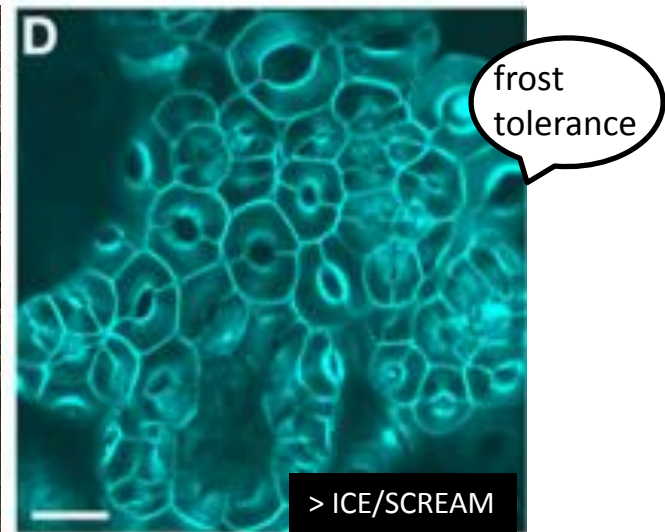
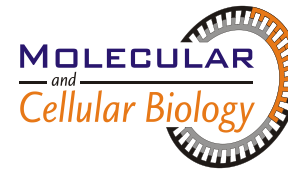


“Do grapes SCREAM for frost tolerance?”

Annette Nassuth

UNIVERSITY
of GUELPH



CCOVI Lecture series, February 12 ,2018

Grapevines in Canada



Vitis riparia

-40°C



Vitis vinifera

-15 to -20°C

► Winter damage may reduce *V. vinifera* yield up to 54%

Plants cannot move



► They need to adapt to their environment!

Freezing tolerance increases in autumn

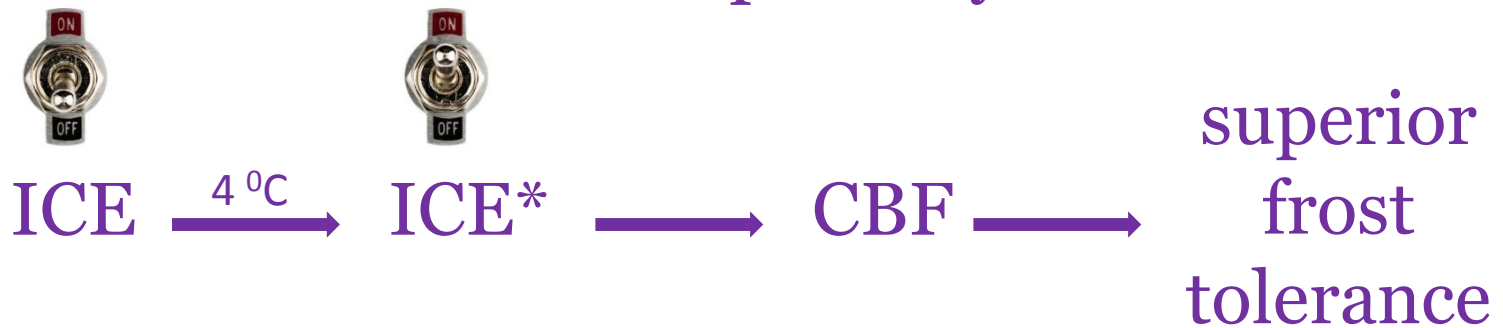
- Shorter days with low, non-freezing temperatures



The CBF pathway contributes to cold acclimation



CBF pathway



Grape: 4 genes

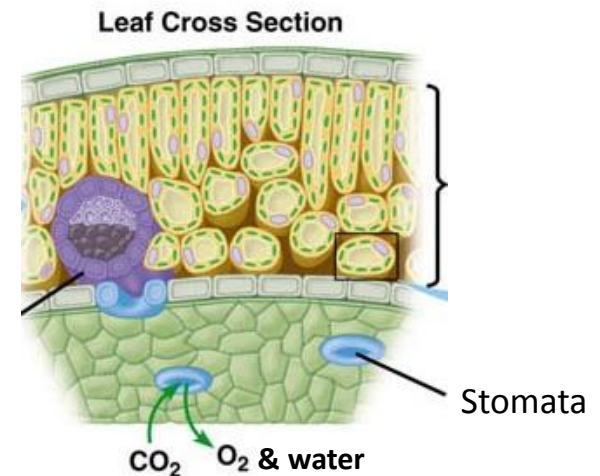
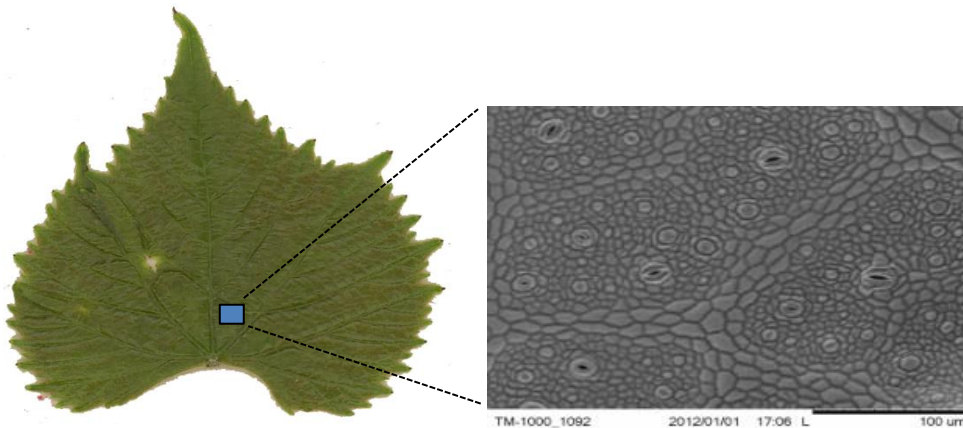
7 genes

ICE = Inducer of CBF Expression

ICE protein has dual function

ICE → frost tolerance

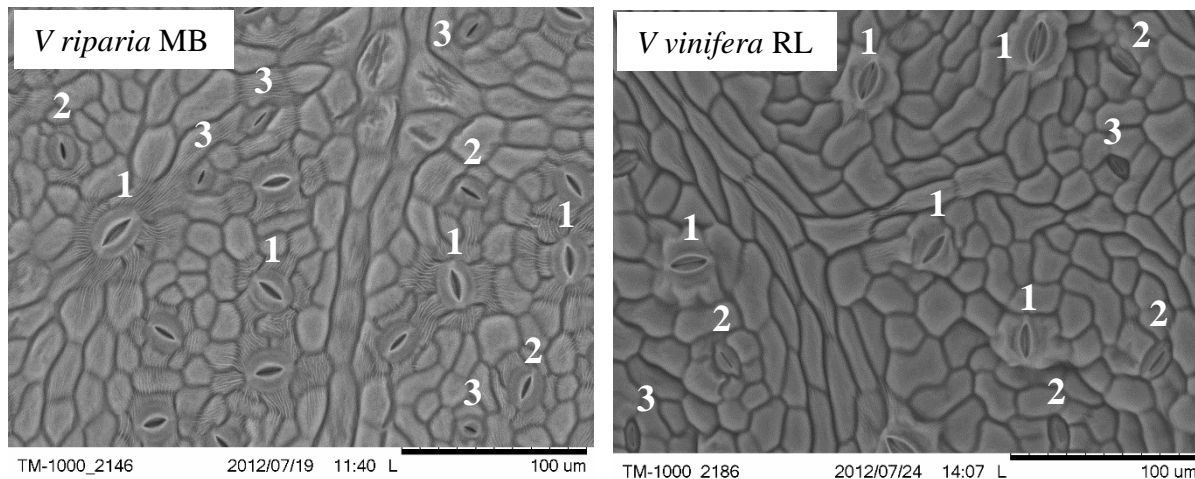
↘ stomata
balance photosynthesis and water loss



Is stomata development correlated to frost tolerance?

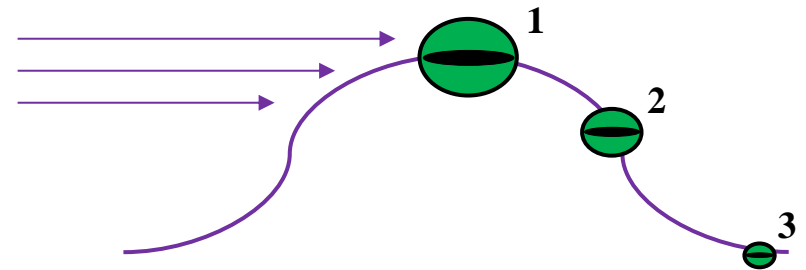
Grape leaves contain different types of stomata

► Grape has 3 types of stomata

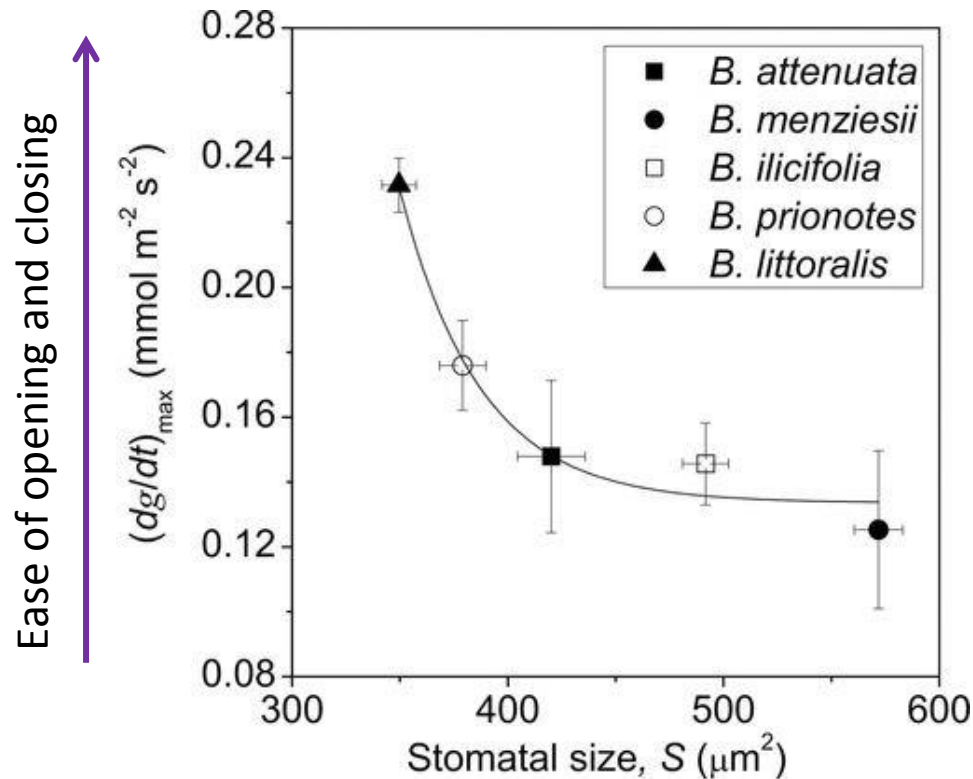


► Differ in size and position

► Sunken stomata are more protected from waterloss due to airflow

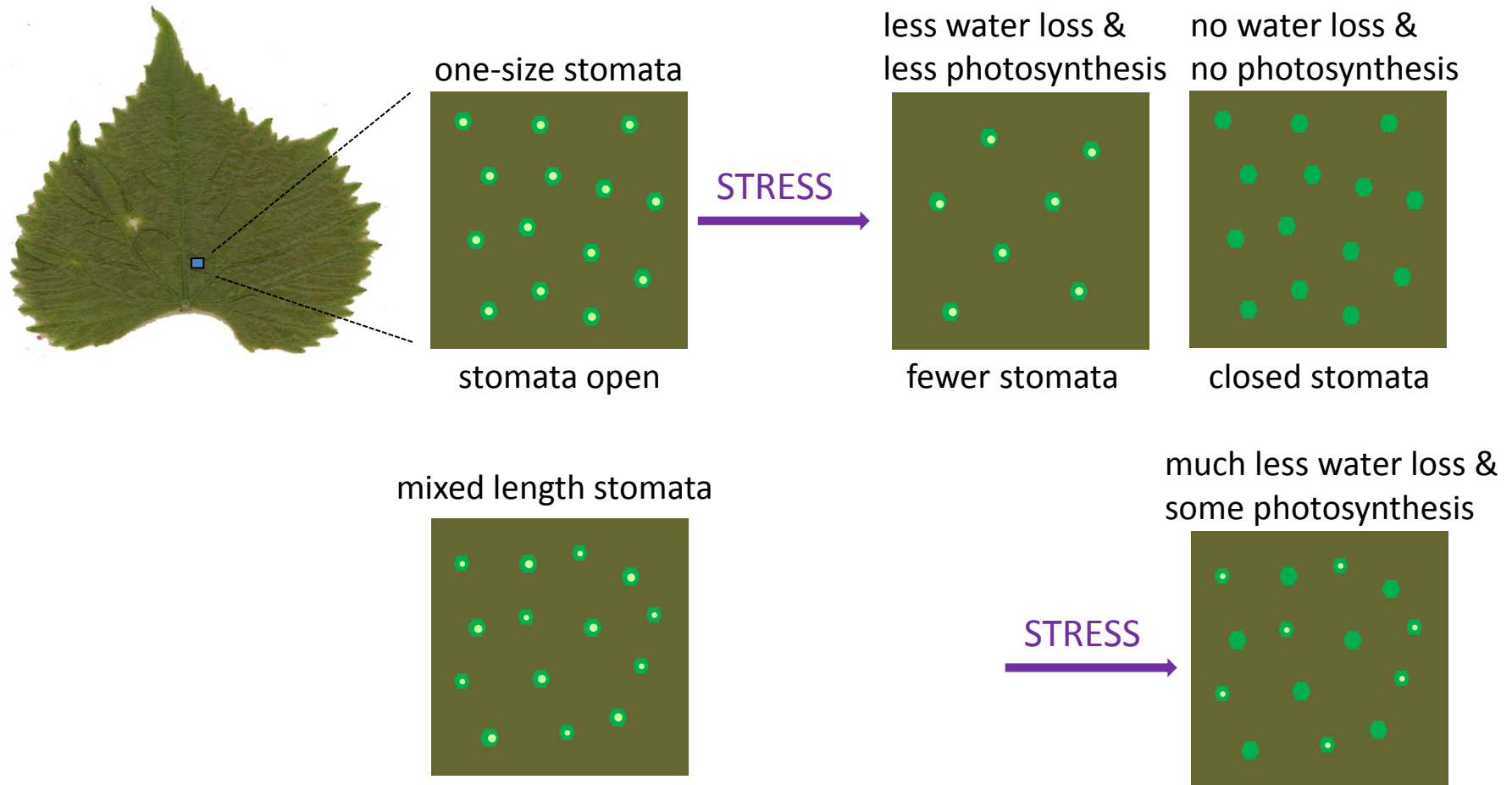


Smaller stomata open and close faster



- Smaller stomata allow better regulation of water loss and photosynthesis

Mixture of stomata is thought to give an advantage



- Sunken stomata lose less water
- Small stomata can open easier under stress conditions

V. riparia has more and smaller stomata than *V. vinifera*

► Stomatal density (# stomata/area)

V. riparia (MB and Guelph) > *V. vinifera* (Riesling)

► Stomatal length

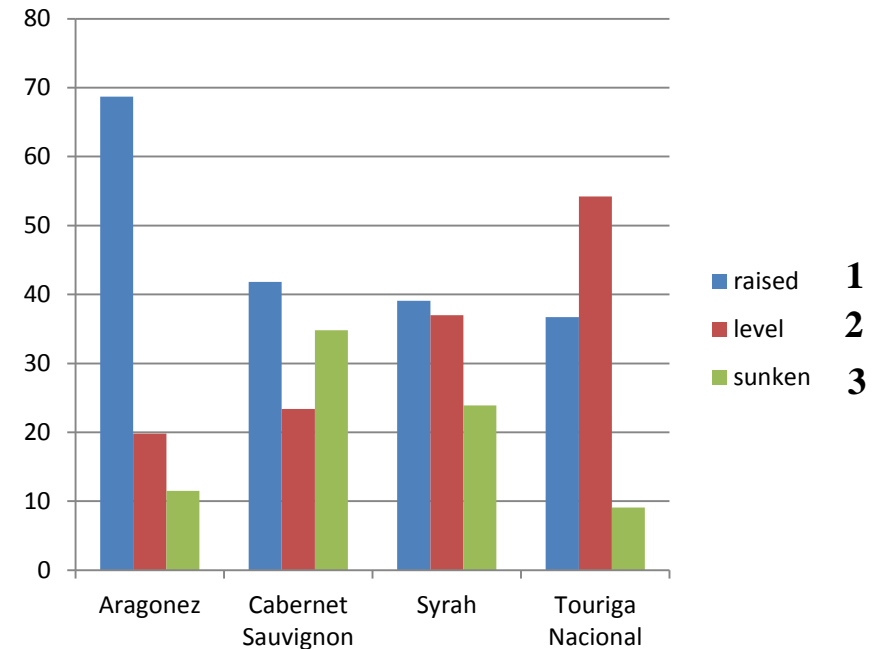
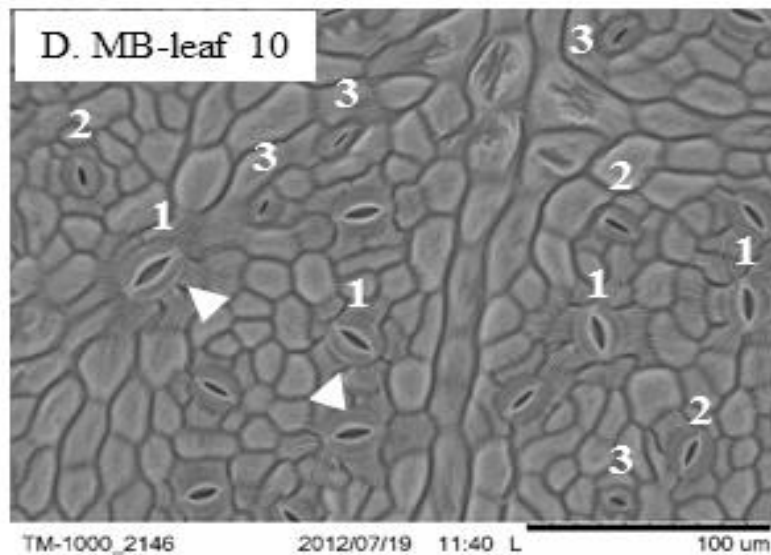
V. riparia (MB and Quebec) < *V. vinifera* (Riesling)

Range	3.8-34.5	4.2-40.4
Average	10.3-26.7	11.3 – 27.1
		13.0 – 21.0

V. riparia is more frost tolerant than *V. vinifera*

What about different cultivars?

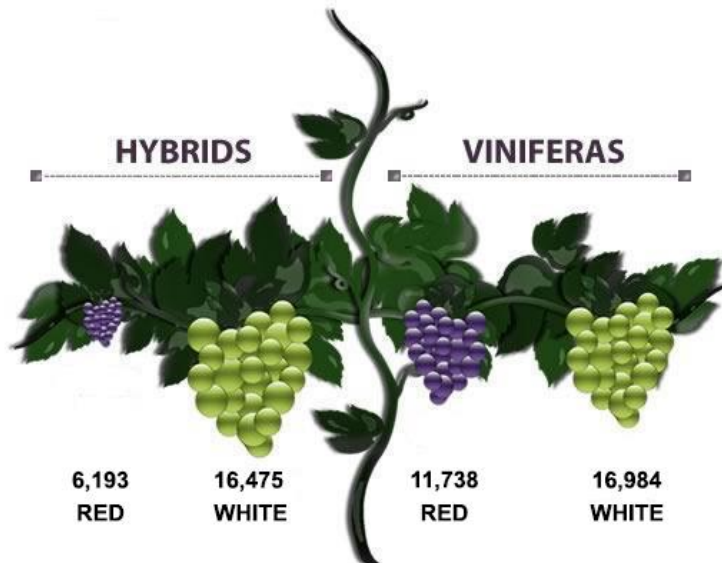
Each grape cultivar has a characteristic number and type of stomata



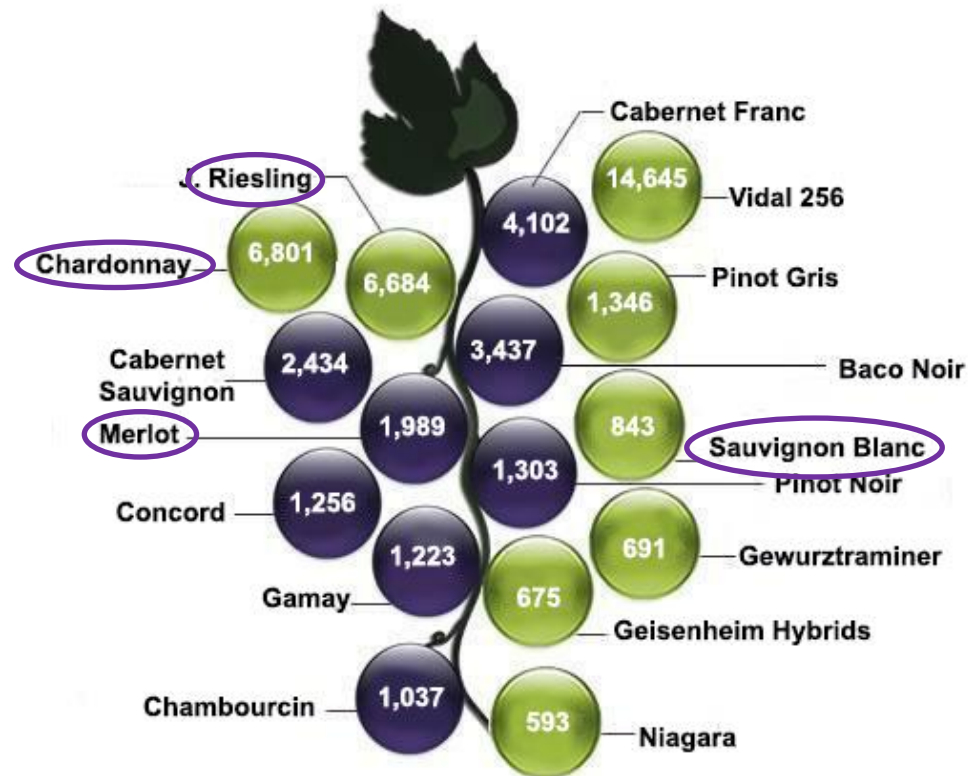
- Aragonez is less drought tolerant than CS and TN
Has fewer smaller, sunken stomata!

Ontario Grape and Wine Industry

Ontario Wine Grape Harvest 2015



Production of Grapes: Top Varieties 2015 (in tonnes)



Ontario Grape and Wine Industry



Stomata analysis of Ontario cultivars

- ▶ Can stomata distinguish between more cold hardy and less cold hardy grape cultivars?

Chardonnay, Riesling (>FT); Merlot, Syrah (<FT)

- ▶ Is this is the case at different times of the year?

3x/year (start till end of growing season)

- ▶ Is this is the case at different sites?

2 different sites (Stratus and CDC)

- ▶ Does it differ between plants grafted on different rootstocks?

3 different rootstocks (3309, SO4, Gloire)

September & July stomatal density (SD) correlate with cultivar, site and rootstock

Vineyard	Cultivar and rootstock	July Stomata/mm ² Mean SE	August Stomata/mm ² Mean ± SE	September Stomata/mm ² Mean ± SE
CDC	Riesling239-3309	306 ± 34	354 ± 47	311 ± 13
CDC	Riesling239-RipG	288 ± 20	328 ± 29	295 ± 33
CDC	Chard548-SO4	281 ± 21	250 ± 23	289 ± 8
CDC	Riesling239-SO4	273 ± 5	?±	271 ± 21
Stratus	Riesling21B-SO4	270 ± 12	333 ± 24	258 ± 29
Stratus	Chard548-3309	251 ± 11	246 ± 36	226 ± 3
CDC	Merlot347-SO4	215 ± 22*	264 ± 20	212 ± 18
CDC	SB530-SO4	207 ± 24	265 ± 23	252 ± 24*
Stratus	SB530-RipG	207 ± 23	220 ± 14	232 ± 9
Stratus	Merlot347-3309	191 ± 23	259 ± 28	226 ± 32
Stratus	Merlot181-RipG	185 ± 21	275 ± 26	219 ± 6
Stratus	Merlot?-SO4	178 ± 11	187 ± 75	176 ± 3

YES

- *outlier

- ▶ Riesling – Chardonnay - Sauvignon blanc – Merlot
- ▶ CDC – Stratus 3309 – Riparia Gloire – SO4

September sunken stomata density
correlates with cultivar, site and rootstock

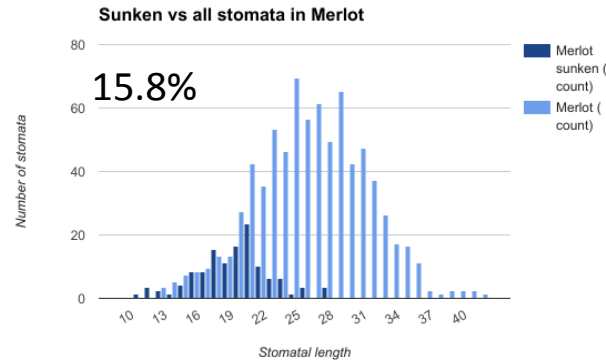
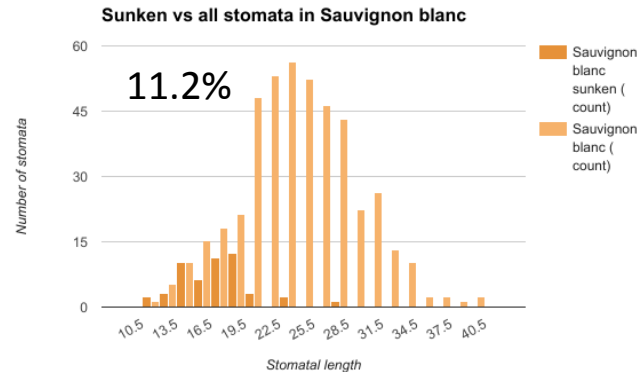
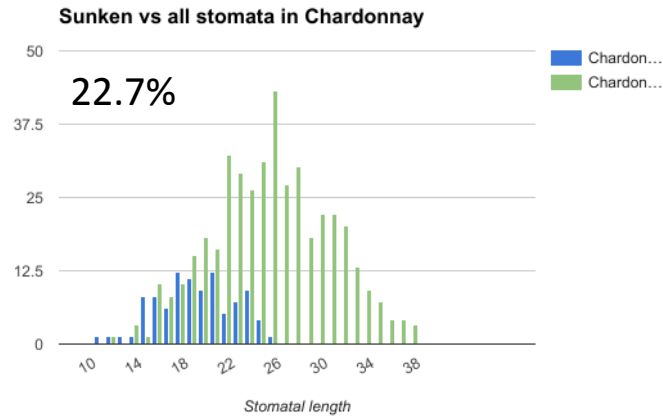
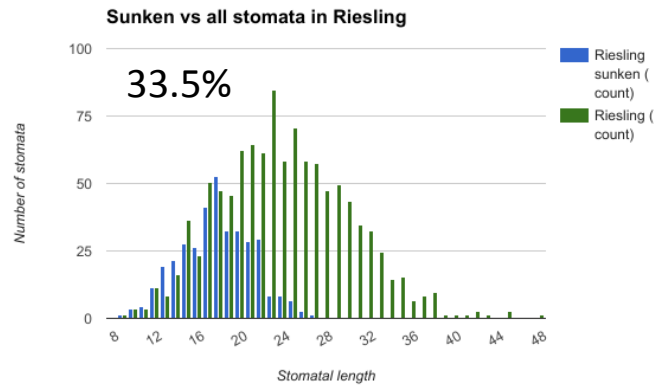
Vineyard	Cultivar	September Stomata/mm ² Mean ± SE	September Sunken stomata/mm ² Mean ± SE	% sunken stomata
CDC	Riesling239-3309	311 ± 13	132 ± 14	43
CDC	Riesling239-RipG	295 ± 33	94 ± 17	32
CDC	Chard548-SO4	289 ± 8	67 ± 13	23
CDC	Riesling239-SO4	271 ± 21	94 ± 17	35
Stratus	Riesling21B-SO4	258 ± 29	71 ± 19	27
Stratus	Chard548-3309	226 ± 3	37 ± 16	16*
CDC	Merlot347-SO4	212 ± 18	42 ± 9	20
CDC	SB530-SO4	252 ± 24	37 ± 9	15
Stratus	SB530-RipG	232 ± 9	17 ± 4	7
Stratus	Merlot347-3309	226 ± 32	44 ± 13	20
Stratus	Merlot181-RipG	219 ± 6	24 ± 8	11
Stratus	Merlot?-S04	176 ± 3	21 ± 5	12

YES

*outlier

- ▶ Riesling – Chardonnay - Merlot & Sauvignon blanc
- ▶ CDC – Stratus 3309 – Riparia Gloire & SO4

Sunken stomata are the small stomata in all CVs



Sunken stomata sizes

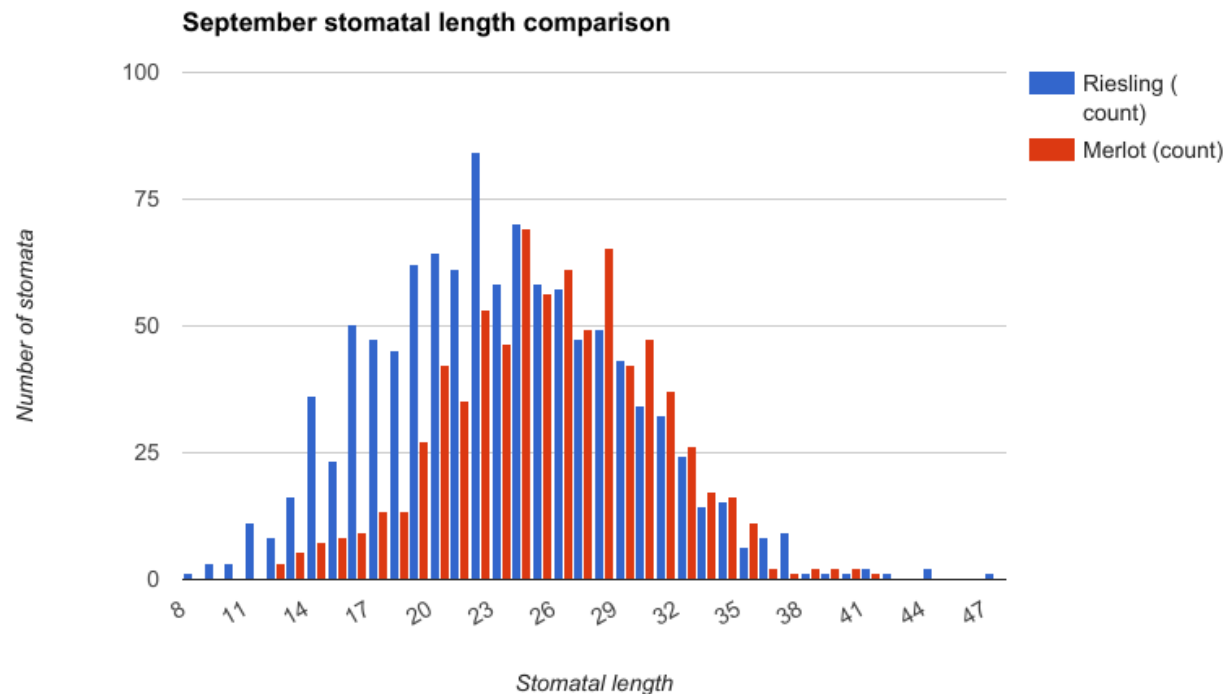
Riesling: 8.9 - 26.4 (vs - 47.2 for all)

Chardonnay: 10.9 - 25.3 (vs - 37.9 for all)

Sauvignon blanc: 10.9 - 28.2 (vs - 39.0 for all)

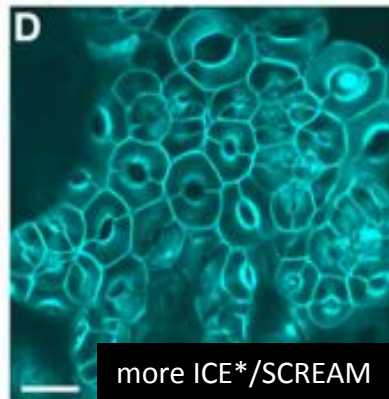
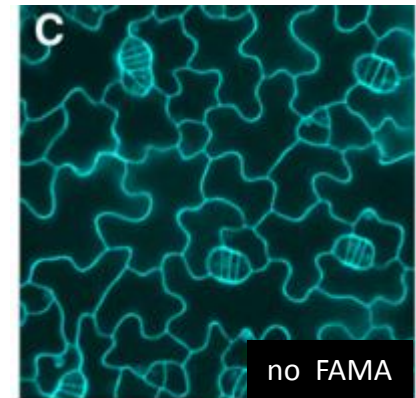
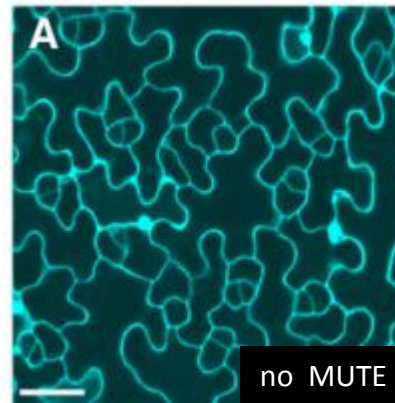
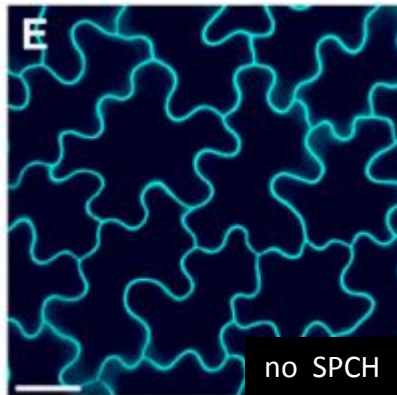
Merlot: 10.5 - 27.6 (vs - 41.2 for all)

Riesling leaves contain more stomata than Merlot; and these are small stomata!

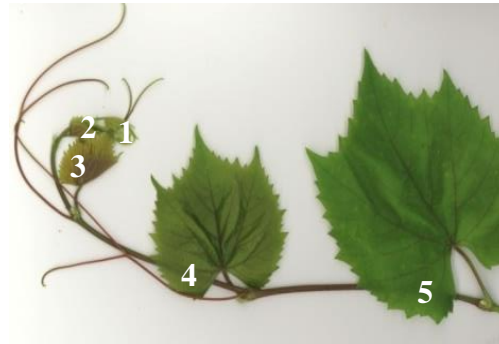







More frost tolerant cultivar has more small, sunken stomata
How is stomata development regulated?

SCREAM = ICE directs the development of stomata in Arabidopsis



ICE pairs with different partners to facilitate steps in the development of stomata



Leaf	1	3	5	11
SPCH				
MUTE				
FAMA(E)				
FAMA(L)				
ICE1,2,3,4				

- ▶ Grape *SPCH*, *MUTE* and *FAMA* genes are expressed as expected for stomata development in grape
- ▶ All 4 grape *ICE* genes are expressed in all leaves

Can they pair with *SPCH*, *MUTE* or *FAMA*?

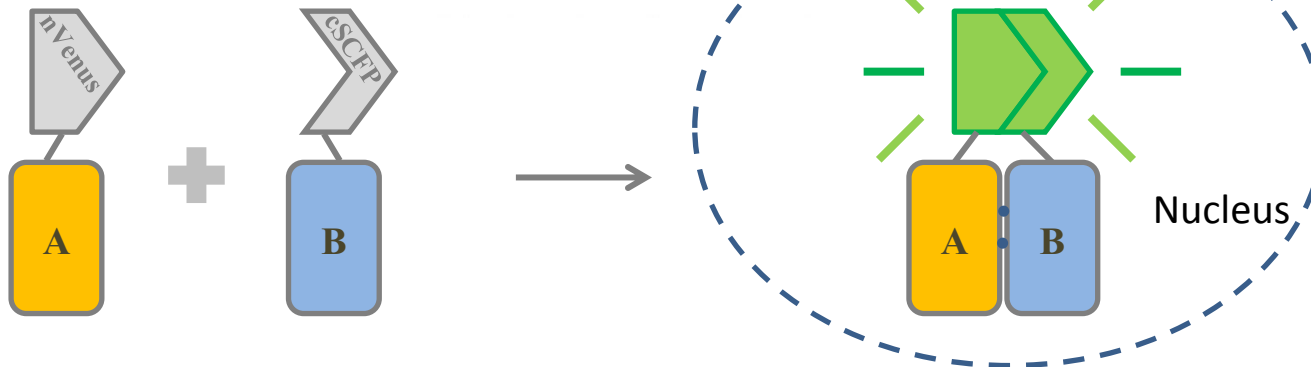
Different pairs have different activities!



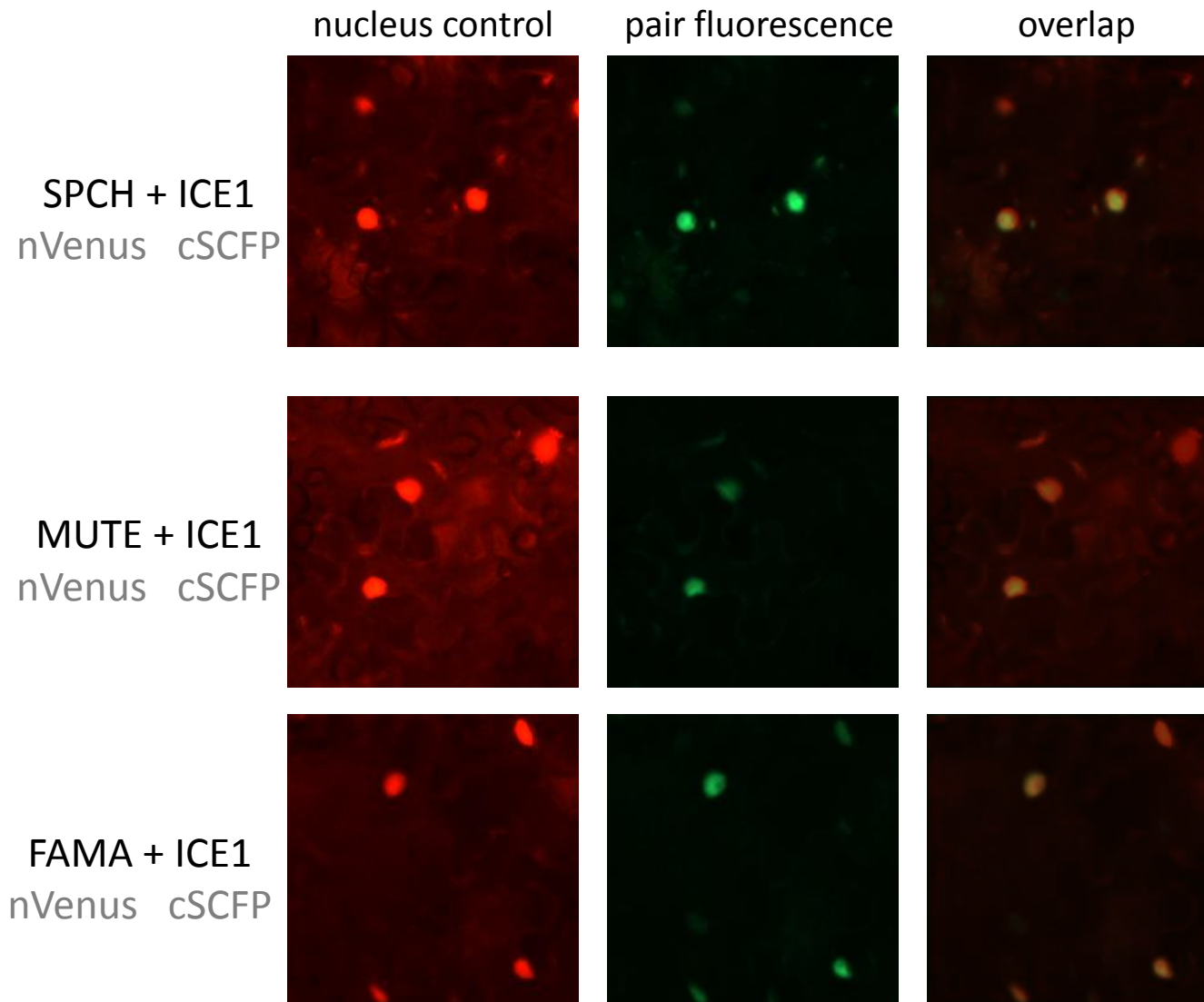
Greek dancing is different from Polish dancing!

Which ICE protein can pair with SPCH, MUTE or FAMA?

Analyzing pair formation between proteins

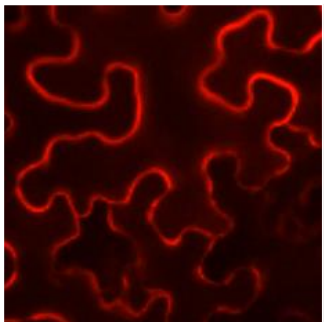
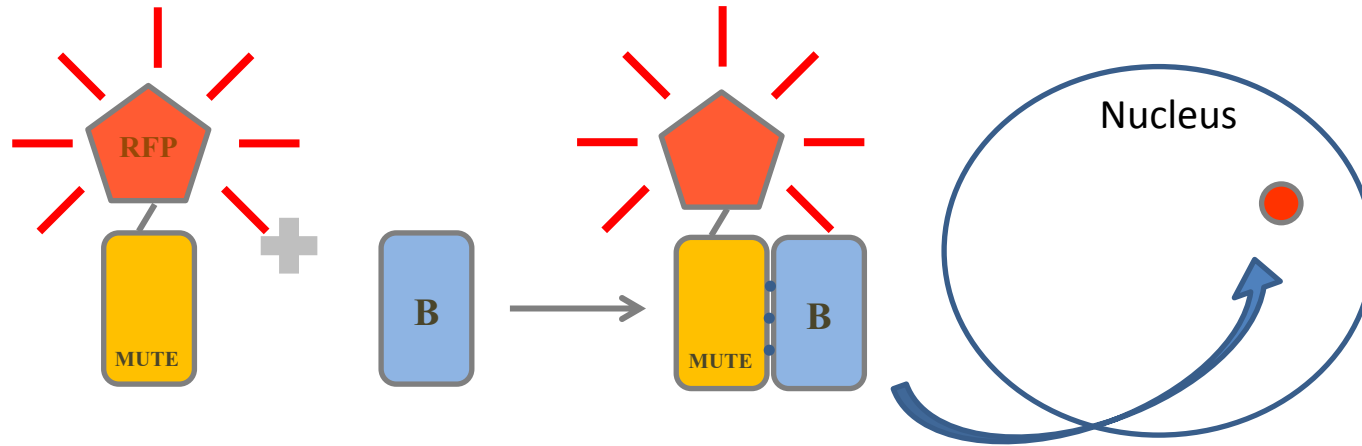


fluorescence in the nucleus indicates an interaction between A and B

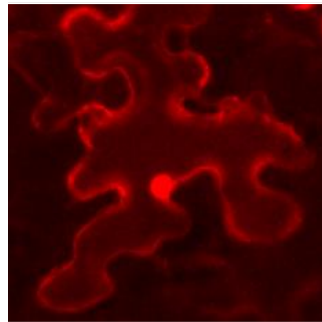


Grape ICE1, ICE2, ICE3 and ICE4 all pair with
SPCH, MUTE and FAMA, in the nucleus

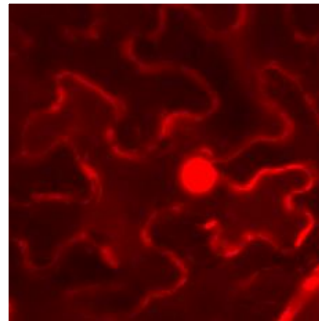
Analyzing pair formation between proteins



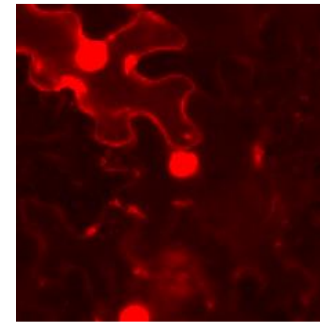
MUTE-RFP
only



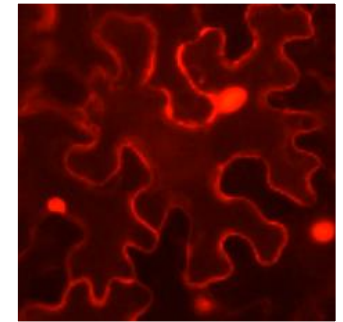
MUTE-RFP
+ ICE1



MUTE-RFP
+ ICE2



MUTE-RFP
+ ICE3



MUTE-RFP
+ ICE4

Grape ICE1, ICE2, ICE3 and ICE4 all pair with MUTE

Summary

- ▶ More frost tolerant grapes have a higher number of small, sunken stomata
- ▶ July and September give best correlation with known cultivar bud hardiness data from CCOVI
- ▶ Site and rootstock might also affect stomata results

WARNING: Data from just 1 year with 1 type of weather!
Data not all statistically significant

Future work

- ▶ Confirm data in other years
(repeatable results, also with different weather?)
- ▶ What effect does low temperature have on stomatal development? (number and type)
- ▶ What is the relation between ICE₁, 2, 3 and 4 and stomata type

Acknowledgements



Chateau des charmes



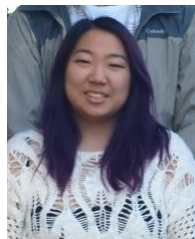
Stratus vineyards



Atik
Rahman



Chevonne
Carlow



Christine
Lee



Ali
Ebadi



Alison
Edge



Layla
Alibabai

Financial assistance from



Thank you!