Supplementary material of the manuscript published in Vitis **60**, 63–67 (2021):

Leaves of more cold hardy grapes have a higher density of small, sunken stomata

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Supplementary Table S1

Stomatal density and index of different grape cultivars /accessions grown under regular (22 °C) and low temperature (4 °C) conditions. Data shown are the mean of three biological replicates (SE = standard error). Values indicated by different letters in the same column are significantly different as determined by Tukey-Kramer's HSD test ($p < 0.05$)

Leaf number	Leaf area (mm^2)	Number of cells·mm^{-2}	Stomatal density (stomata·mm^{-2}) Mean	SE	Stomatal index (*100) Mean	SE	Number of cells·mm^{-2}	Stomatal density (stomata·mm^{-2}) Mean	SE	Stomatal index (*100) Mean	SE
Manitoba (MB)											
Leaf 1	86.7 f	20855.0 abc	30.4 f	11.5	14.1 i	4.8	18303.0 a	65.1 c	10.6	107.2 d	72.0
Leaf 2	186.1 f	25894.1 a	130.2 ef	32.8	49.2 hi	9.2	14783.0 abc	395.0 abc	54.4	210.5 cd	53.9
Leaf 3	292.6 f	24401.0 ab	247.4 c-f	19.9	111.3 ghi	7.3	8181.4 abc	527.3 ab	16.0	511.4 a-d	110.6
Leaf 4	658.5 f	23068.6 ab	525.2 abc	11.5	229.4 e-i	15.3	4969.6 bc	334.2 abc	61.2	611.2 abc	29.8
Leaf 5	1755.8 ef	10503.5 c-h	449.2 b-e	69.1	402.7 c-g	119.3	3350.7 c	225.7 bc	23.0	781.3 ab	44.4
Leaf 9	13699.6 ab	3177.1 e-h	227.9 c-f	38.6	699.4 abc	86.5					
Leaf 10	15478.8 a	2777.8 h	191.0 def	26.4	683.5 abc	20.1					
Quebec (QC)											
Leaf 1	165.2 f	24865.5 ab	342.9 c-f	24.2	138.1 ghi	3.3	17469.6 ab	468.8 ab	162.8	292.9 cd	113.8
Leaf 2	557.0 f	15746.5 a-e	729.2 a	81.3	478.9 b-f	53.5	10694.4 abc	638.0 a	64.2	657.0 abc	128.5
Leaf 3	1275.7 f	9192.7 d-h	787.8 ab	111.6	724.5 abc	55.8	6189.2 abc	447.1 ab	50.1	736.9 ab	56.6
Leaf 4	2698.1 ef	5672.7 e-h	499.1 a-d	176.3	763.6 ab	40.2	4409.7 c	347.2 abc	78.2	773.2 ab	42.3
Leaf 5	4391.1 e	3971.4 fgh	316.8 c-f	37.8	827.3 ab	45.8	3880.2 c	308.2 abc	11.5	801.6 a	47.9
Leaf 9	10077.8 cd	3802.1 fgh	308.2 c-f	8.7	866.2 a	24.4					
Leaf 10	12234.6 bc	3103.3 h	243.1 c-f	11.5	784.6 ab	43.1					
Riesling (RL)											
Leaf 1											
Leaf 2											
Leaf 3	258.6 f	17209.2 a-d	123.7 ef	5.3	74.7 ghi	14.5	11480.0 abc	191.0 bc	24.2	253.9 cd	117.8
Leaf 4	587.5 f	17161.5 a-d	130.2 ef	10.6	77.6 ghi	11.1	11744.8 abc	277.8 bc	44.1	287.1 cd	79.4
Leaf 5	1349.6 f	14027.8 b-h	234.4 c-f	0.0	165.9 f-i	35.8	9205.7 abc	286.5 bc	37.6	367.0 b-d	81.0
Leaf 9	8227.2 d	4097.2 fgh	217.0 c-f	5.3	566.7 a-d	84.8					
Leaf 10	9671.1 cd	4392.4 e-h	182.3 c-f	10.6	485.4 b-f	64.4					
Chardonnay (CH)											
Leaf 1											
Leaf 2											
Leaf 3	361.3 f	15230.0 a-f	195.3 def	56.8	152.8 ghi	71.9					
Leaf 4	1037.4 f	15056.4 a-g	429.7 a-e	39.8	349.2 d-h	106.7					
Leaf 5	2041.5 ef	9674.5 c-h	347.2 b-f	69.4	398.1 c-g	62.9					
Leaf 9	7954.8 d	3663.2 gh	208.3 c-f	15.0	644.5 a-d	15.9					
Leaf 10	10191.7 cd	3085.9 h	186.6 def	17.4	566.1 a-e	18.1					

Supplementary Table S2

Size of stomata and pavement cells, and number of pavement cells between stomata, for different grape cultivars/ accessions. The length of stomata includes the lengths of all three different sized stomata: sunken, same level and raised. Values indicated by different letters in the same column are significantly different as determined by Tukey-Kramer's HSD test ($p < 0.05$) (SE = standard error)

Grape cultivar/accession	Leaf number	Stomata length (µm)	Length of pavement cells (µm)	Number of pavement cells between stomata
		Mean ±SE	Mean ± SE	Mean ± SE
Manitoba (MB)	Leaf 1	11.5 ± 0.8 d-g		
	Leaf 2	11.5 ± 0.9 fg		
	Leaf 3	10.3 ± 0.4 g	4.7 ± 0.1 c	7.0 ± 0.3 ab
	Leaf 4	11.0 ± 0 .3 fg		
	Leaf 5	13.6 ± 0.2 d-g	5.9 ±0.3c	3.4 ± 0.5 cd
	Leaf 9	26.7 ± 1.1 ab		
	Leaf 10	25.9 ± 1.1 ab	18.2 ± 1.8a	3.0 ± 0.2 d
Quebec (QC)	Leaf 1	11.3 ± 0.5 fg		
	Leaf 2	12.2 ±1.2 efg		
	Leaf 3	16.5 ± 1.9 c-g	9.2 ± 2.1 bc	2.8 ± 0.1 d
	Leaf 4	20.9 ± 2.6 a-d		
	Leaf 5	24.4 ± 2.2 abc	9.9 ± 2.3 bc	3.2 ± 0.2 d
	Leaf 9	26.3 ± 0.2 ab		
	Leaf 10	27.1 ± 0.9 a	15.5 ± 0.4 ab	3.3 ± 0.2 cd
Riesling (RL)	Leaf 1			
	Leaf 2			
	Leaf 3	13.0 ± 2.7 d-g	5.3 ± 0.3 c	7.8 ± 1.0 a
	Leaf 4	18.7 ± 1.7 b-f		
	Leaf 5	21.0 ± 1.3 a-d	6.3 ± 0.6c	5.7 ± 0.7 a-d
	Leaf 9	20.8 ± 1.1 a-d		
	Leaf 10	20.4 ± 1.9 a-e	12.6 ± 2.5 abc	3.7 ± 0.2 cd
Chardonnay (CH)	Leaf 1			
	Leaf 2			
	Leaf 3	17.0 ±1.5 c-g	5.4 ±1.1 c	6.4 ±1.6 abc
	Leaf 4	15.0 ±1.9 d-g		
	Leaf 5	20.4 ±0.6 a-e	9.3 ±2.5 bc	4.3 ± 0.3 bcd
	Leaf 9	24.6 ±1.5 abc		
	Leaf 10	24.1 ±1.1 abc	15.0 ±1. ab	3.0 ± 0.2 d

Supplementary Table S3

Summary of multi-factor ANOVA for stomatal density and stomatal index of mature leaves (10th leaf) of grape cultivars 'Riesling', 'Chardonnay', 'Sauvignon blanc' (SB) and 'Merlot' grown on different rootstocks and sites, when the data for three different times (July, August and September) were combined. These grape cultivars were grown on rootstock 3309, Riparia Gloire (RipG) or SO4 in the vineyards of Chateau des Charmes (CDC) and Stratus.

Summary of Multi-factor ANOVA for stomatal density and stomatal index

Source	Stomatal density (stomata·mm^{-2})				Stomatal index (×100)			
	df	MS	F	p	df	MS	F	p
Cultivar	3	42782	35.405	4.90e-14 ***	3	4.974	6.251	0.000803 ***
Time	2	17367	14.372	5.89e-06 ***	2	2.771	3.482	0.036179 *
Vineyard	1	3655	3.025	0.0864^{NS}	1	7.007	8.806	0.004106 **
Rootstock	2	3953	3.271	0.0438 *	2	0.721	0.906	0.408913^{NS}
Interactions								
Cultivar x Vineyard	3	1223	1.012	0.3926^{NS}	3	3.137	3.943	0.011696 *
Time x Vineyard	2	3915	3.240	0.0451 *	2	2.199	2.763	0.069983^{NS}
Cultivar x Time x Vineyard	6	3054	2.528	0.0284 *	6	2.735	3.437	0.004947 **

Level of significance: *** 0.001, ** 0.01, * 0.05; df = degrees of freedom; MS = mean square and NS = not significant.
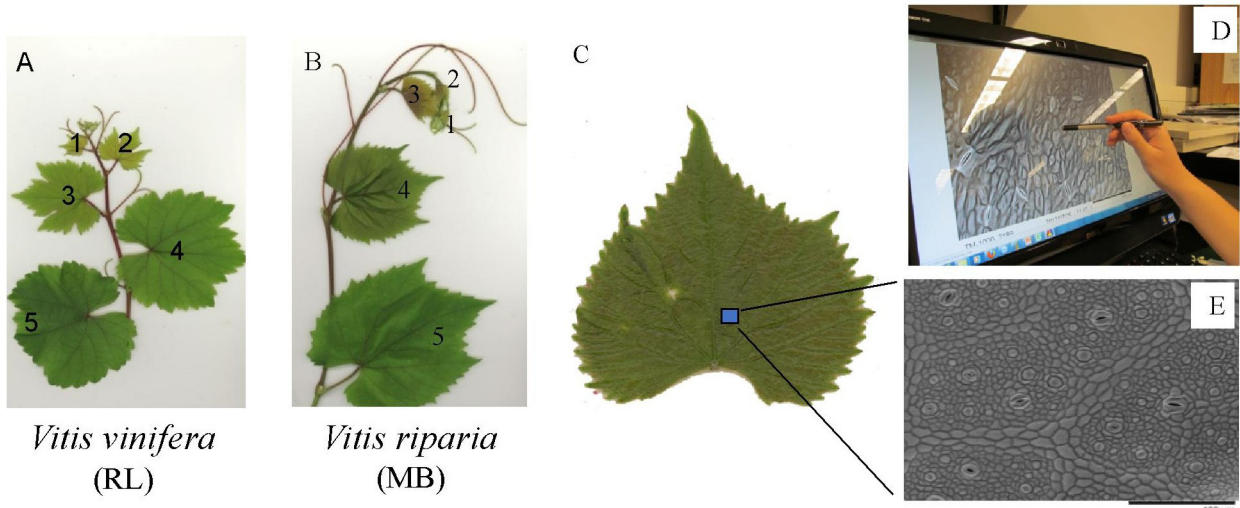
Supplementary Table S4

Summary of post-hoc Tukey HSD test for multiple comparison of means for stomatal density and stomatal index of mature leaves (10th leaf) of grape cultivars 'Riesling', 'Chardonnay', 'Sauvignon blanc' (SB) and 'Merlot' at 95 % family-wise confidence level, when the data for three different times (July, August and September) were combined. These grape cultivars were grown on different rootstocks (3309, Riparia Gloire (RipG) or SO4) in the vineyards of Chateau des Charmes (CDC) and Stratus

Summary of Tukey HSD test for stomatal density and stomatal index

| Source	Multiple comparisons	Stomatal density (stomata·mm^{-2})	Stomatal index (×100)
		Adjusted p value	Adjusted p value
Cultivar	Merlot-Chardonnay	0.0000680 ***	0.0010585 **
	Riesling-Chardonnay	0.0121405 *	0.3614432
	Sauvignon Blanc-Chardonnay	0.0028371 **	0.7660088
	Riesling-Merlot	0.0000000 ***	0.0395294 *
	Sauvignon Blanc-Merlot	0.9550581^{NS}	0.0308629
	Sauvignon Blanc-Riesling	0.0000000 ***	0.9494106
Time	July-August	0.0000280 ***	0.5949632
	September-August	0.0000646 ***	0.0303411 *
	September-July	0.9708286^{NS}	0.2280985
Vineyard	CDC-Stratus	NS	0.0078931 **
Rootstock	3309-RipG	NS	NS
	SO4-RipG	NS	NS
	SO4-3309	NS	NS

Level of significance: *** 0.001, ** 0.01, * 0.05; df = degrees of freedom; MS = mean square and NS = not significant.

Supplementary Fig. S1: Procedure followed for the examination of grape leaves. *V. vinifera* (**A**) and *V. riparia* (**B**) leaves were collected from different nodes, a sample was cut out from the indicated area, and SEM pictures from that area were analyzed on the computer. RL = 'Riesling', MB = Manitoba Note that the leaves are numbered from young to more mature (1-5 in this figure).
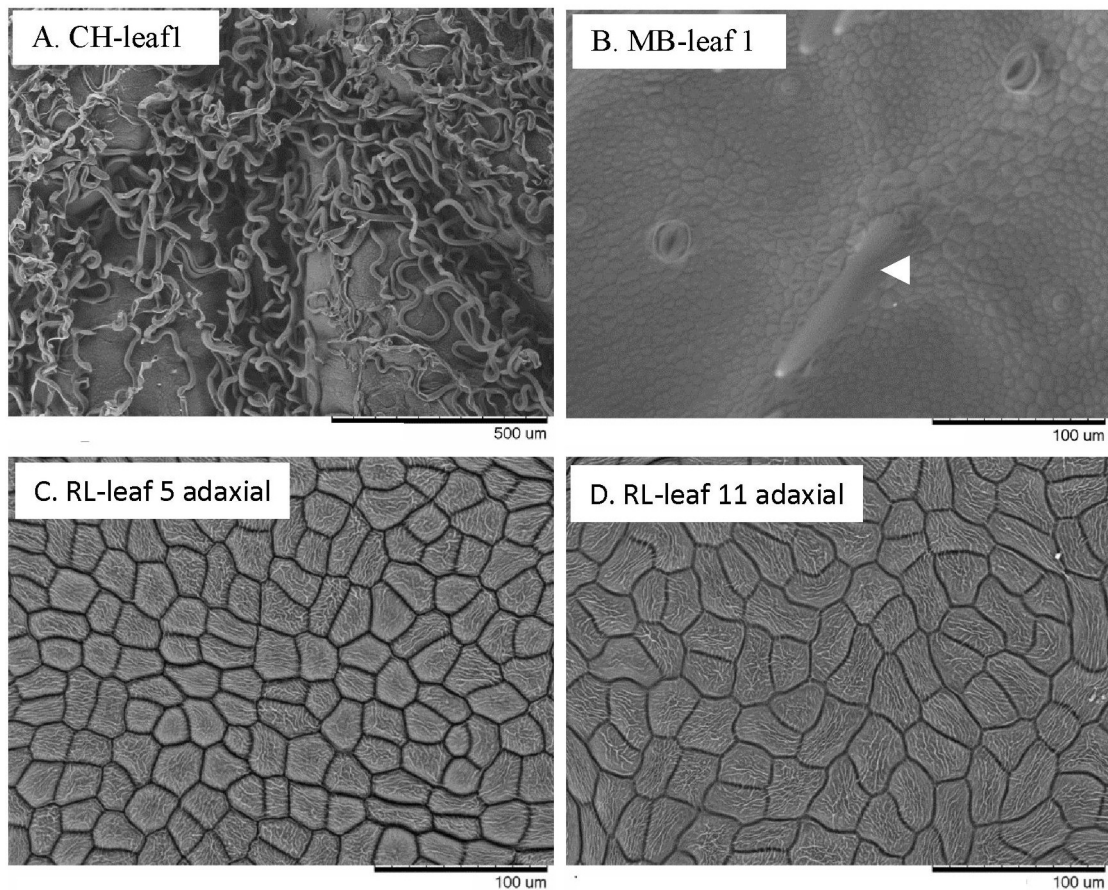
Supplementary Fig. S2: SEM pictures of grape leaves. The pictures illustrate the presence on the abaxial surface of young grape leaves of long coiled prostate hairs in the case of *V. vinifera* 'Chardonnay' (**A**), and of trichomes (erect hairs) in the case of *V. riparia* Manitoba (**B**), and the absence of stomata on the adaxial surfaces of younger or older leaves (**C** and **D**). CH = 'Chardonnay', MB = Manitoba, RL = 'Riesling'.
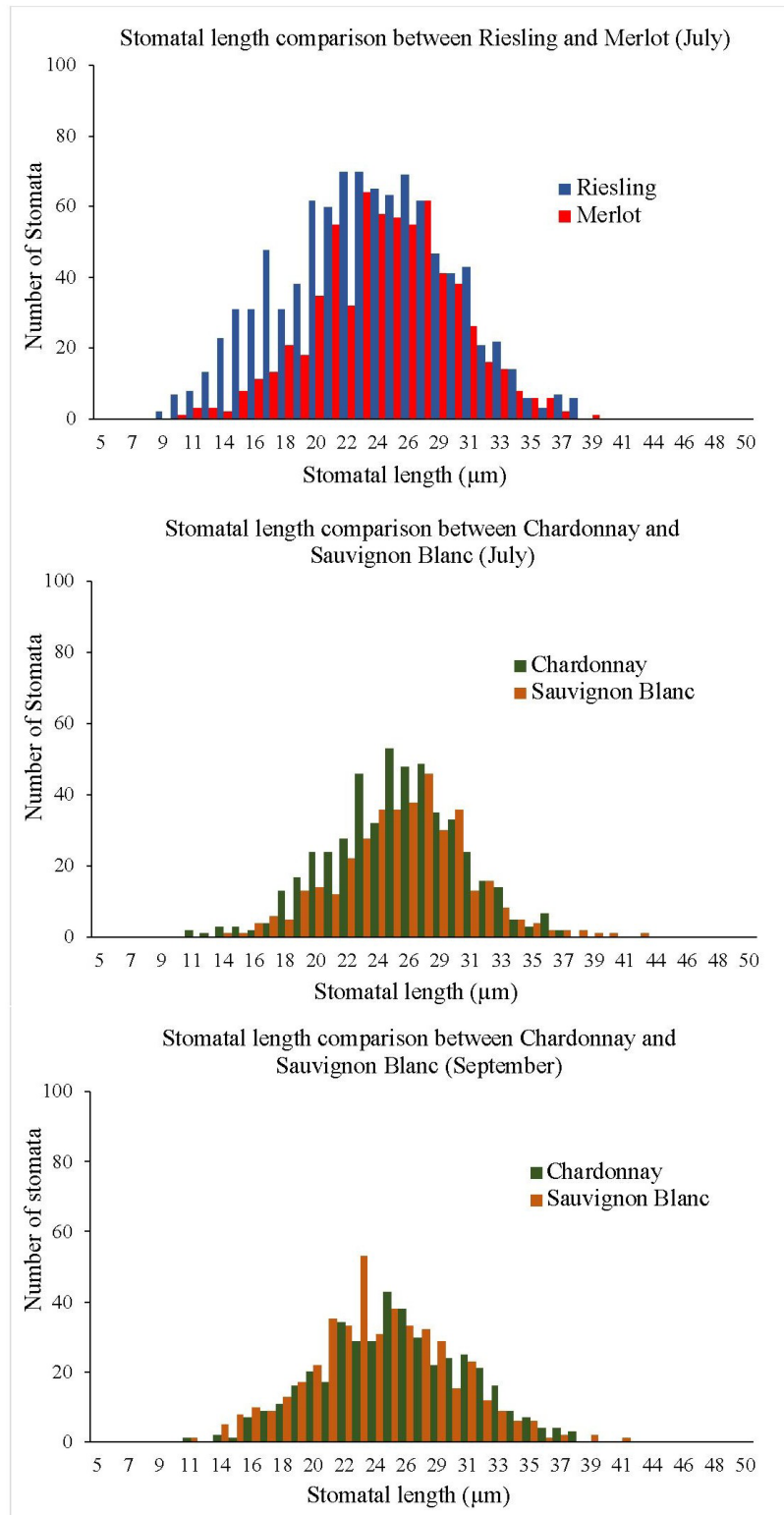
Supplementary Fig. S3: Length of stomata present in the 10th leaves from vineyard-grown *V. vinifera* cultivars. Stomata were analyzed from (top) July leaves of 'Riesling' (n = 963) and 'Merlot' (n = 656), (middle) July leaves of 'Sauvignon Blanc' (n = 383) and 'Chardonnay' (n = 489) and (bottom). September leaves of of 'Sauvignon Blanc' (n = 446) and 'Chardonnay' (n = 422).
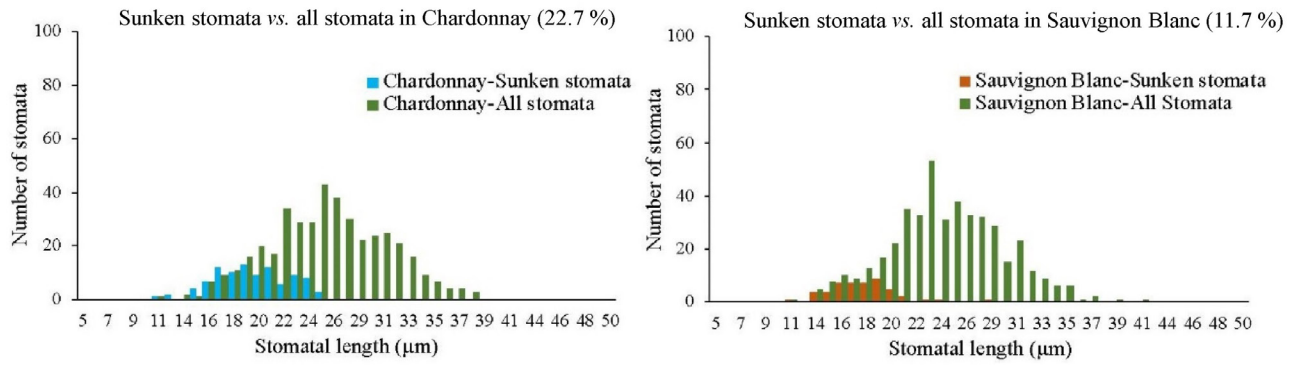
Supplementary Fig. S4: Sunken stomata are small. The length of sunken stomata was determined by an independent re-analysis of the September pictures used for Fig. S3 and the results were superimposed on the initial data for all stomata.