10.1071/FP15008_AC

Supplementary Material: *Functional Plant Biology*, 2015, 42(8), 737–745.

Supplementary Material

Spatial heterogeneity in stomatal features during leaf elongation: an analysis using *Rosa hybrida*

Dimitrios Fanourakis[A,D,E], *Ep Heuvelink*[A] *and Susana M. P. Carvalho*[A,B,C]

[A]Wageningen University, Department of Plant Sciences, Horticulture and Product Physiology Group, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

[B]CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Dr António Bernardino Almeida, 4200-072 Porto, Portugal.

[C]Faculty of Sciences, University of Porto, Rua do Campo Alegre 687, 4169-007 Porto, Portugal.

[D]Present address: Institute of Viticulture, Floriculture and Vegetable Crops, Hellenic Agricultural Organisation 'Demeter' (NAGREF), PO Box 2228, GR 71003, Heraklio, Greece.

[E]Corresponding author. Email: dimitrios.fanourakis82@gmail.com

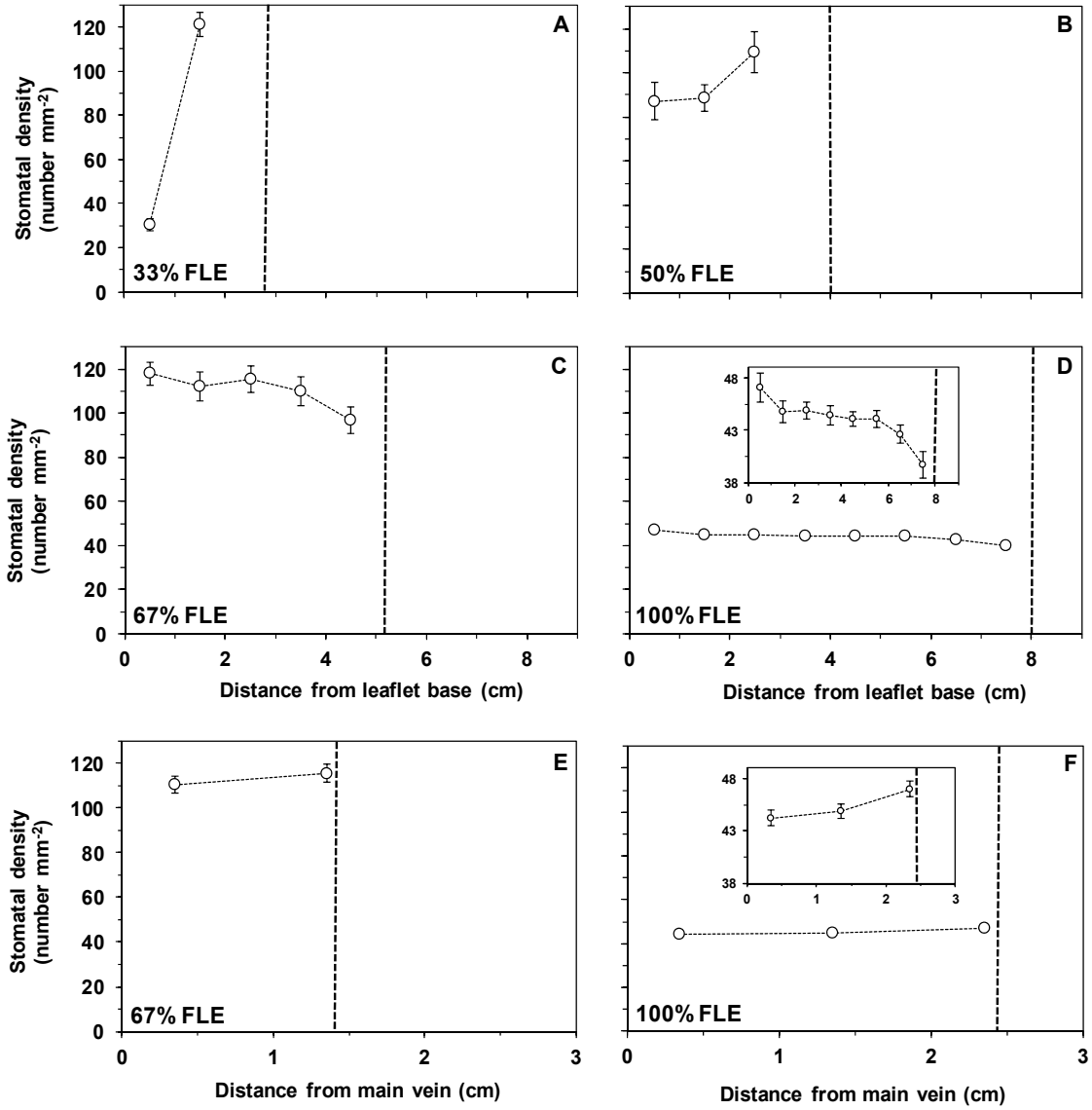

Fig. S1. Gradients in stomatal density over the leaflet of *Rosa hybrida* cv. Pink Prophyta. Stomatal density is given as a function of the distance from the leaflet base (i.e. close to the petiole-leaflet lamina junction) and the leaflet tip (A, B, C, D) and the distance between the main vein (i.e. midrib) and the leaflet edge (E, F), at various percentages of full leaflet elongation (FLE; leaflet length relative to its final length): A: 33%, B: 50%, C, E: 67%, D, F: 100%. Values are the average of all sampling areas at a given distance from the leaflet base or from the main vein (i.e. the rows or the columns in Fig. 1A, respectively). The vertical lines depict the leaflet length (A, B, C, D) or the distance between the leaflet edge and the main vein (E, F) at each percentage of FLE. The data are based on measuring the whole leaflet surface (sampling method in Table S1). The inserts represent the same data as the main figures, using a different scale. Data are means ± s.e. When the s.e. bar is not visible, the s.e. is smaller than the symbol.

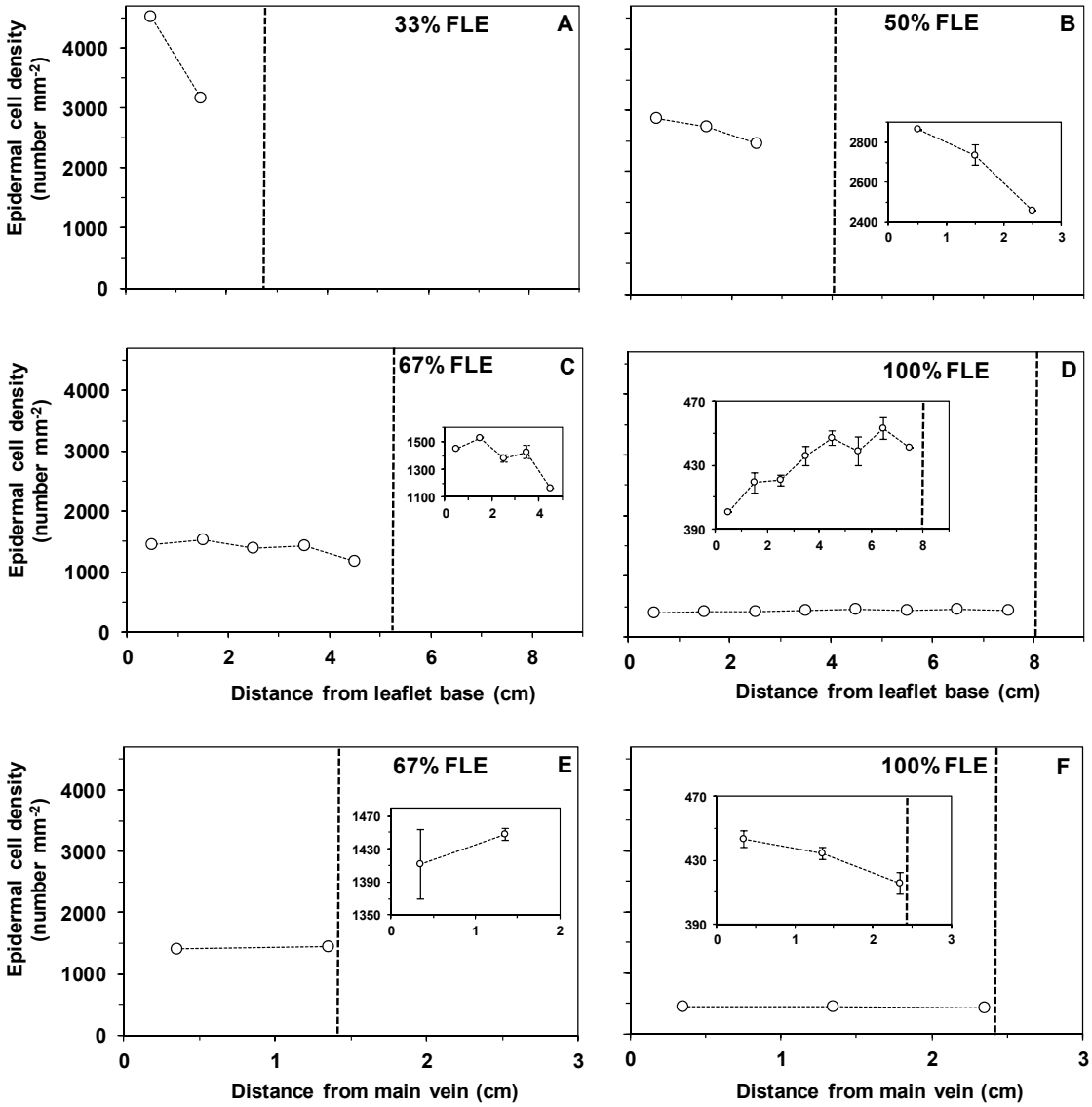

Fig. S2. Gradients in epidermal cell density over the leaflet of *Rosa hybrida* cv. Pink Prophyta. Epidermal cell density is given as a function of the distance from the leaflet base (i.e. close to the petiole-leaflet lamina junction) and the leaflet tip (A, B, C, D) and the distance between the main vein (i.e. midrib) and the leaflet edge (E, F), at various percentages of full leaflet elongation (FLE; leaflet length relative to its final length): A: 33%, B: 50%, C, E: 67%, D, F: 100%. Values are the average of all sampling areas at a given distance from the leaflet base or from the main vein (i.e. the rows or the columns in Fig. 1A, respectively). The vertical lines depict the leaflet length (A, B, C, D) or the distance between the leaflet edge and the main vein (E, F) at each percentage of FLE. The data are based on measuring the whole leaflet surface (sampling method in Table S1). The inserts represent the same data as the main figures, using a different scale. Data are means ± s.e. When the s.e. bar is not visible, the s.e. is smaller than the symbol.

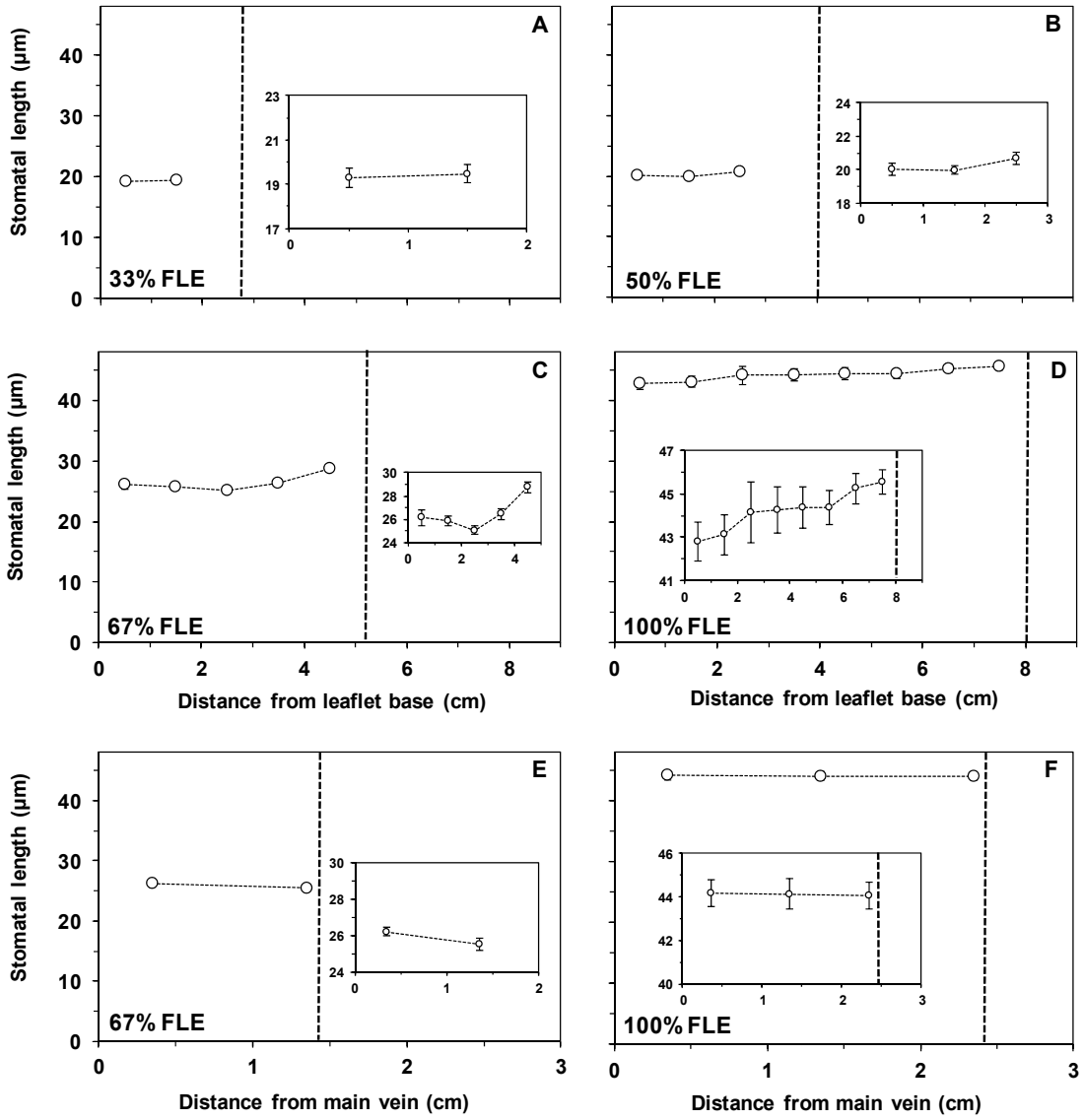

Fig. S3. Gradients in stomatal length over the leaflet of *Rosa hybrida* cv. Pink Prophyta. Stomatal length is given as a function of the distance from the leaflet base (i.e. close to the petiole-leaflet lamina junction) and the leaflet tip (A, B, C, D) and the distance between the main vein (i.e. midrib) and the leaflet edge (E, F), at various percentages of full leaflet elongation (FLE; leaflet length relative to its final length): A: 33%, B: 50%, C, E: 67%, D, F: 100%. Values are the average of all sampling areas at a given distance from the leaflet base or from the main vein (i.e. the rows or the columns in Fig. 1A, respectively). The vertical lines depict the leaflet length (A, B, C, D) or the distance between the leaflet edge and the main vein (E, F) at each percentage of FLE. The data are based on measuring the whole leaflet surface (sampling method in Table S1). The inserts represent the same data as the main figures, using a different scale. Data are means ± s.e. When the s.e. bar is not visible, the s.e. is smaller than the symbol.

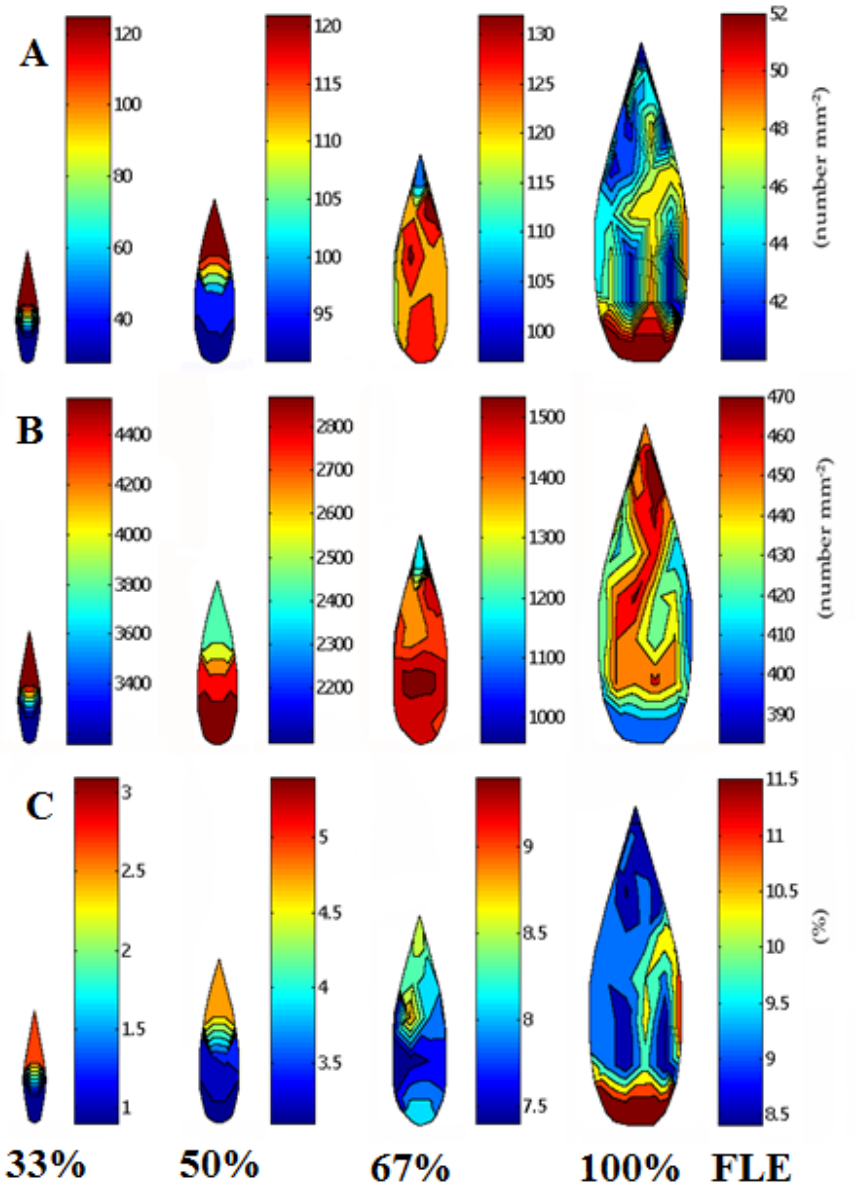

Fig. S4. Contour maps showing spatial heterogeneity in stomatal density (A), epidermal cell density (B) and stomatal index (C) over the leaflet of Rosa hybrida cv. Pink Prophyta at various percentages of full leaflet elongation (FLE; leaflet length relative to its final length). Columns at the right of each FLE denote the range of values. The data are based on measuring the whole surface of one representative leaflet. The sampling method is described in Table S1.

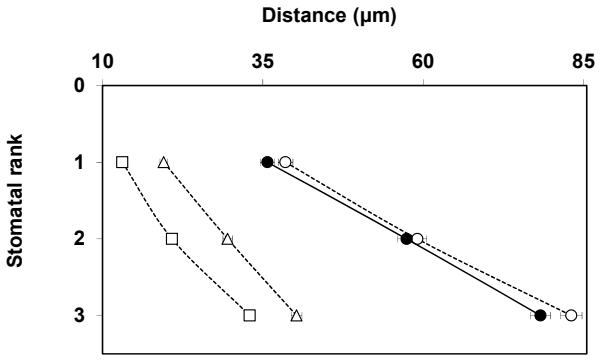

Fig. S5. Stomatal distribution on leaflets of *Rosa hybrida* cv. Pink Prophyta grown at 60 or 95% relative air humidity (open and closed symbols, respectively). Average distances (from edge to edge) are given from a reference stoma to three closest neighbour stomata in three directions, each direction covering an angle of 120°. These have been ranked 1 (nearest stoma) through 3 (most distant stoma). Data refer to various percentages of full leaflet elongation (leaflet length relative to its final length; 50%: square; 67%: triangle; 100%: circle). Values are the mean distances to a total of 250 reference stomata ± s.e.per elongation stage. When the s.e. bar is not visible, the s.e. is smaller than the symbol.

Table S1. Stomatal and epidermal cell features at increasing percentages of full leaflet elongation (FLE; leaflet length relative to its final length) in *Rosa hybrida* cv. Pink Prophyta grown at 60 or 95% relative air humidity (RH)

Minimum and maximum values are given when comparing sampling areas, as well as the coefficient of variation at the macro (between sampling areas within the leaflet) and micro (between fields of view within the sampling area) scale. A comparison is also made between the means of the areas separated by the midrib (referred as symmetry). Values are the means of one representative leaflet up to 12 leaflets ± s.e. The data are based on measuring the whole leaflet surface. The number of sampling areas per leaflet was 2, 4, 10 and 28 for 33, 50, 67 and 100% FLE, respectively. Per sampling area, 100, 75, 50 and 40 stomata were evaluated at 33, 50, 67 and 100% FLE, respectively. Nine or four fields of view per sampling area were assessed for stomatal and epidermal cell densities, respectively (Fig. 1B). Different letters indicate significant differences between FLE (capitals) or RH means according to Fisher's protected LSD test (comparison in columns)

RH (%)	FLE (%)	Number of assessed leaflets	Min ± s.e.	Max ± s.e.	Coefficient of variation (%)		Symmetry main vein (%)
					Macro scale	Micro scale	
			Stomatal density[1] (mm^{-2})				
60	33	4	31 ± 3^{C}	121 ± 5^{A}	85	30	–
	50	4	83 ± 7^{A}	109 ± 9^{A}	12	9	3
	67	4	95 ± 6^{A}	123 ± 5^{A}	7	5	4
	100	12	$38 \pm 1^{B, a}$	$51 \pm 1^{B, a}$	8	6	4
95	100	12	40 ± 1^{a}	52 ± 1^{a}	9	6	3
			Epidermal cell density[2] (mm^{-2})				
60	33	1	3126	4557	21	8	–
	50	1	2457	2866	6	4	0
	67	1	1116	1536	7	3	1
	100	1	400	470	5	3	0
95	100	1	383	464	5	4	1
			Stomatal index[3] (%)				
60	33	1	0.9	2.6	69	–	–
	50	1	3.2	4.7	18	–	1
	67	1	7.5	8.8	5	–	1
	100	1	8.4	11.4	7	–	4
95	100	1	8.7	11.3	6	–	2
			Stomatal length[4] (µm)				
60	33	1	19	19	0	22	–
	50	1	20	21	0	15	–
	67	1	25	29	0	16	–
	100	6	41 ± 1^{b}	48 ± 1^{b}	4	8	–
95	100	6	48 ± 1^{a}	55 ± 1^{a}	3	9	–
			Stomatal width[5] (µm)				
60	33	1	17	17	0	17	–
	50	1	17	18	0	12	–
	67	1	18	22	0	12	–
	100	6	31 ± 1^{b}	36 ± 1^{b}	4	8	–
95	100	6	34 ± 0^{a}	39 ± 0^{a}	3	9	–
			Pore length[4] (µm)				
60	100	6	23 ± 1.0^{b}	27 ± 1.0^{b}	5	10	–
95	100	6	29 ± 0.5^{a}	34 ± 0.5^{a}	4	10	–
			Pore aperture[5] (µm)				
60	100	3	3.9 ± 0.4^{b}	5.3 ± 0.7^{b}	8	19	–
95	100	3	6.4 ± 1.0^{a}	10.5 ± 1.0^{a}	11	24	–

[1] number of stomata per unit area; [2] number of epidermal cells per unit area; [3] number of stomata per total number of cells; [4] longest diameter; [5] shortest diameter.