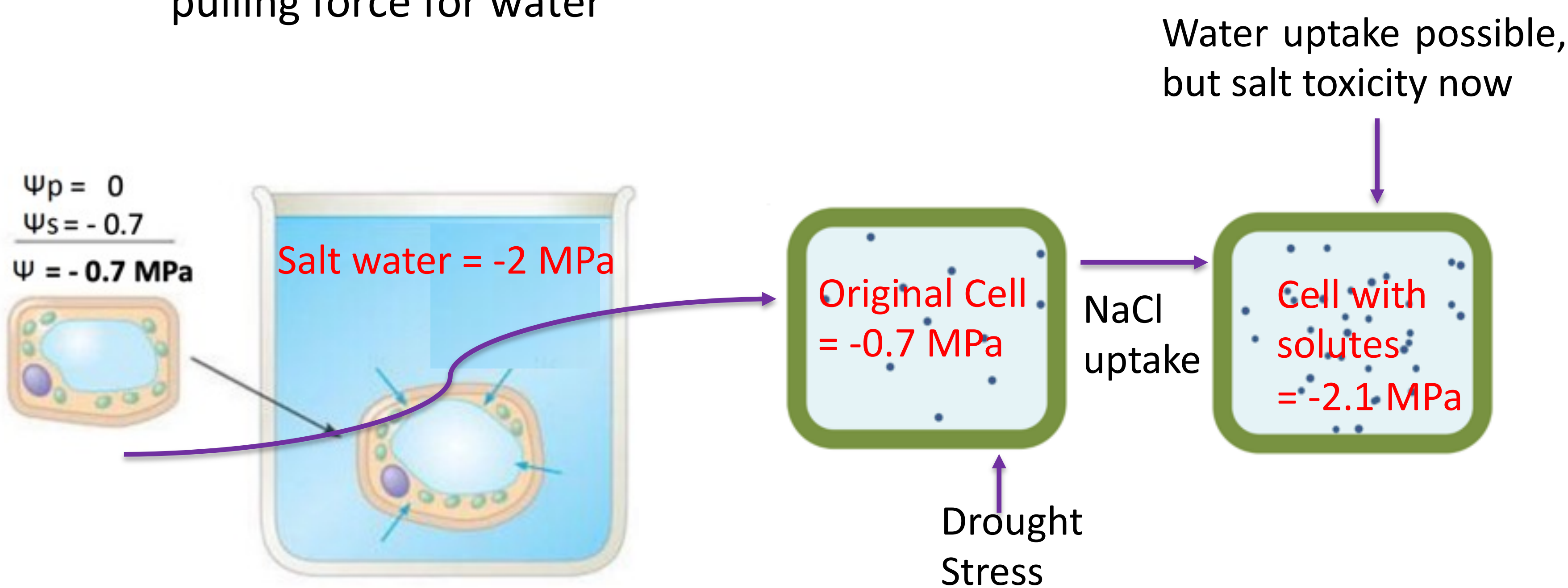
- **Why salt-induced stress differs**
  - Two-stage responses:
    1. Osmotic shock ≈ drought stress
    2. Toxicity: accumulation of salt ions results in toxic response from the plant

Water Potentials and Solutes

Osmotic potential

- Cells are filled with dissolved solutes
  Creates a concentration gradient that attracts water molecules

- A higher concentration = a stronger pulling force for water



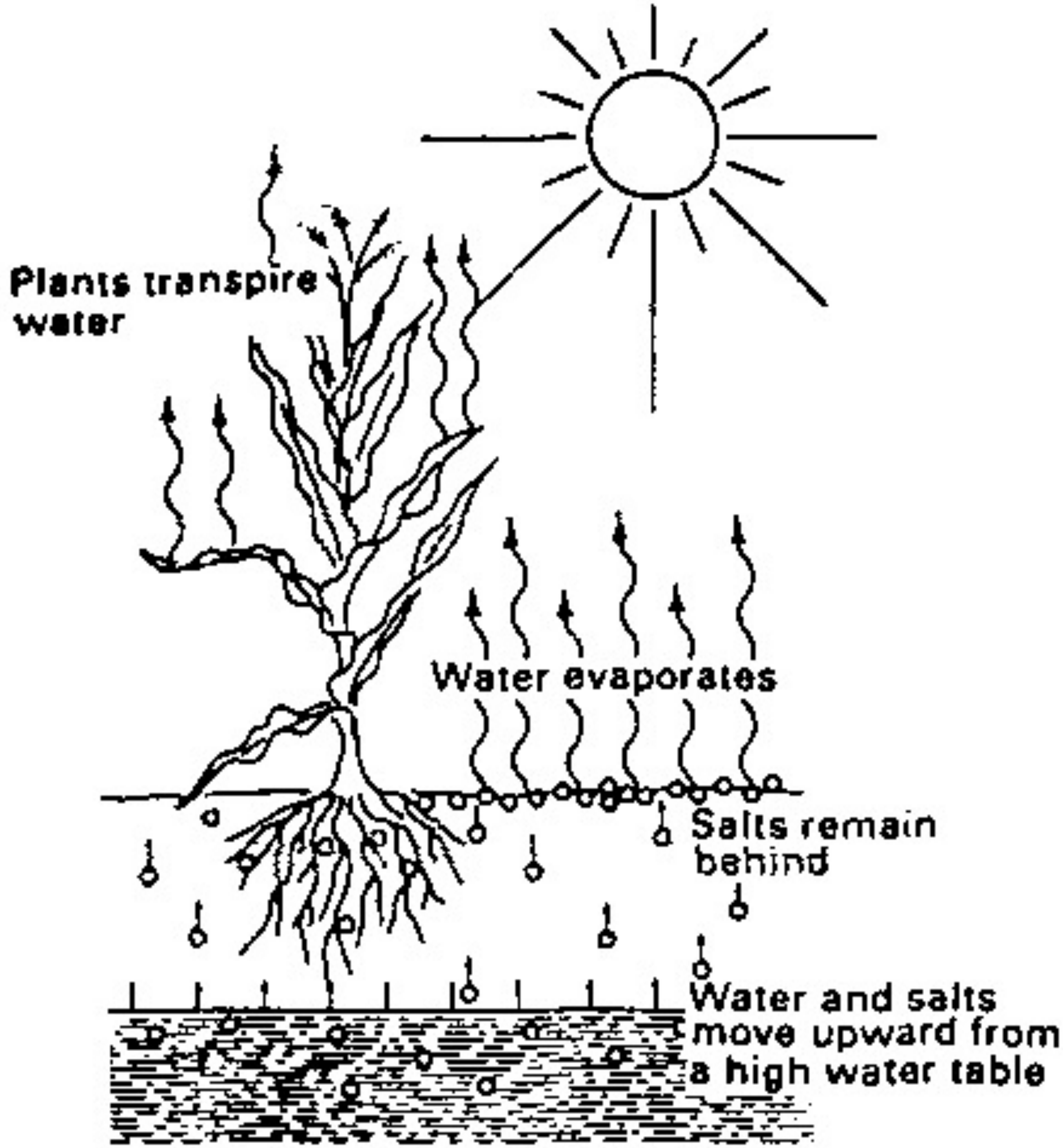
Salt and Drought

How are they related?

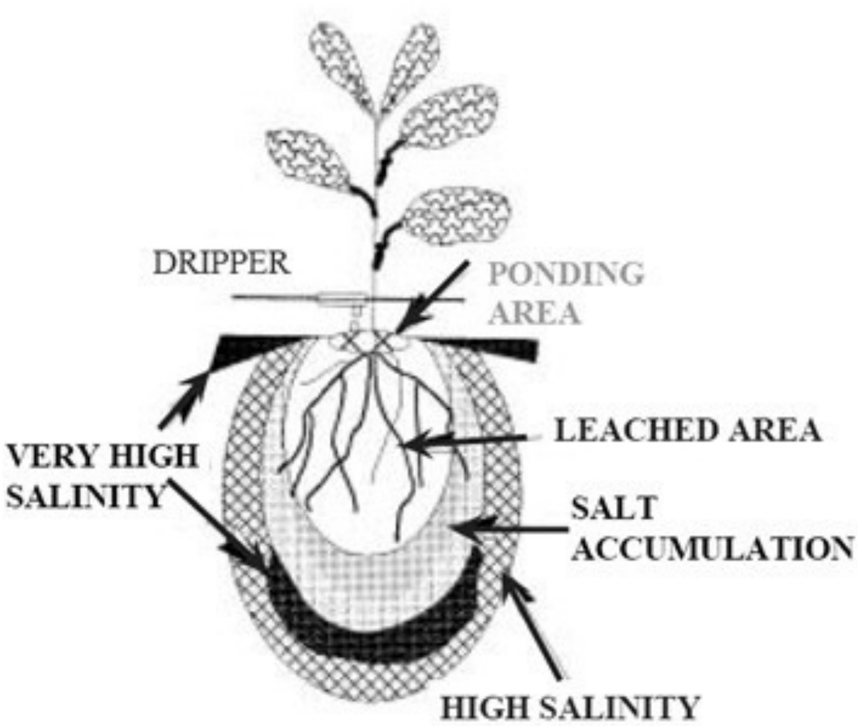
- Drought often leads to less available water
  - Less water for irrigation
  - Drip and micro-sprinkler irrigation often take the place of traditional, ‘high- input’ irrigation methods

- These developments often lead to both drought and salt –induced stresses

Salt sources



Salt patterns



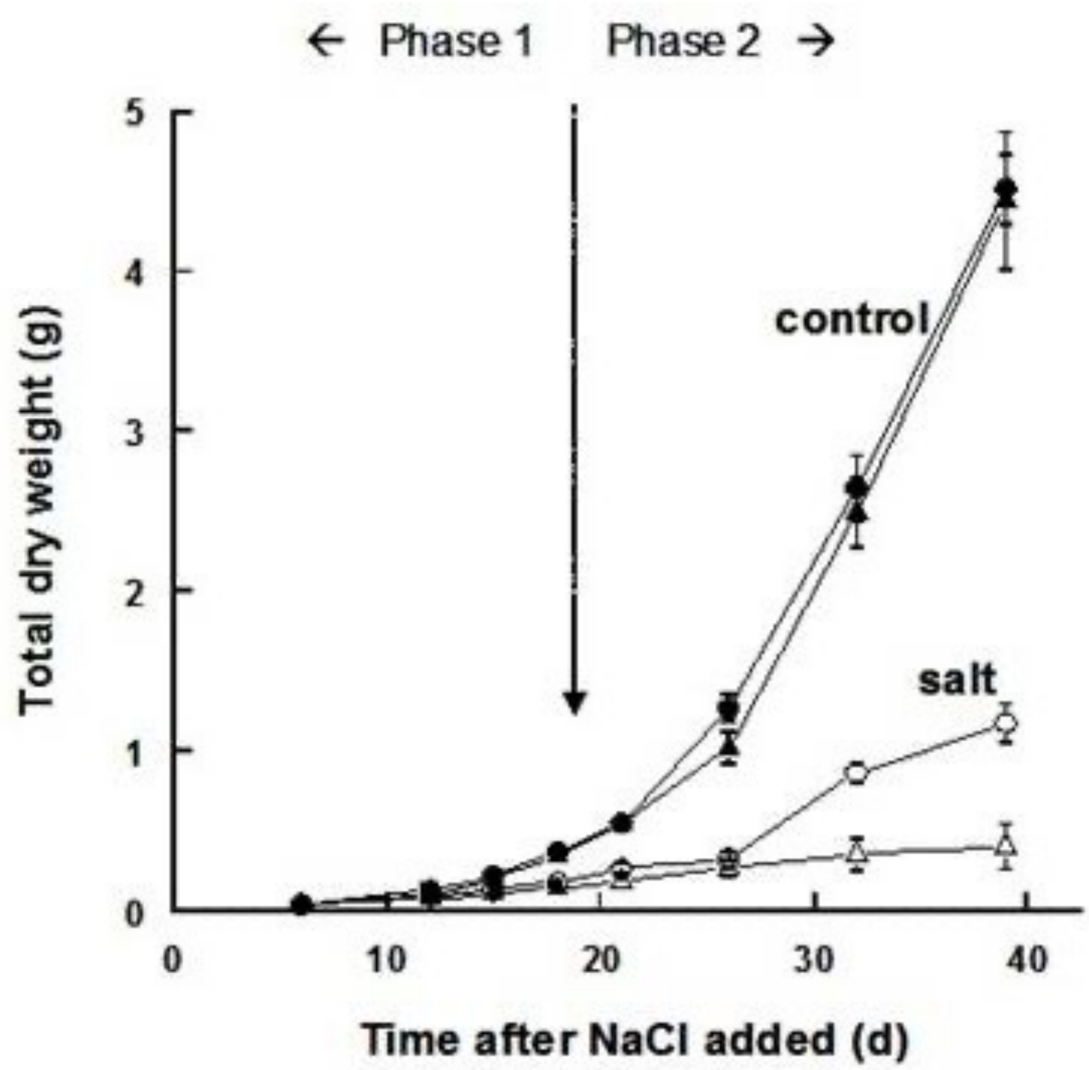
Drip irrigation

- A relatively new practice
- Flood irrigation (previously)

Flood Irrigation

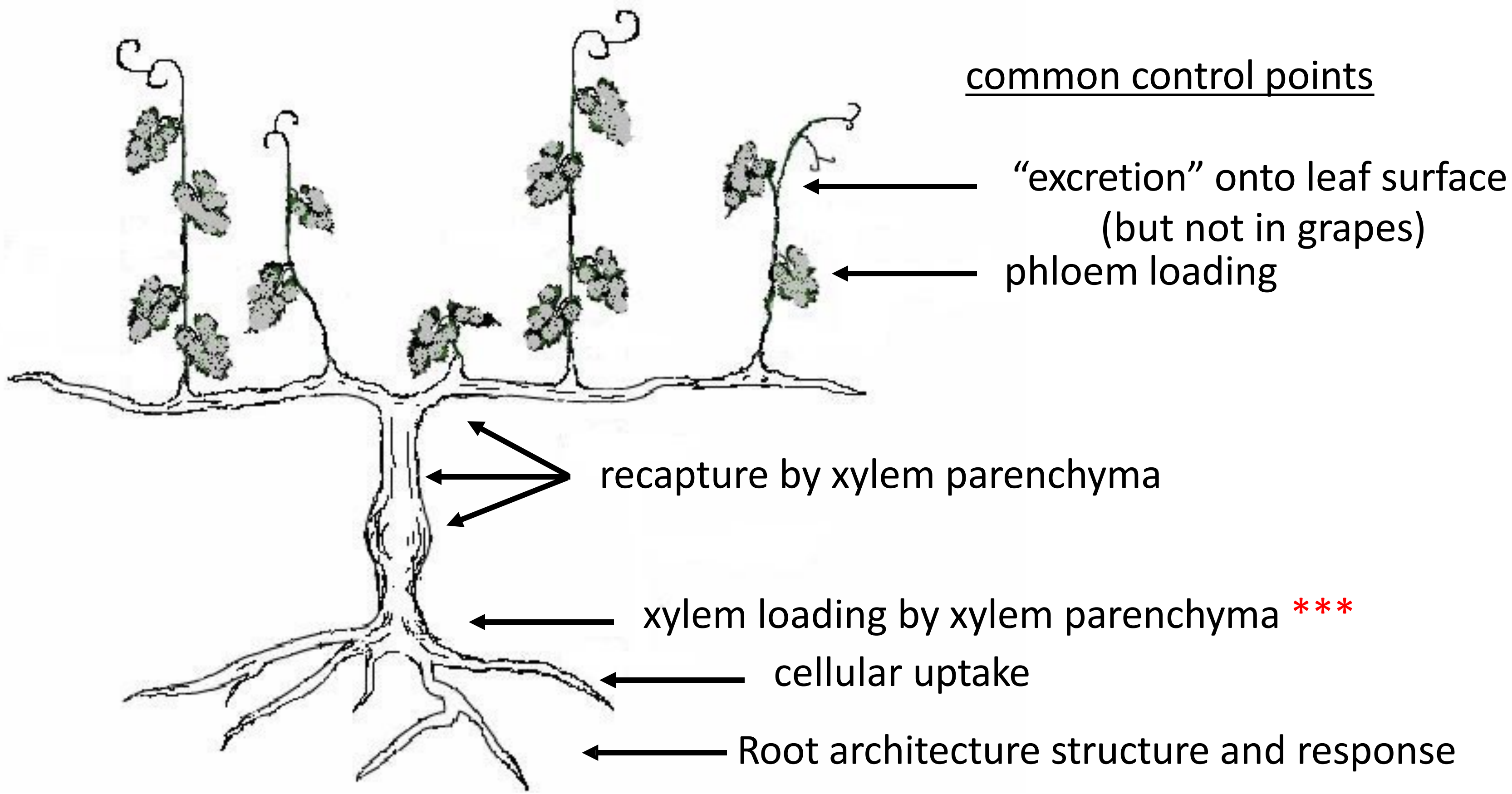
- Salinity accumulation less of a problem with flood
  - Why?
  - Natural flooding controlled for now

NaCl is a problem of accumulation



R. Munns et al. 2002

What exactly is salt tolerance?



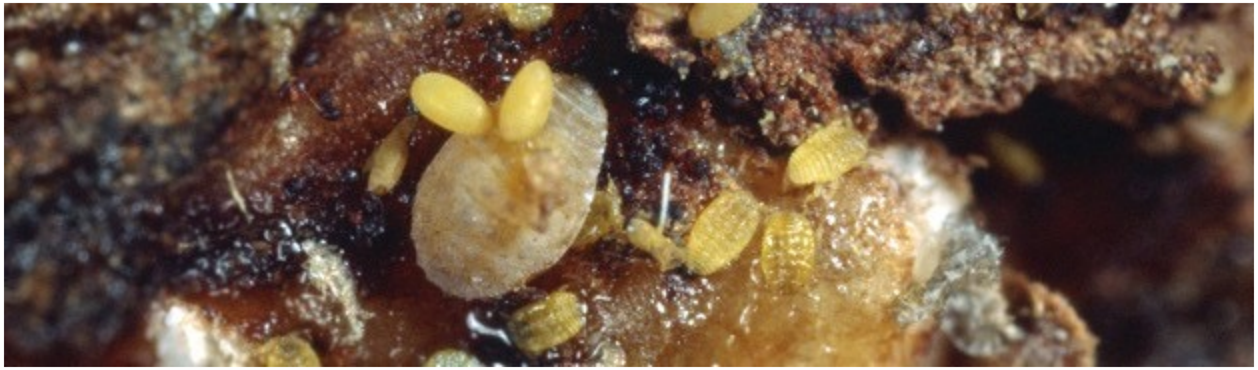
Answer: A complex trait, composed of exclusion, recapture, excretion, and avoidance

Mangroves

- Mangroves are possibly the most salt-tolerant perennial plant on land.
- By sequestering NaCl in roots and shedding the salt-laden roots they have been nicknamed “the walking trees”

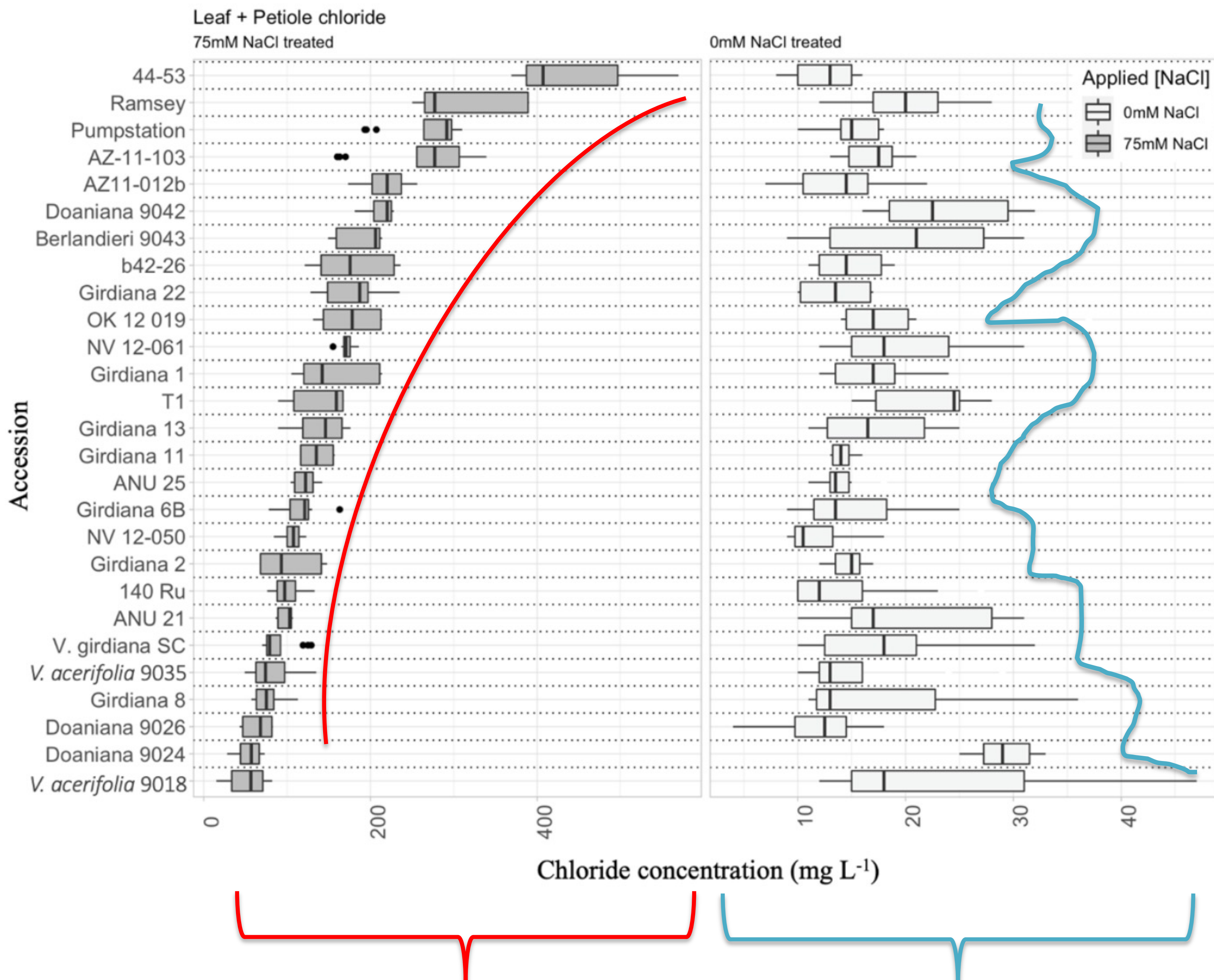

Rootstocks!

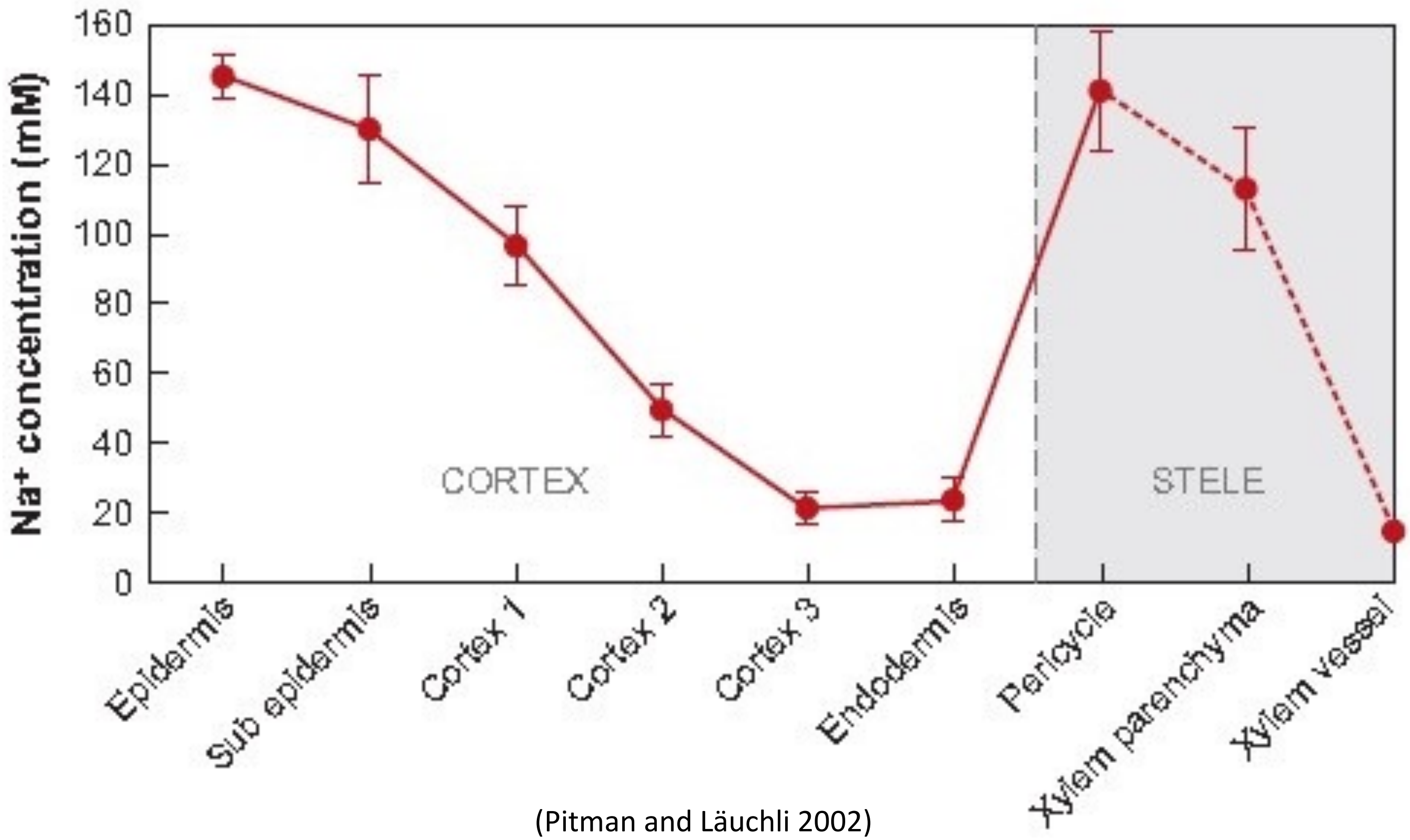


Phylloxera image source: UC ANR IPM

Rootstock	Vitis parentage	Phylloxera resistance	Nematode Resistance		Tolerance				Influence on scion		Soil adaptation	Ease of propagation	Other characteristics
			Root knot	Dagger (*Xiphinema index*)	Drought	Wet soil	Salinity	Lime	Vigor	Mineral nutrition[1]			
Riparia Gloire	riparia	High	Low	Med.	Low	Low	Med.	Low	Low–med.	N, P: low K, Mg: low–med.	Deep, well-drained, fertile, moist soils	High	Early maturation; scions tend to overbear
St. George (*Rupestris du lot*)	rupestris	High	Low	Low	Low–med. in shallow soils; high in deep soils	Low–med.	Med.–high	Med.	High	N: high P: low on low-P soils, high on high-P soils K: high	Deep soils	High	Fruit set problems with some scions; latent virus tolerant
SO4 (Selection Oppenheim)	berlandieri × riparia	High	Med.–high	Low–med.	Low–med.	Med.–high	Low–med.	Med.	Low–med.	N: low–med. P: med. K: med.–high Mg: med.	Moist, clay soils	Med.	Noted as a cool-region rootstock
5BB (Kober)	berlandieri × riparia	High	Med.–high	Med.	Med.	Low	Med.	Med.–high	Med.	N: med.–high P, K, Zn: med. Ca, Mg: med.–high	Moist, clay soils	High	Susceptible to phytophthora root rot; adapted to high-vigor varieties
5C (Teleki)	berlandieri × riparia	High	Med.–high	Low–med.	Low	Low–med.	Med.	Med.	Low–med.	N: low P, K: med. Mg: med.–high Zn: low–med.	Moist, clay soils	High	—
420A (Millardet et de Grasset)	berlandieri × riparia	High	Med.	Low	Med.	Low–med.	Low	Med.–high	Low	N, P, K: low Mg: med. Zn: low–med.	Fine-textured, fertile soils	Med.	Scions tend to overbear when young
99R (Richter)	berlandieri × rupestris	High	Med.–high	Low–med.	Med.–high	Low	Med.	Med.	Med.–high	P: med. K: high Mg: med.	Tolerant of acid soil	Med.	Young scions may develop slowly
110R (Richter)	berlandieri × rupestris	High	Low–med.	Low	High	Low–med.	Med.	Med.	Med.	N: med. P: high K: low–med. Mg, Zn: med.	Hillside soils; acid soils	Low–med.	Develops slowly in wet soils

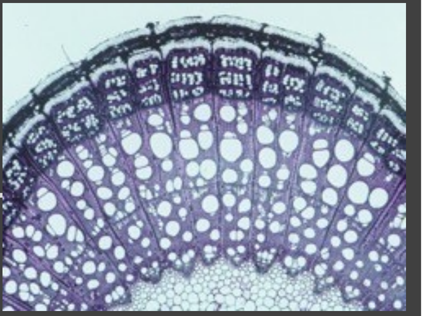


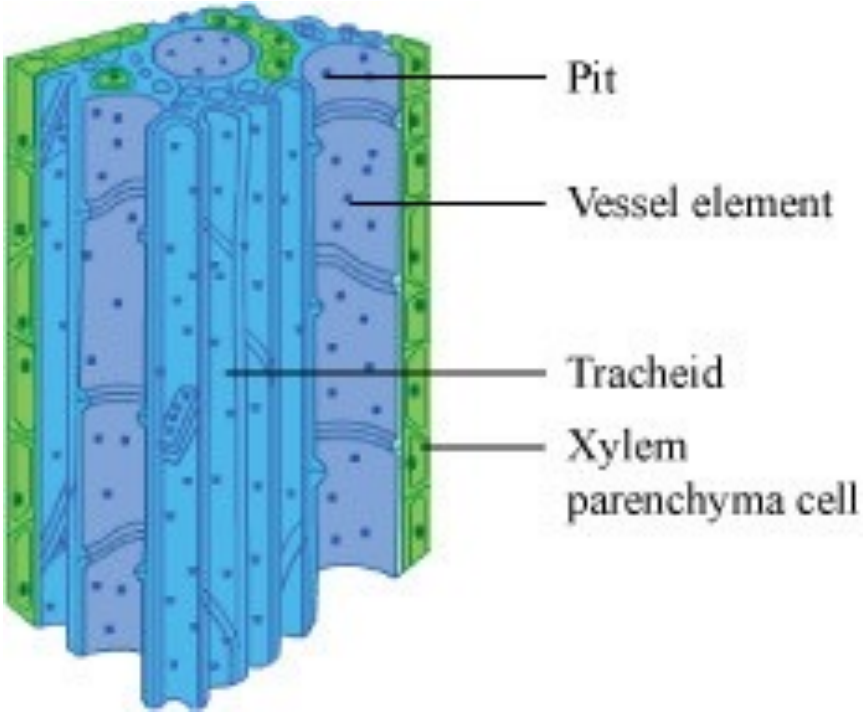
Difference in scale

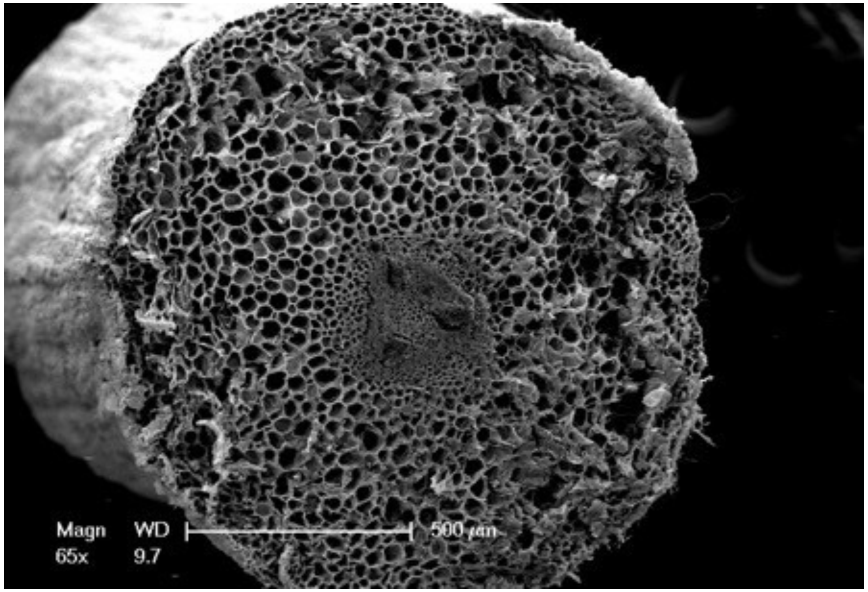


(Pitman and Läuchli 2002)

Xylem parenchyma regulation







Xylem parenchyma

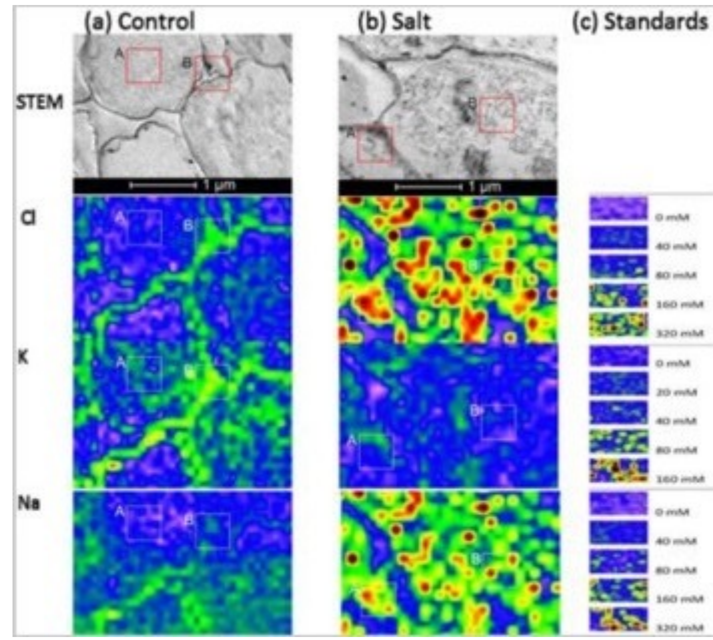
Elemental Compartmentalization

Ion Subcellular Compartmentalization:

- Cl^-, Na^+, K^+
- (Ca^{2+} and Mg^{2+})

TEM – EDX

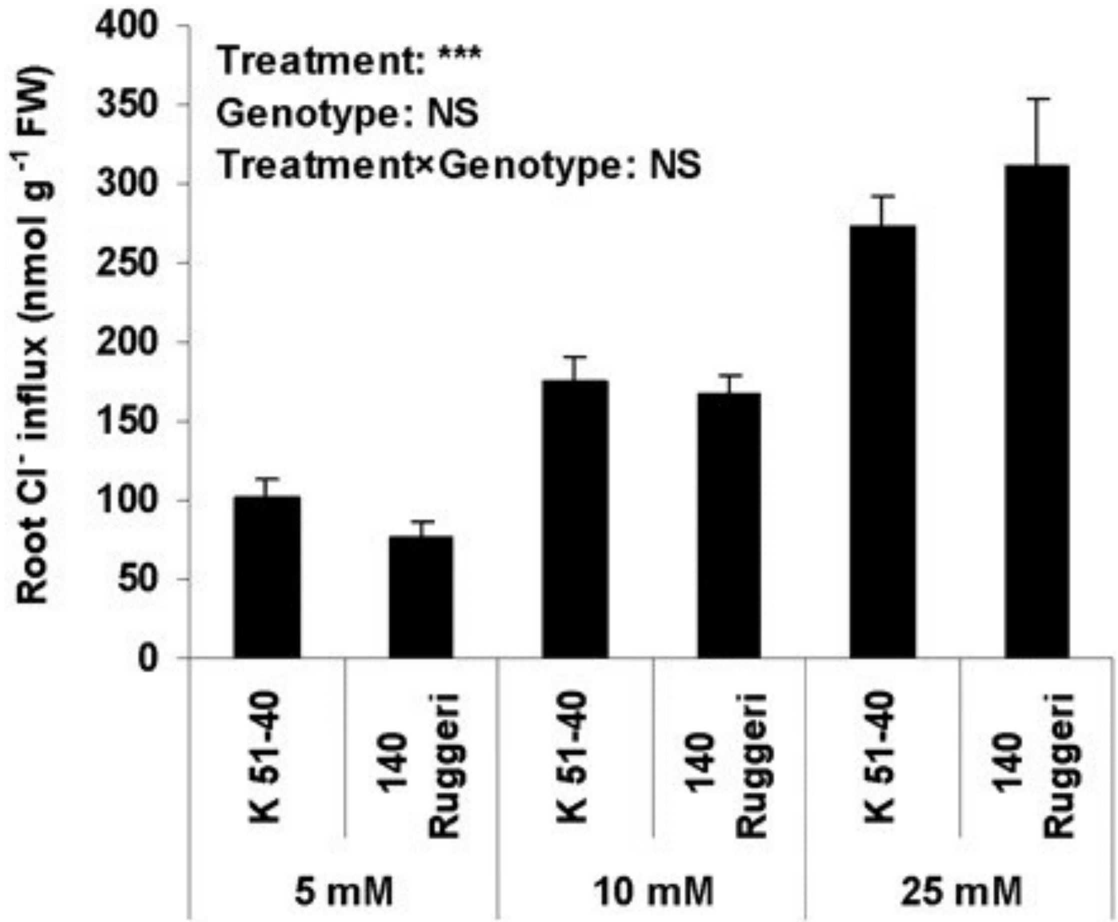
- Analyze and quantify ion content in the different root cell-types at subcellular level



TEM-EDX imaging of elemental ion composition in cortical cells of *P. euphratica. Credit: (Chen et al. 2014)*

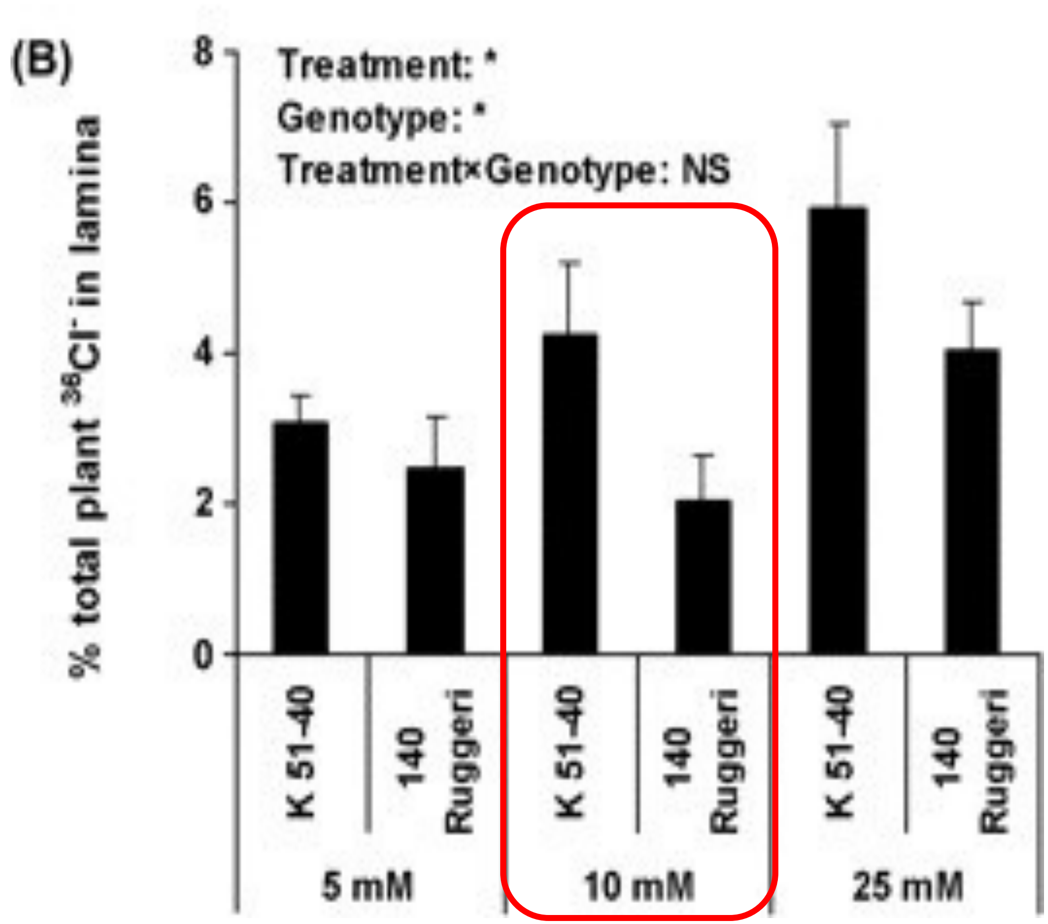
Previous work: Xylem parenchyma regulation model

Part I:
No differential exclusion at the level of chloride uptake into the root



Gong et al. 2011

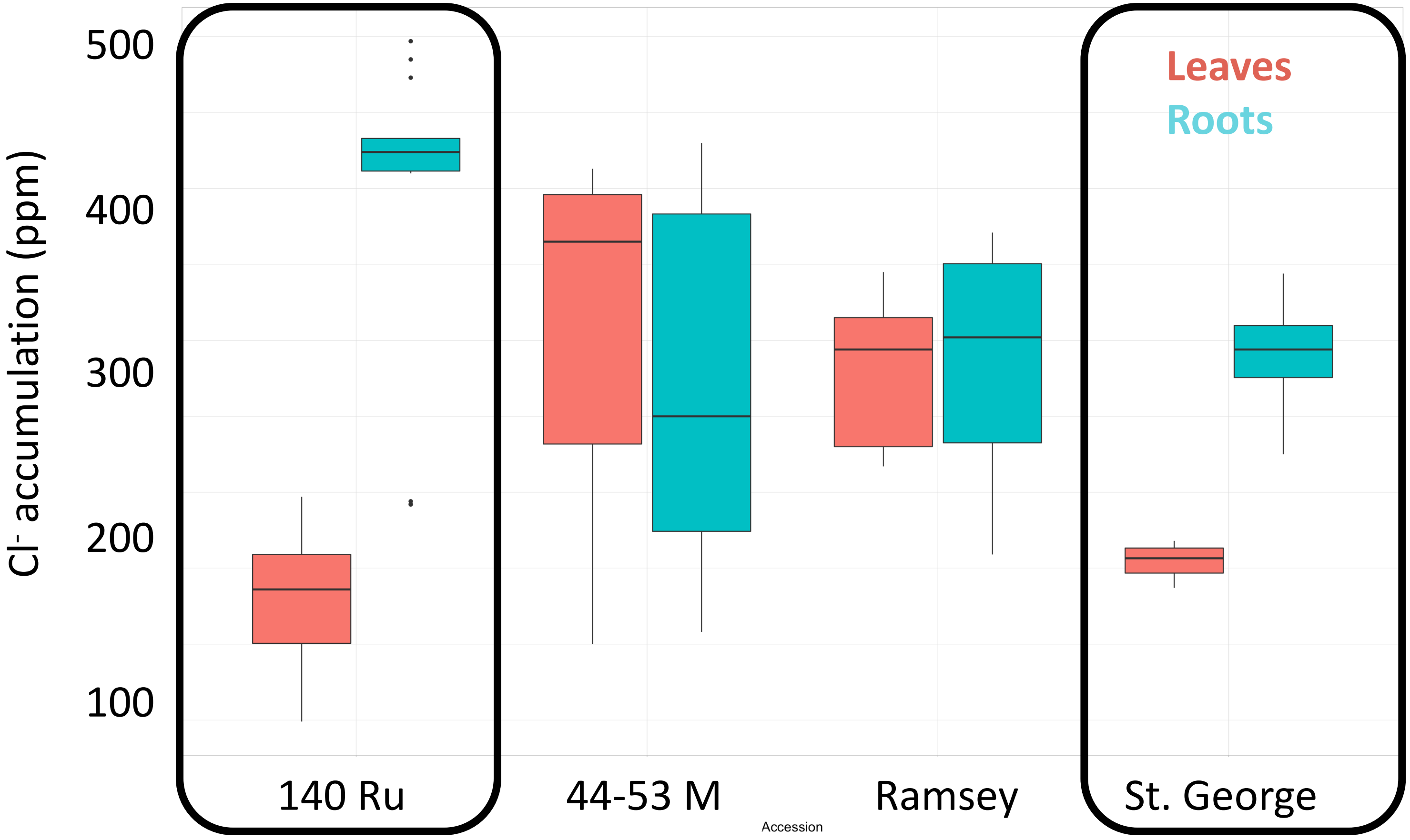
Part II: Differential exclusion *was* observed at the level of chloride translocation from root to shoot



Gong et al. 2011

More Evidence

- During my PhD trials, I found evidence for this delineation also
- As in the previous slides, more delineation was observed in Leaf + Petiole chlorine levels than in Roots.



- When separated into Post-hoc groups:
  - Leaf and petiole tissue under 75 mM NaCl
    - 10 distinct groups
  - Root tissue chloride
    - One group

- The trait of salt-tolerance in grapevines likely occurs during the **long-distance transport** of chloride from root to shoot.

- Continuous variability suggests Cl-tolerance may be a **complex trait**

	Leaf and Petiole tissue $[Cl^-]$ 75 mM NaCl		Root tissue $[Cl^-]$ 75 mM NaCl	
Accession	$[Cl^-](mg \cdot L^{-1})$	Post hoc	$[Cl^-]$ $(mg \cdot L^{-1})$	Post hoc
18113-077	98.00 ± 28.2	a	327.67 ± 82.36	a
18113-008	85.67 ± 26.2	ab	257.5 ± 91.3	a
18113-055	83.75 ± 27.3	abc	286.42 ± 70.6	a
18113-038	81.59 ± 19.7	abcd	366.00 ± 42.4	a
18113-018	78.67 ± 20.3	abcde	294.75 ± 34.5	a
18113-046	76.42 ± 12.2	abcdef	298.92 ± 26.2	a
18113-058	75.83 ± 35.7	abcdefg	355.83 ± 122.9	a
18113-076	73.67 ± 21.1	abcdefgh	345.75 ± 42.5	a
GRN3	67.67 ± 26.4	abcdefghi	145.5 ± 27.8	a
18113-048	66.5 ± 22.6	abcdefghi	256.17 ± 45.2	a
18113-043	47.83 ± 15.6	bcdefghij	371.08 ± 93.1	a
18113-026	36.09 ± 8.8	cdefghij	234.92 ± 58.9	a
18113-007	33.58 ± 5.2	defghij	228.92 ± 97.0	a
18113-027	32.34 ± 7.5	efghij	404.92 ± 127.9	a
18113-051	28.67± 5.8	fghij	265.58 ± 61.6	a
18113-034	26.92 ± 4.4	ghij	340.67 ± 74.6	a
18113-024	26.42 ± 6.1	hij	288.67 ± 37.3	a
18113-001	23.99 ± 6.8	ij	322.67 ± 96.9	a
V. acerifolia '9018'	15.92 ± 1.6	j	165.5 ± 34.5	a
p value	2.20^{-16} ***		0.054	

Table 1.4. Accumulated leaf + petiole combined tissue, and root tissue, Cl^- concentration at harvest following 21 day application period for 75 mM NaCl applied treatment; representative accessions from each Tukey posthoc group

Grapevine Breeding for Salinity Tolerance: a potential solution

Why does salinity pose such a difficult problem for plant breeders?

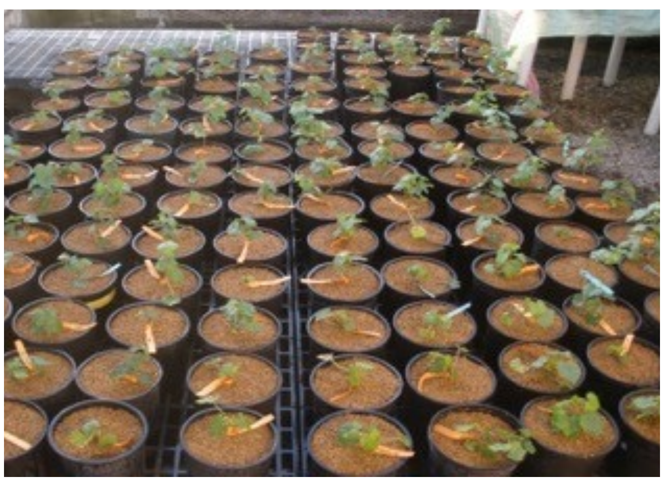
T.J. Flowers *, S.A. Flowers

Developing a rapid & reliable assay




Propagation

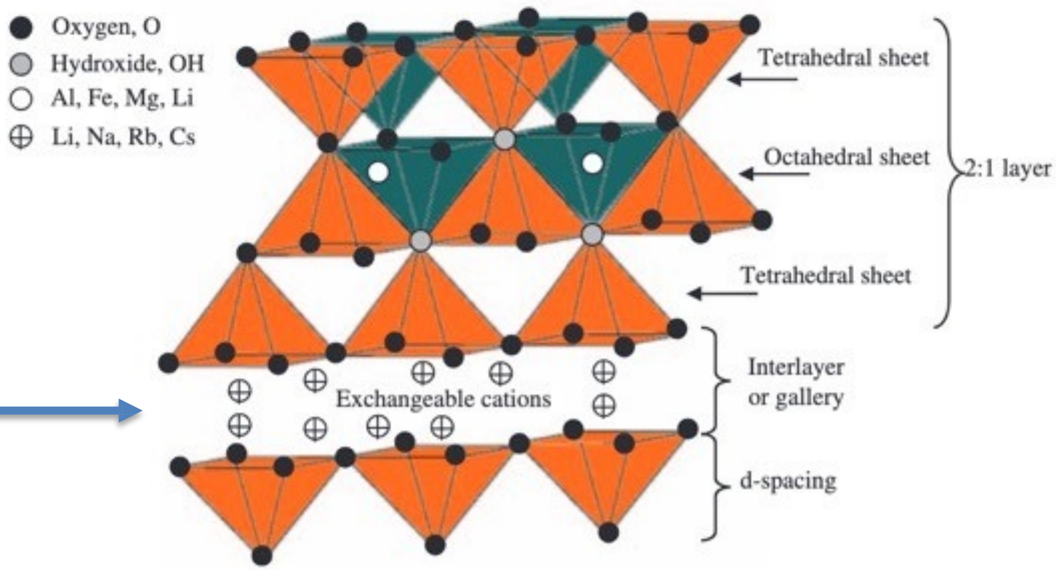
- Type of propagule:
  - Hardwood
  - Herbaceous
- This affects the uptake of the cuttings
  - Carbohydrate reserves differ
  - Only herbaceous cuttings are actively growing at time of excision
- Does this limit us to testing during growing season?



Fritted-clay

Medium: Fritted Clay

- Calcined, non-swelling illite clay
  - 2:1 layer silicate
- Porosity
  - 74% total (39% micro;35% macro)
  - Bulk Density = 600 kg • m^{-3}
- CEC
  - 30 ± 5 mEq • 100 g^{-1}
- pH range: 5.5 - 7.5
- Helps prevent losses to hydraulic conductivity while retaining nutrients
- Potassium retainer
  - (K+ trapped in interlayer between tetrahedral layers)



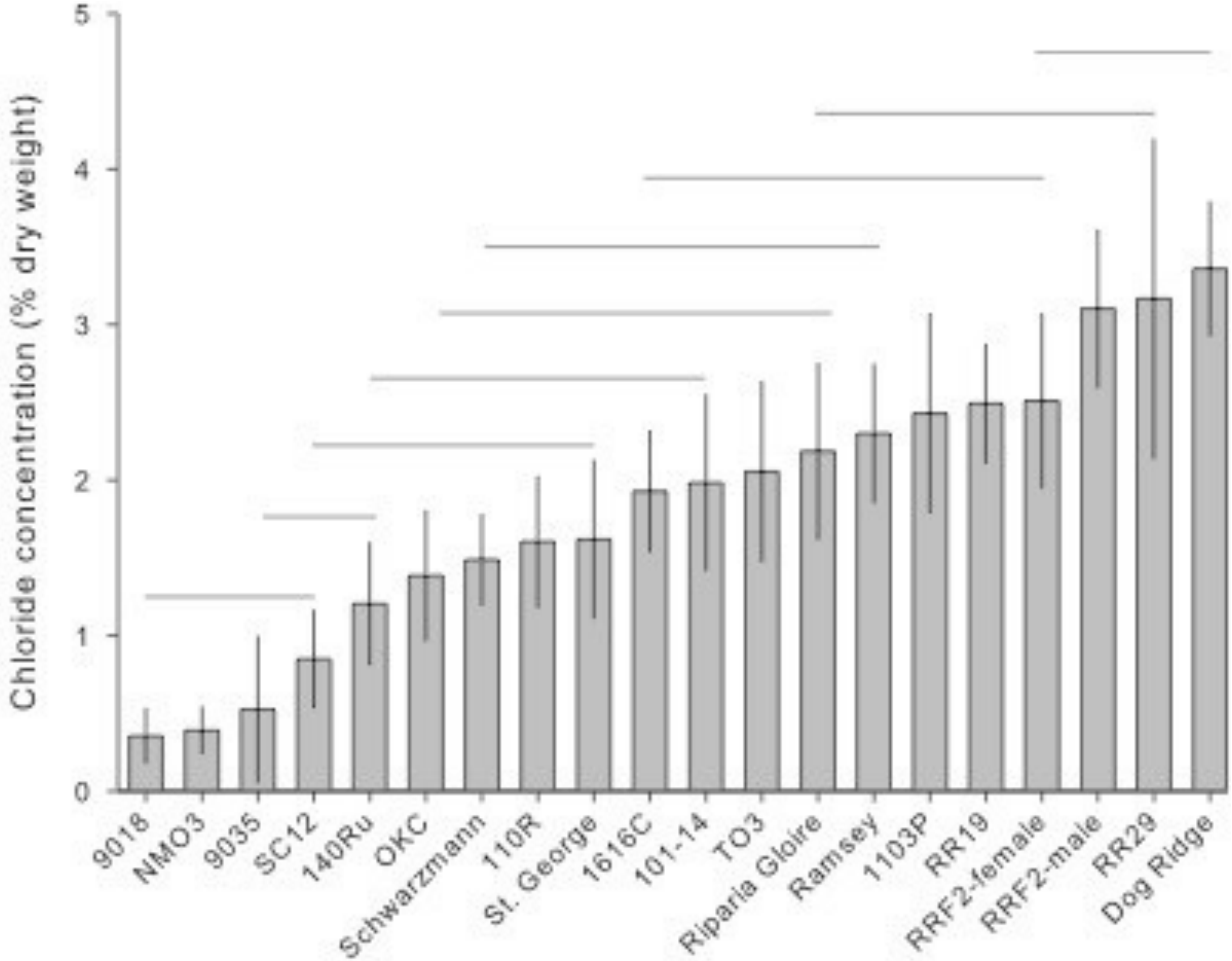
Multi-stage screening for chloride exclusion

1. Greenhouse acclimation
2. Growth and root development
3. NaCl application

Consistent methodology provides more consistent results across trials

Leaf margin burn and nutrient deficiency symptoms

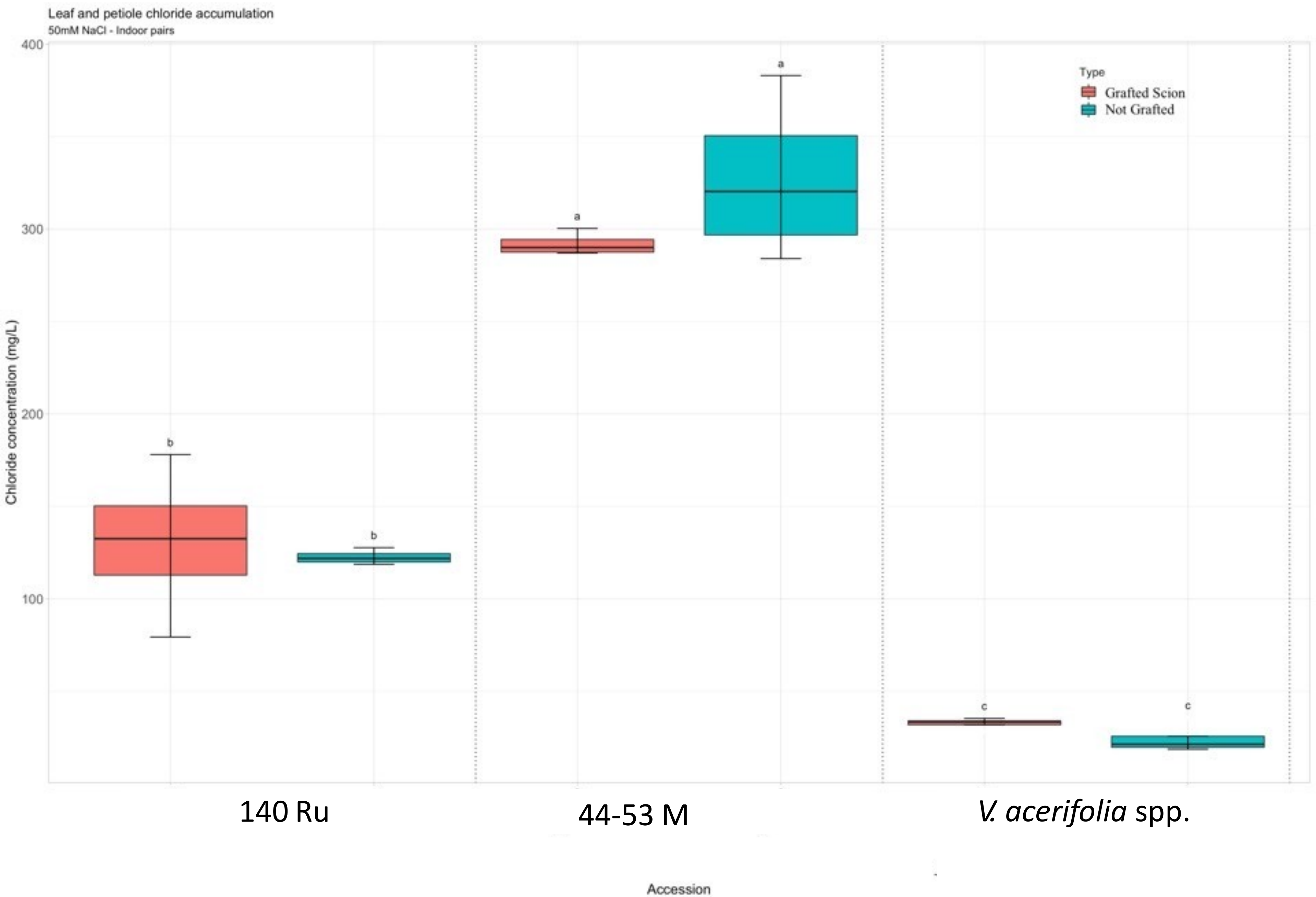
Chloride concentration in leaves (% dry weight)



Applying salt tolerance to scions

Looking for new, salt-tolerant rootstocks is great but:

1. Can they impart their tolerance to a grafted scion?
2. Tandem Indoor and outdoor grafted trials conducted to explore this



Leaf and petiole chloride accumulation
50mM NaCl - Indoor pairs
Chloride concentration (mg/L)
400
300
200
100
a
a
b
b
c
c
Type
Grafted Scion
Not Grafted
140 Ru
44-53 M
V. acerifolia spp.
Accession

What’s next?

1. **Field Trials**
   - *Are they functional in a real-world setting?*

2. **Horticultural evaluation**
   - *Yield, quality, and consistency*

3. **Foundation Plant Service**
   - *Clean material needed*

4. **Nursery propagation**

Rootstock Recommendations

Strong Salt Excluders	140 Ru, Schwarzmann, St. George, 99R
Lower Potential Salt Exclusion (yield maintenance)	1103 P, 110 R
Poor Exclusion Potential (yield mostly maintained)	Ramsey (a.k.a. 'Salt Creek')
Poor Salt Excluders (yield reductions)	039-16, 44-53 M, Dog Ridge, V. vinifera (own roots)

- Some of these rootstocks may be difficult to find at a nursery
- Be sure to check that you're getting the rootstock you wanted
- Avoid *V. riparia*-based rootstocks in saline environments; yield declines
  - e.g. 101-14, 5C, Riparia 'Gloire'

Thanks for Listening

Contact me: codchen@ucanr.edu