Identification of the primary lesion of toxic aluminum (Al) in plant roots

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Table SI. Aluminum toxicity symptoms observed in roots of soybean growing in solutions containing 10, 30, or 75 µM Al. Using light microscopy, two symptoms of Al toxicity were evident as: (i) radial swellings behind the apex, and (ii) rupturing of the rhizodermis and outer cortex. Previous experiments using scanning electron microscopy (Kopittke et al., 2008), however, had shown that rupturing begins in ≤ 2 h.

	Radial swelling		**Rupturing**	
	Observed?	**Typical time before formation (h)**	**Observed?**	**Typical time before formation (h)**
10 µM Al	Yes	4-8	No	-
30 µM Al	Yes	6-10	Yes	24-36
75 µM Al	No	-	Yes	6-10

Table SII. Aluminum concentration (fresh mass basis) in apical tissues of soybean roots grown in solutions containing 1 mM Ca, 5 µM B, and 30 µM Al at pH 4.8 for 0, 1.5, 2, 5, or 12 h before being cut into segments 0 to 3 mm and 3 to 10 mm from the apex. Values are presented also for the entire 0 to 10 mm apical tissues based on the Al concentration and fresh mass of the individual segments.

Time	**Al (µg/g)**		
	0-3 mm	**3-10 mm**	**0-10 mm**
0	3.8	2.2	2.8
1.5	68	22	38
5	150	83	110
12	200	230	220

Table SIII. Concentrations of various Al species in both the simple nutrient solution ('Simple') and complete nutrient solution ('Complete') in which soybean plants were grown calculated using PhreeqcI v3.1.2 using the Minteq database with relevant constants (Nordstrom and May, 1996).

	10 µM Al			**30 µM Al**			**75 µM Al**	
	Simple	**Complete**		**Simple**	**Complete**		**Simple**	**Complete**
Al^{3+} (µM)	5.9	5.0		18	15		45	38
$AlOH^{2+}$ (µM)	2.9	2.5		8.6	7.5		21	19
$Al(OH)_2^{+}$ (µM)	1.2	1.1		3.5	3.0		8.8	7.9
$AlSO_4^{+}$ (µM)	0	1.4		0	4.0		0	10

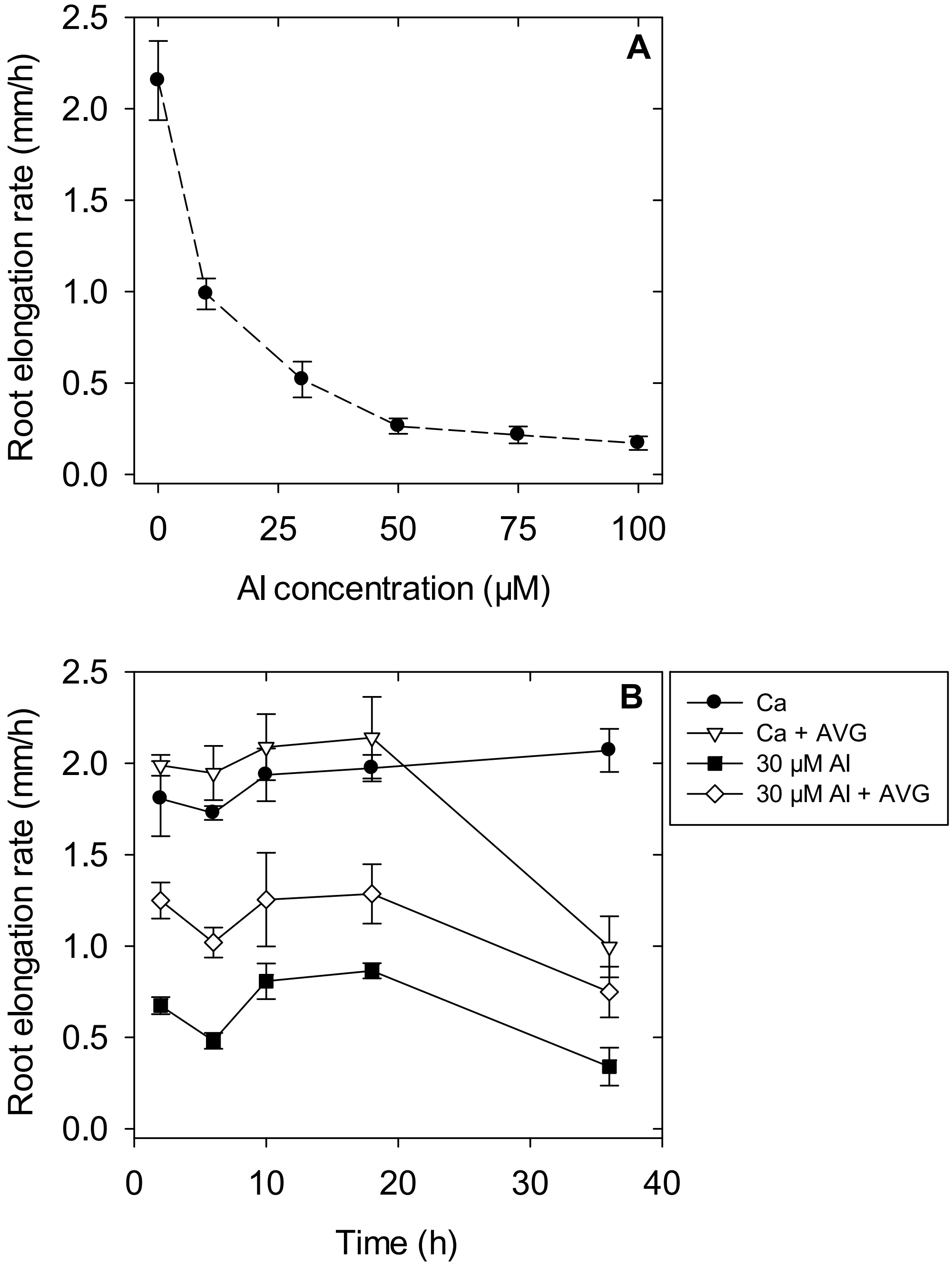

Figure S1. (A) The effect of Al concentration on the elongation rate of soybean roots over 48 h in solutions containing 0 to 100 μM Al. (B) The effect of 4 μM AVG (an ethylene synthesis inhibitor) on the elongation rate of soybean roots in solutions with or without 30 μM Al. All solutions contained 1 mM $CaCl_2$ and 5 μM H_3BO_3; for (B), data are presented halfway between their measurement times at 0, 4, 8, 12, 24, and 48 h.

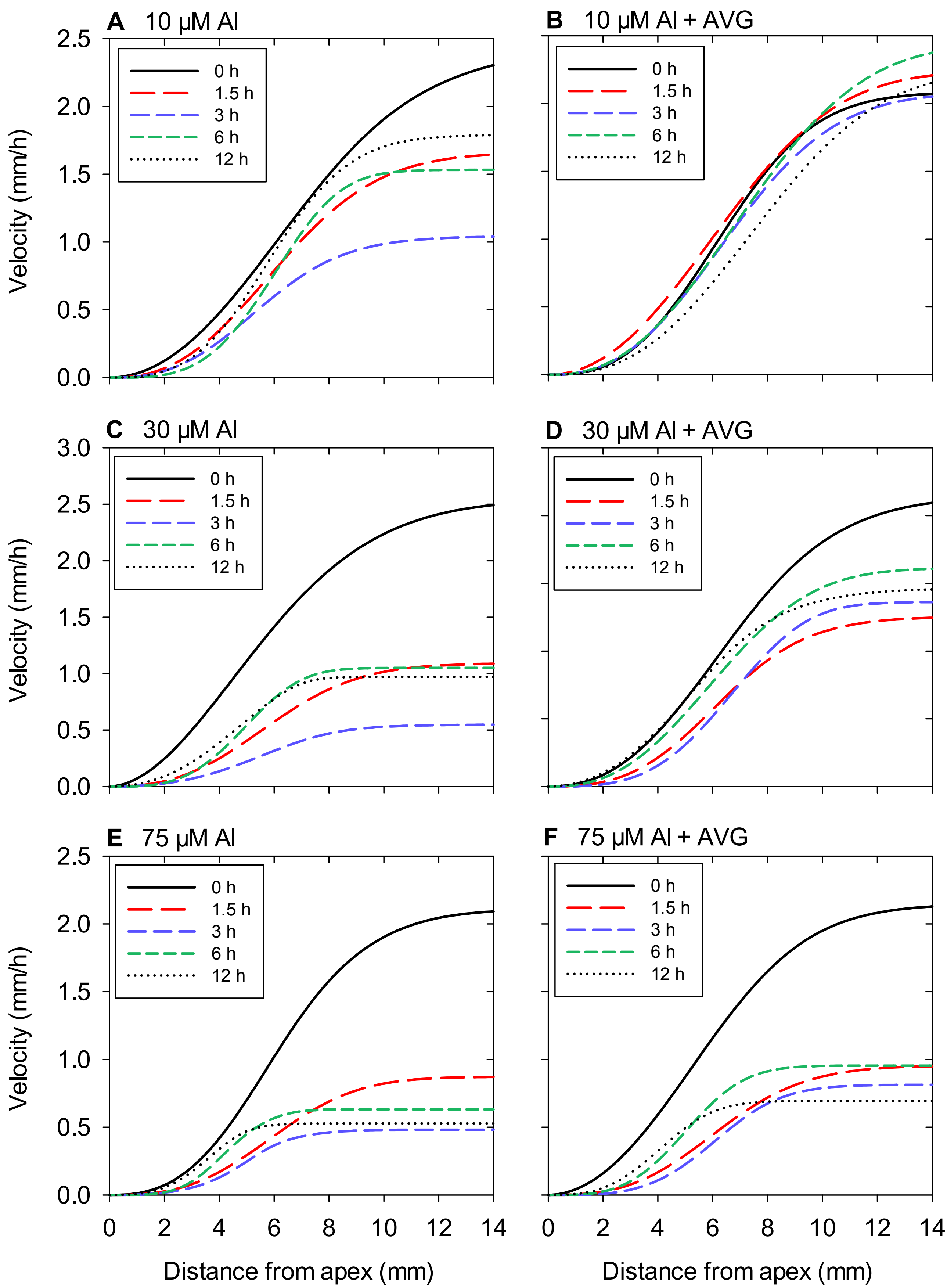

Figure S2. Profiles of velocity along the elongation zone of soybean roots exposed to 10, 30, or 75 µM Al without or with 4 µM AVG (an ethylene synthesis inhibitor) for up to 12 h.

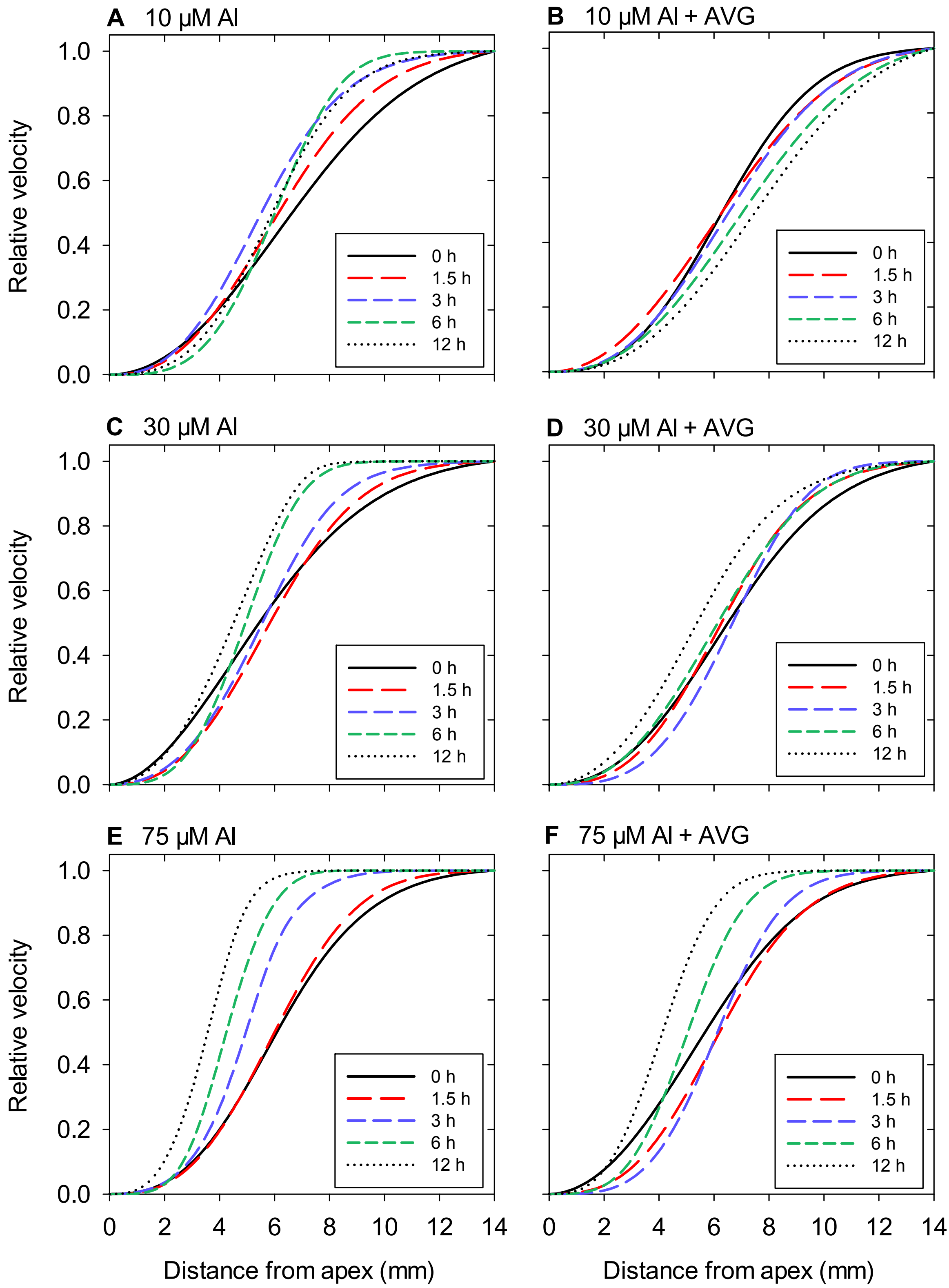

Figure S3. Profiles of relative velocity along the elongation zone of soybean roots exposed to 10, 30, or 75 µM Al without or with 4 µM AVG (an ethylene synthesis inhibitor) for up to 12 h. The length of the elongation zone was calculated as the region between 10 to 90 % of the relative velocity profile (Figure S2) (i.e. in which 80 % of root elongation occurs).

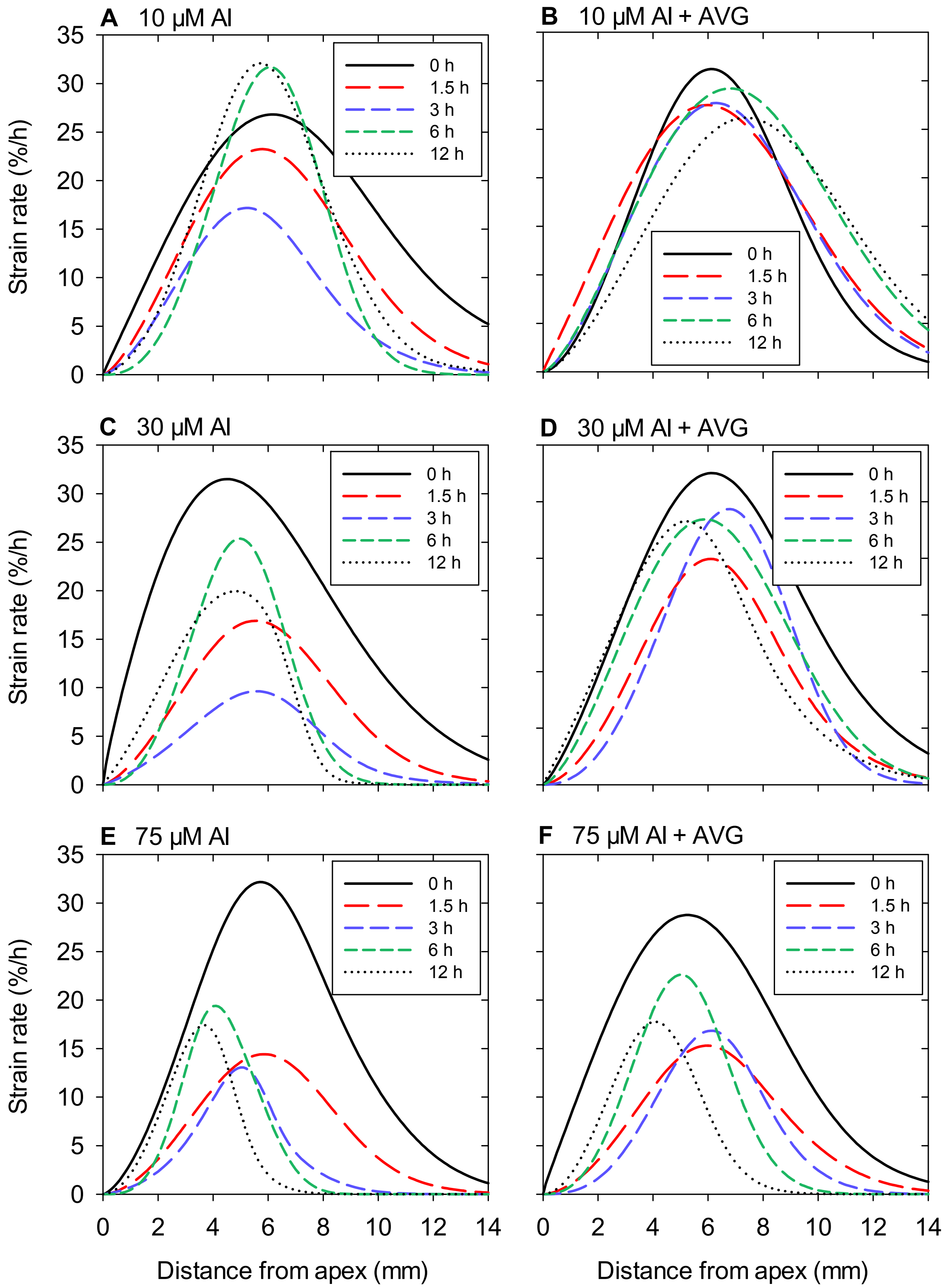

Figure S4. Rate of cell expansion along the elongation zone of soybean roots exposed to 10, 30, or 75 µM Al without or with 4 µM AVG (an ethylene synthesis inhibitor) for up to 12 h. For clarity, standard deviations are not shown although they are presented in Figure 3 for the relative maximum elemental elongation rate (i.e. the maximum strain rate for each curve). The profiles of velocity from which these curves are calculated are shown in Supplementary Figure S3.

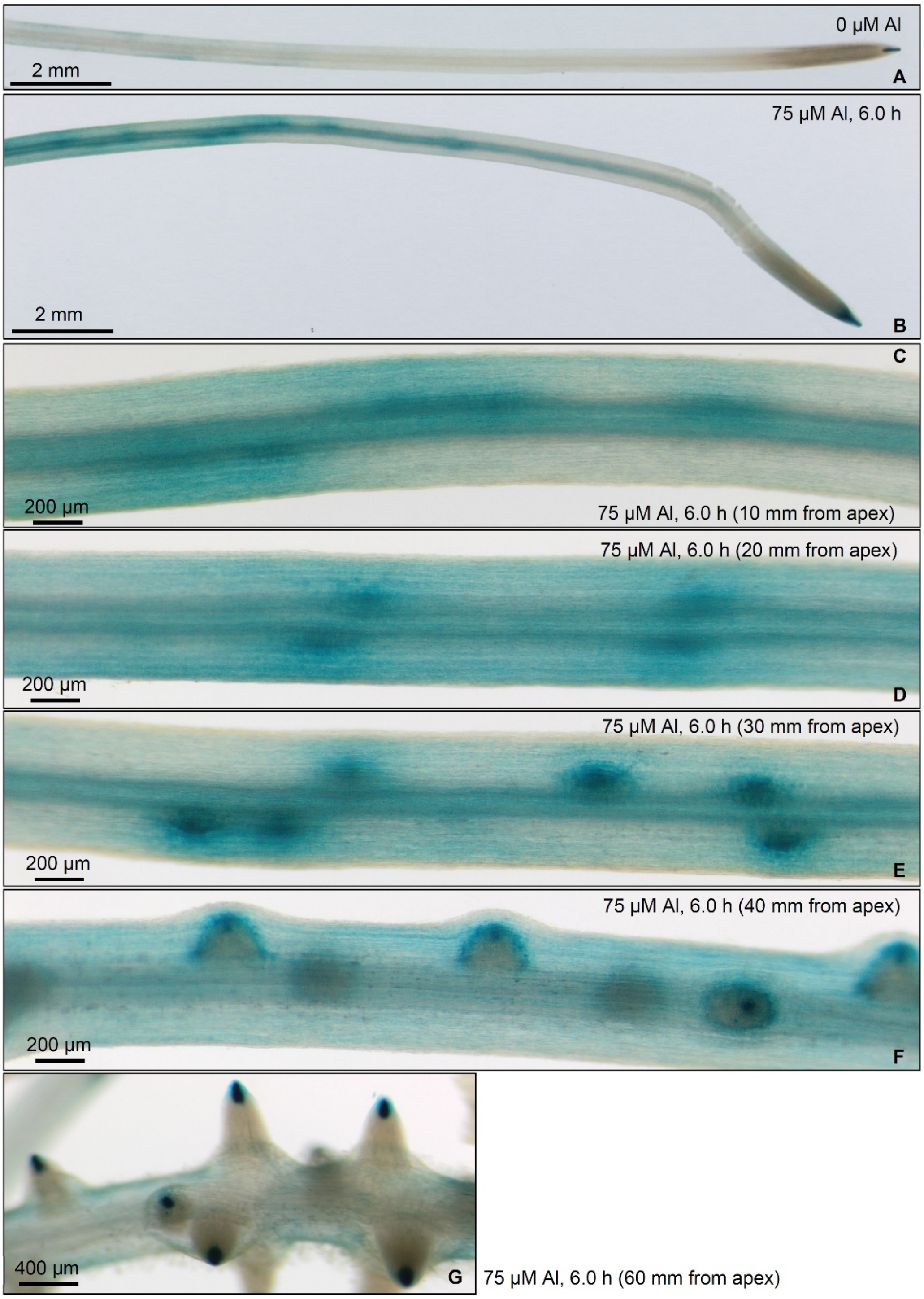

Figure S5. Effects of Al on the expression of the GUS reporter gene fused to a minimal promoter and the DR5 auxin-responsive promoter element (DR5::GUS) in the apical ca. 20 mm section of transgenic soybean roots. Images are of roots treated with 0 µM Al (a) or 75 µM Al for 6.0 h (b) with ruptures 2.8 – 4.3 mm from the apex, as is increased proximal GUS expression in the stele. Subsequent images (c – g) show the expression of GUS in the stele and developing lateral roots at an increasing distance from the apex (the scale bars indicate the approximate distance from the centre of the image to the apex which is always to the right of the image).

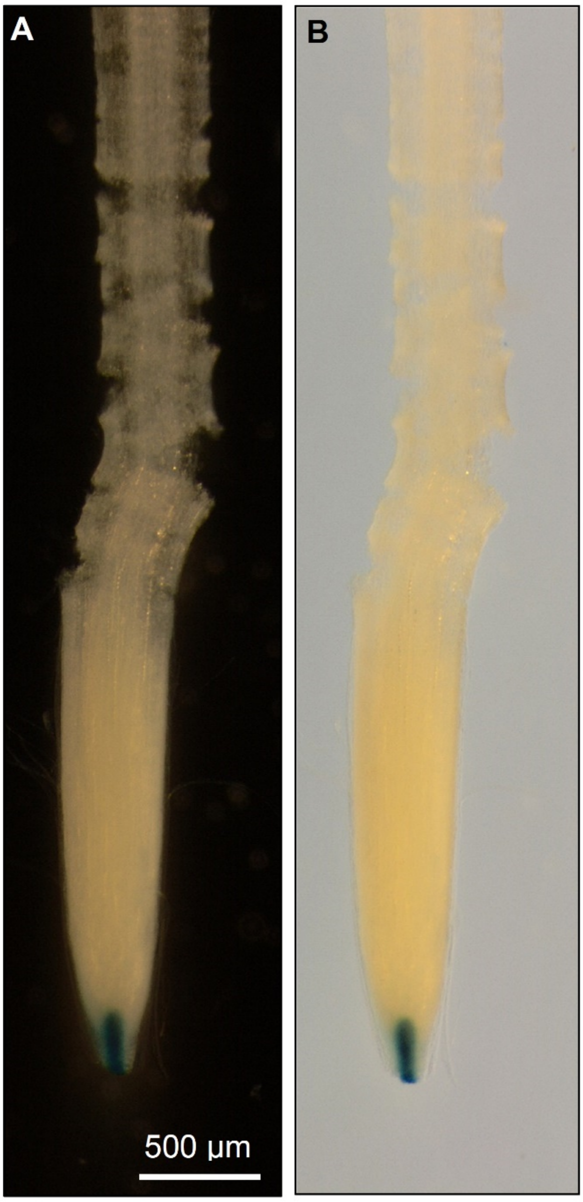

Figure S6. Effects of Al on the expression of GUS fused to a minimal promoter and the auxin-responsive promoter element DR5 after exposure to 30 µM Al and 4 µM AVG (an ethylene synthesis inhibitor) for 6.0 h. The two images are of the same root, with (a) showing the ruptures clearly and (b) showing the expression of GUS more clearly. Note that the formation of ruptures occurs despite the distribution of auxin (as evident from expression of GUS) being similar to that in solutions containing 0 µM Al (Figure S5).

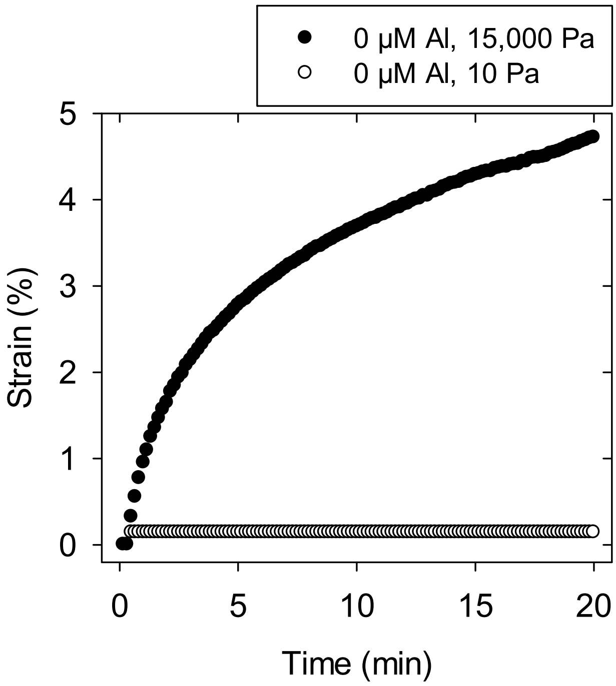

Figure S7. Extension of cell walls of fresh soybean roots grown in solutions containing 1 mM $CaCl_2$ and 5 μM H_3BO_3 without Al. Roots were compared using a stress of 10 or 15,000 Pa. When a stress of only 10 Pa was applied, roots ceased to elongate following clamping (crushing) of the apex. Therefore, the creep measured following the application of 15,000 Pa stress was not due to the continued 'natural' elongation of the fresh roots. See Cosgrove (1993) for more details.

Supplementary References

Cosgrove DJ (1993) Tansley Review No. 46. Wall extensibility: Its nature, measurement and relationship to plant cell growth. New Phytologist **124:** 1-23

Kopittke PM, Blamey FPC, Menzies NW (2008) Toxicities of soluble Al, Cu, and La include ruptures to rhizodermal and root cortical cells of cowpea. Plant and Soil **303:** 217-227

Nordstrom DK, May HM (1996) Aqueous equilibrium data for mononuclear aluminum species. *In* G Sposito, ed, The Environmental Chemistry of Aluminum, Ed 2nd. CRC/Lewis Publishers, Boca Raton, pp 39-80