Synchrotron-based Techniques Shed Light on Mechanisms of Plant Sensitivity and Tolerance to High Manganese in the Root Environment

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Supplemental Results

µ-XRF analysis of Mn in the Control treatment

The original, overview image of Mn distribution, uncorrected for variations in tissue thickness using Compton scatter (Fig. S4A) of leaves in the Control treatment of 0.5 µM Mn, indicates high Mn in small, localized areas in soybean, towards the leaf edge in white lupin, and towards the tip in narrow-leafed lupin. In sunflower, however, there was little Mn accumulation; that which did was in small, localized areas. The effect of normalization to the Compton scatter (Fig. S4B), which adjusts µ-XRF fluorescence for leaf thickness, was especially evident in narrow-leafed lupin in which part of the leaf tip was folded. The localization of Mn was clearly evident in soybean and there was high Mn near the leaf edge in white lupin and to some extent the leaf tip in narrow-leafed lupin. In sunflower, Mn was generally below the detection limit. At higher magnification, a small amount of Mn was present at the base of some non-glandular trichomes (NGT) (data not shown).

At 0.5 µM Mn, there were localized areas of high Ca in soybean against an even background of low to moderate Ca (Fig. S4C). In contrast to soybean, Ca was evenly distributed in leaf blades of the two lupin species. The distribution of Ca in sunflower differed markedly from that of the other three species, accumulating at high concentration in the NGT. Focusing on Mn and Ca, the three-color image of Mn, Ca, and Zn distribution provides a good illustration of the localization of these three nutrients in leaf blades (Fig. S4D). Both Mn and Ca appeared to accumulate in small localized areas in soybean, with both nutrients associated with the chlorotic areas visible in this treatment (Fig. S1C). At this magnification, however, it was not possible to ascertain whether or not Mn and Ca are co-located. In sunflower, there was a striking accumulation of Ca in the NGT but the low Mn concentration precluded assessment of their possible co-location.

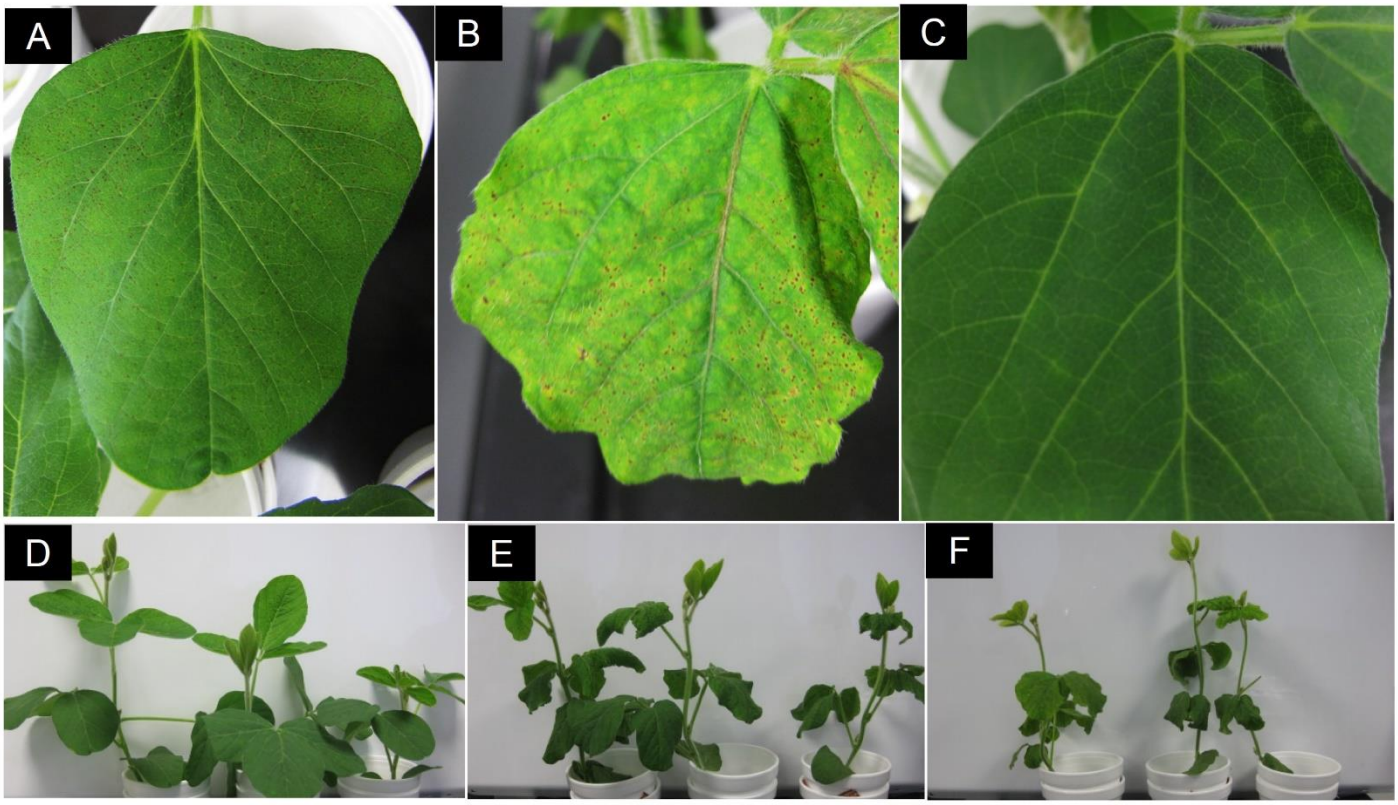

Figure S1. Symptoms of Mn toxicity on soybean leaves and effects on plant growth. A, Necrotic spots on a unifoliate leaf of soybean grown for 3 d at 100 µM Mn. B, Necrotic spots, chlorosis, and distortion of a lateral trifoliate leaflet of soybean grown for 16 d at 100 µM Mn. C, Chlorotic areas on a lateral trifoliate leaflet of soybean grown for 16 d at 0.5 µM Mn. Soybean plants grown for 16 d in solution containing (D) 0.5, (E) 30, or (F) 100 µM Mn.

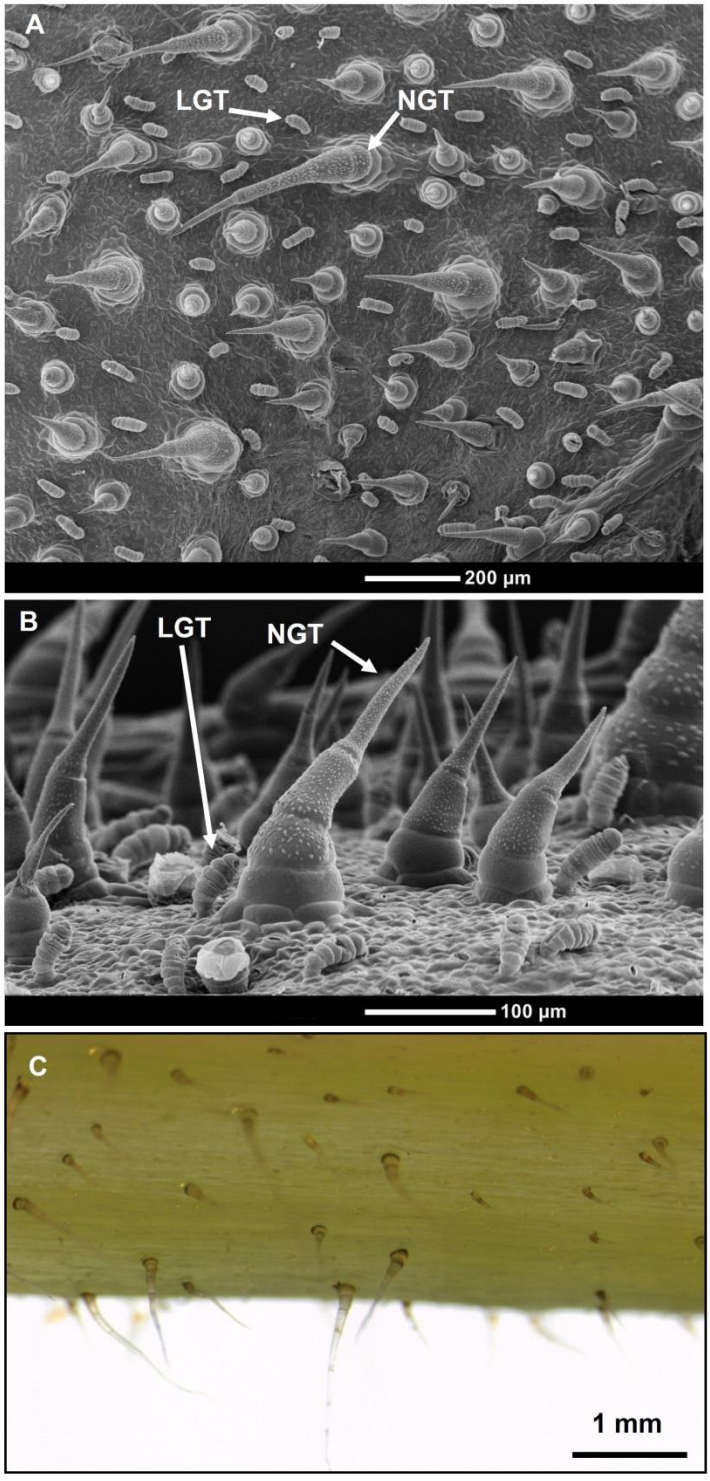

Figure S2. Sunflower trichomes. A,B, Scanning electron micrographs of linear glandular trichomes (LGT) and non-glandular trichomes (NGT) on a leaf blade at 0.5 µM Mn. C, Light micrograph of a petiole of a sunflower plant grown for 8 d at 100 µM Mn illustrating the darkened NGT which are characteristic when grown at elevated Mn in solution.

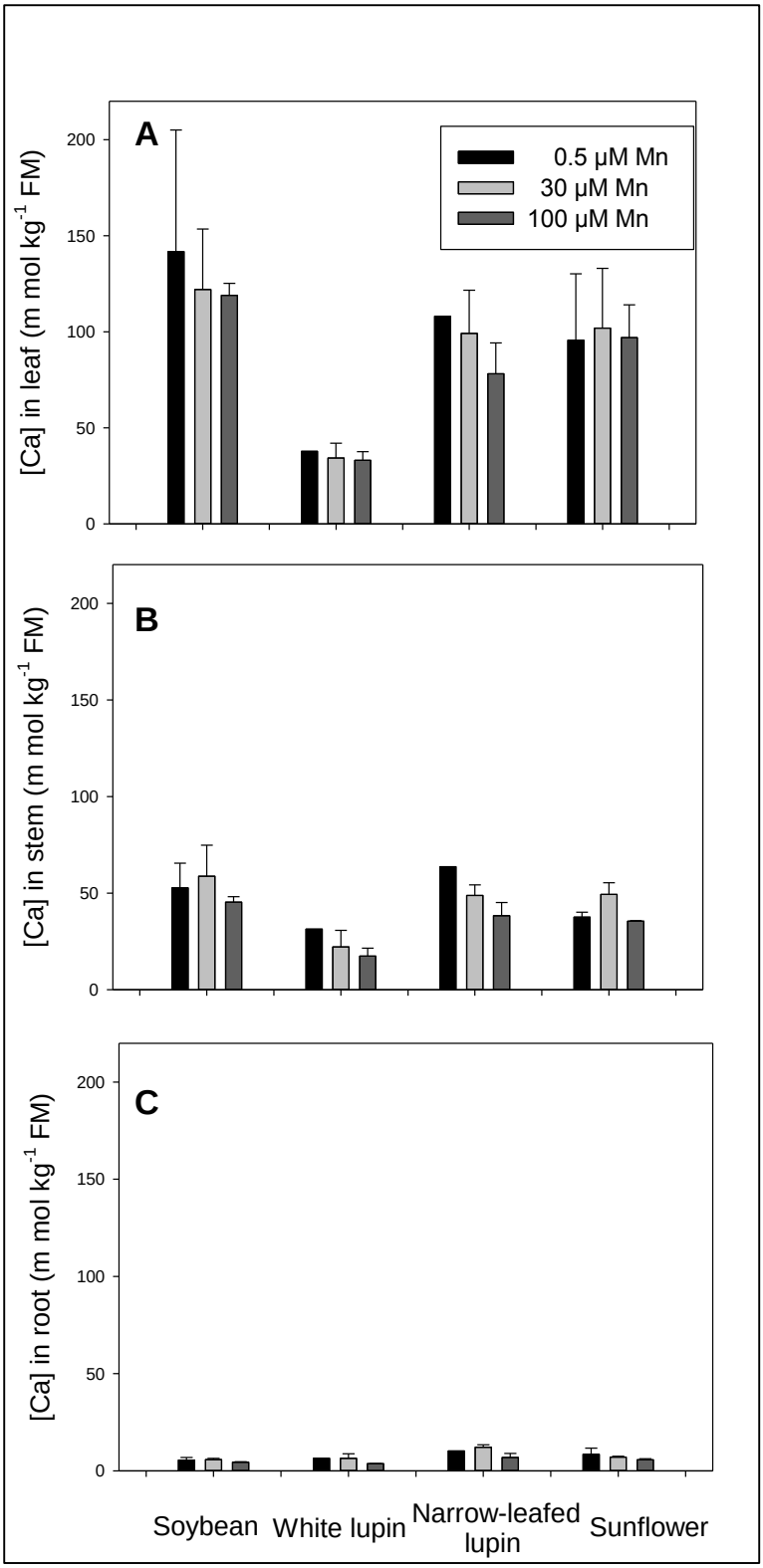

Figure S3. Effects of Mn in solution on the concentration of Ca on a fresh mass (FM) basis in leaves, stems, and roots of soybean, white lupin, narrow-leafed lupin, and sunflower grown for 16 d in solutions containing 0.5, 30, or 100 µM Mn. Values are means ± SE (n = 2).

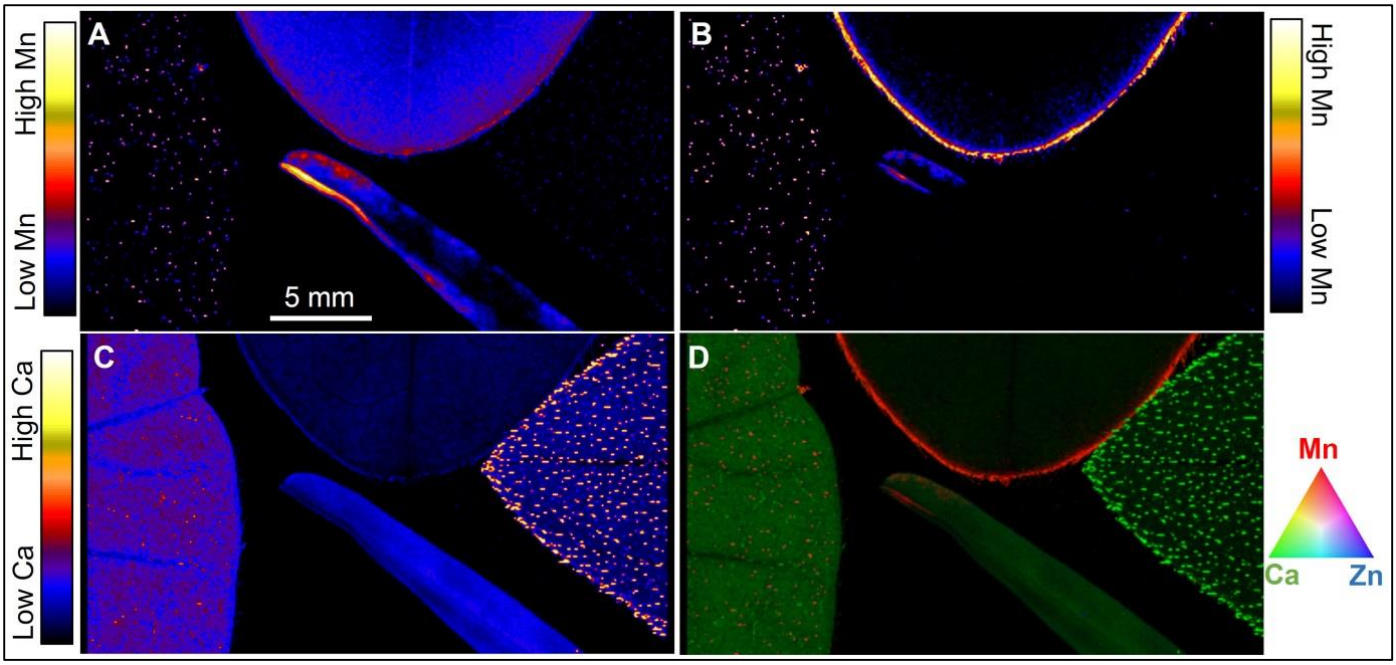

Figure S4. µ-XRF images of leaves of plants grown at 0.5 µM Mn in solution. A, Mn distribution (clockwise from the left) in soybean, white lupin, sunflower, and narrow-leafed lupin leaves (not corrected for Compton fluorescence). B, Compton-corrected Mn distribution. C, Compton-corrected Ca distribution. D, Compton-corrected distribution of Mn (red), Ca (green), and Zn (blue). The leaves were examined in a single scan and concentrations can be compared among the four species within each scan.

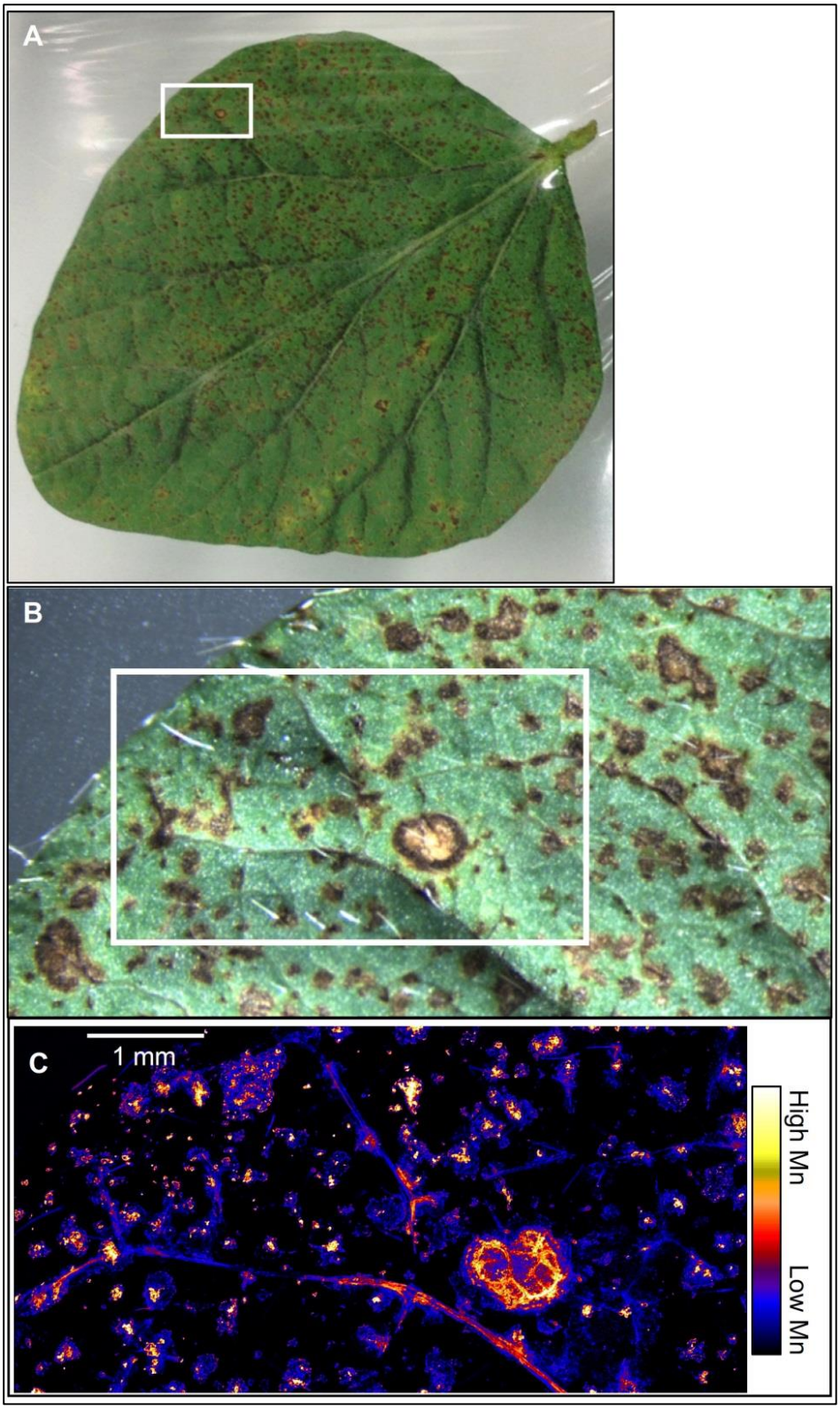

Figure S5. A leaf blade from a soybean plant grown at 100 µM Mn. A, Light micrograph illustrating necroses near the veins and the large and small necrotic lesions in the interveinal areas. B, High magnification light micrograph of the region identified by the white rectangle in A. C, µ-XRF image of part of the leaf blade identified by the white rectangle in B illustrating high Mn accumulation near the veins and in the interveinal necrotic lesions.

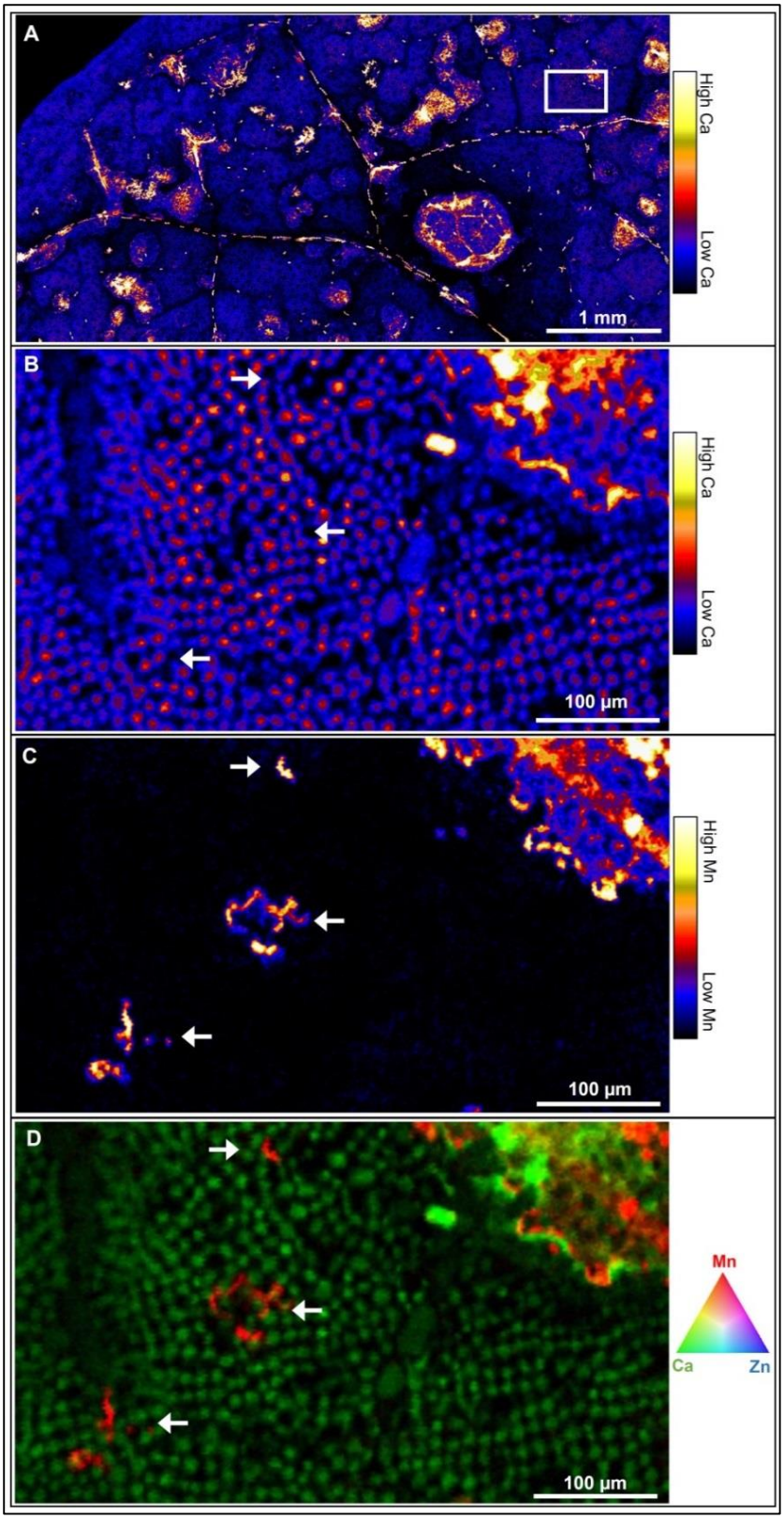

Figure S6. Distributions of Ca, Mn, and Zn in part of leaf of soybean grown at 100 µM Mn. A, Part of a leaf illustrating the distribution of Ca. B, Distribution of Ca in part of a leaf identified by the white rectangle in A. C, Distribution of Mn which is confined to visible (top right) and incipient necrotic lesions (white arrows) in part of a leaf identified by the white rectangle in A. D, Distributions of Ca (green), Mn (red), and Zn (blue) in part of a leaf identified by the white rectangle in A; Ca and Mn were not co-located in the undamaged area of the leaf. The white arrows in B, C, and D indicate the same point in each image to identify the incipient necrotic lesions.

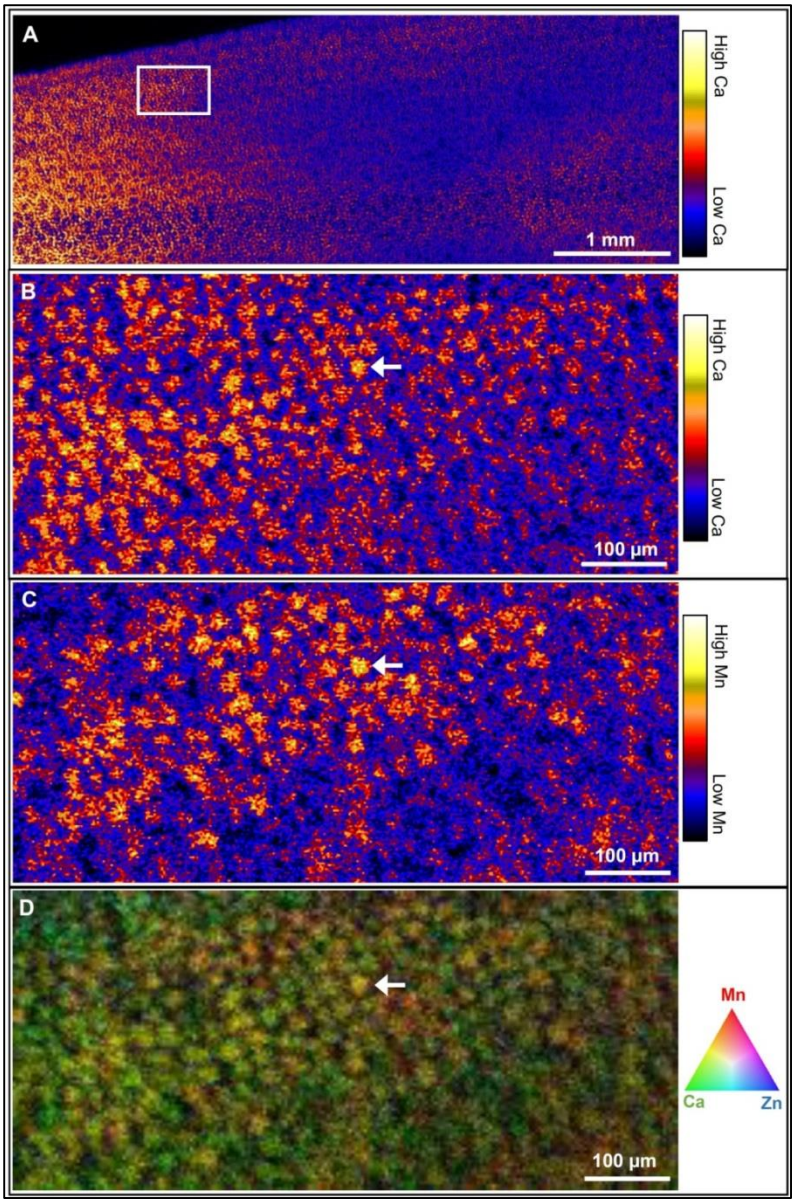

Figure S7. Distributions of Ca, Mn, and Zn in part of leaf of narrow-leafed lupin grown at 100 µM Mn. A, Part of a leaf illustrating the distribution of Ca. B, Distribution of Ca in part of a leaf identified by the white rectangle in A. C, Distribution of Mn in part of a leaf identified by the white rectangle in A. D, Distribution of Ca (green), Mn, (red), and Zn (blue) in part of a leaf identified by the white rectangle in A; Ca and Mn are co-located in vacuoles as shown by the yellow color. The white arrows in B, C, and D identify the same vacuole in each image.

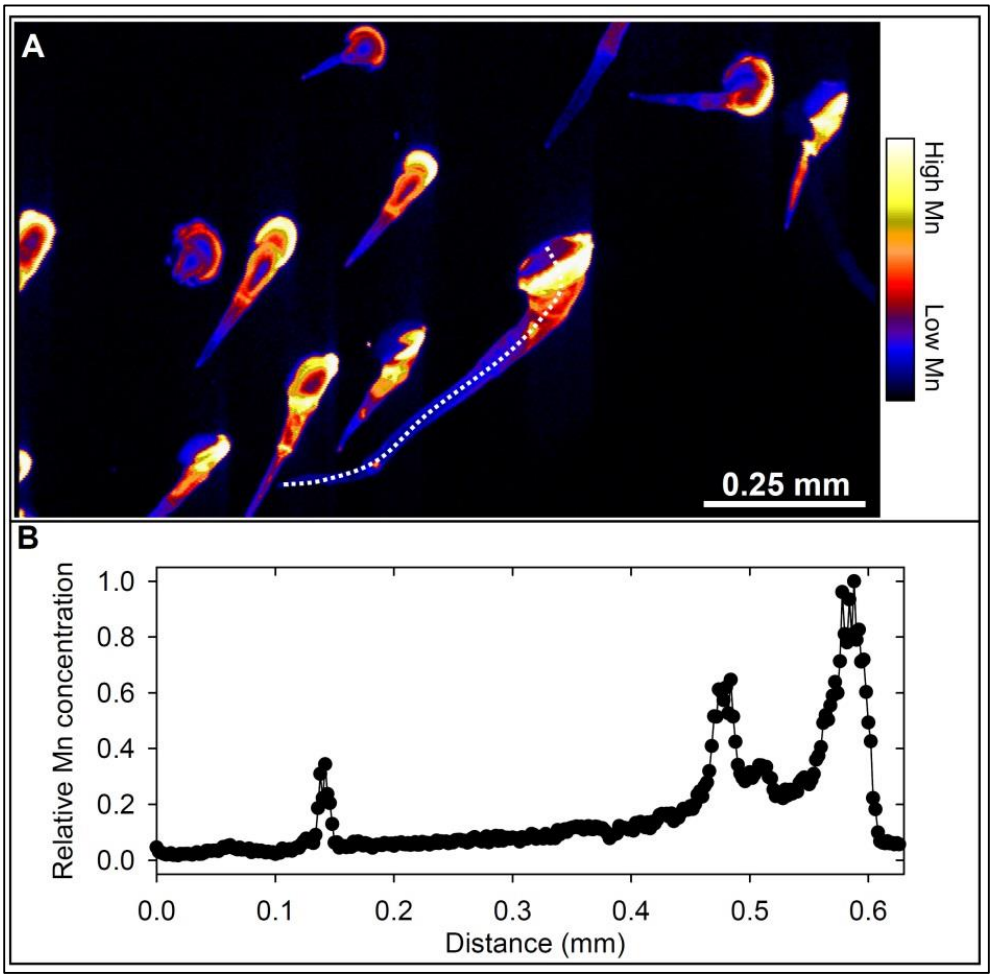

Figure S8. Relative Mn concentration in a sunflower non-glandular trichome. A, μ-XRF image of Mn in sunflower NGT of a plant grown for 16 d at 100 μM Mn. B, Relative Mn concentration along a traverse of one NGT identified in A as a dotted trace.

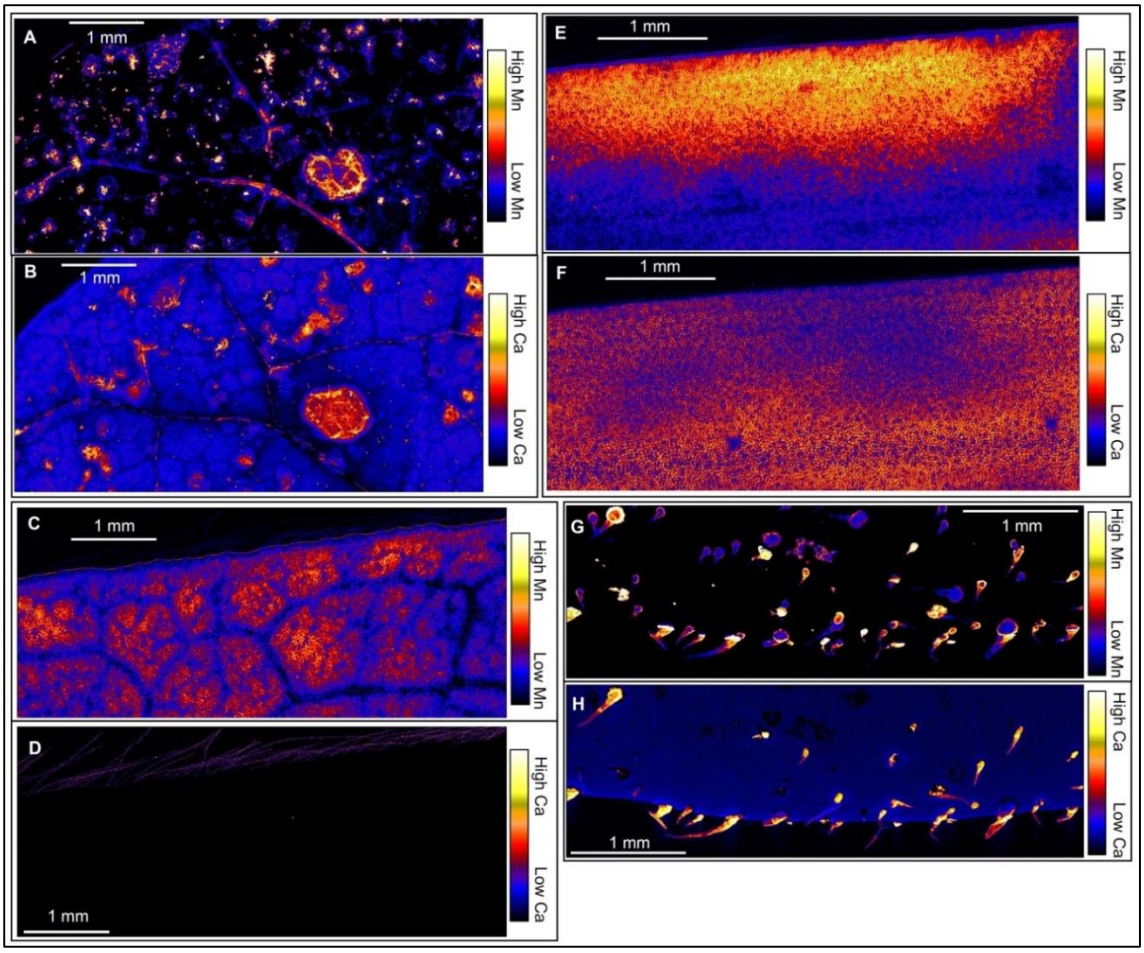

Figure S9. µ-XRF images of Mn and Ca distributions in areas of leaves of plants grown at 100 µM Mn in solution. A,B, soybean. C,D, white lupin. E,F, narrow-leafed lupin. G,H, sunflower.

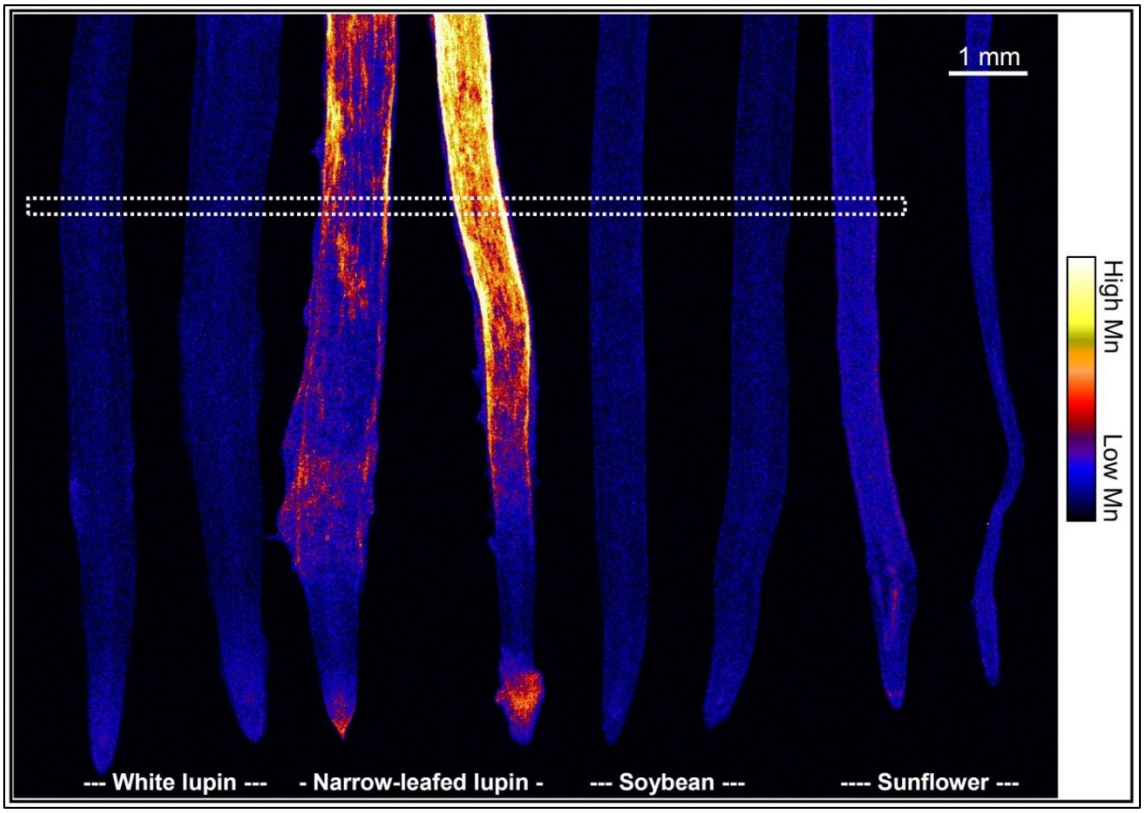

Figure S10. µ-XRF image of roots of white lupin, narrow-leafed lupin, soybean, and sunflower grown for 16 d at 100 µM Mn in solution culture. All roots were examined in a single scan and concentrations can be compared among roots. The right-hand sunflower root was dehydrated prior to scanning and should not be used for comparisons. The dotted white box identifies the region in which the predicted volumetric Mn concentration was determined (Supplemental Fig. S11).

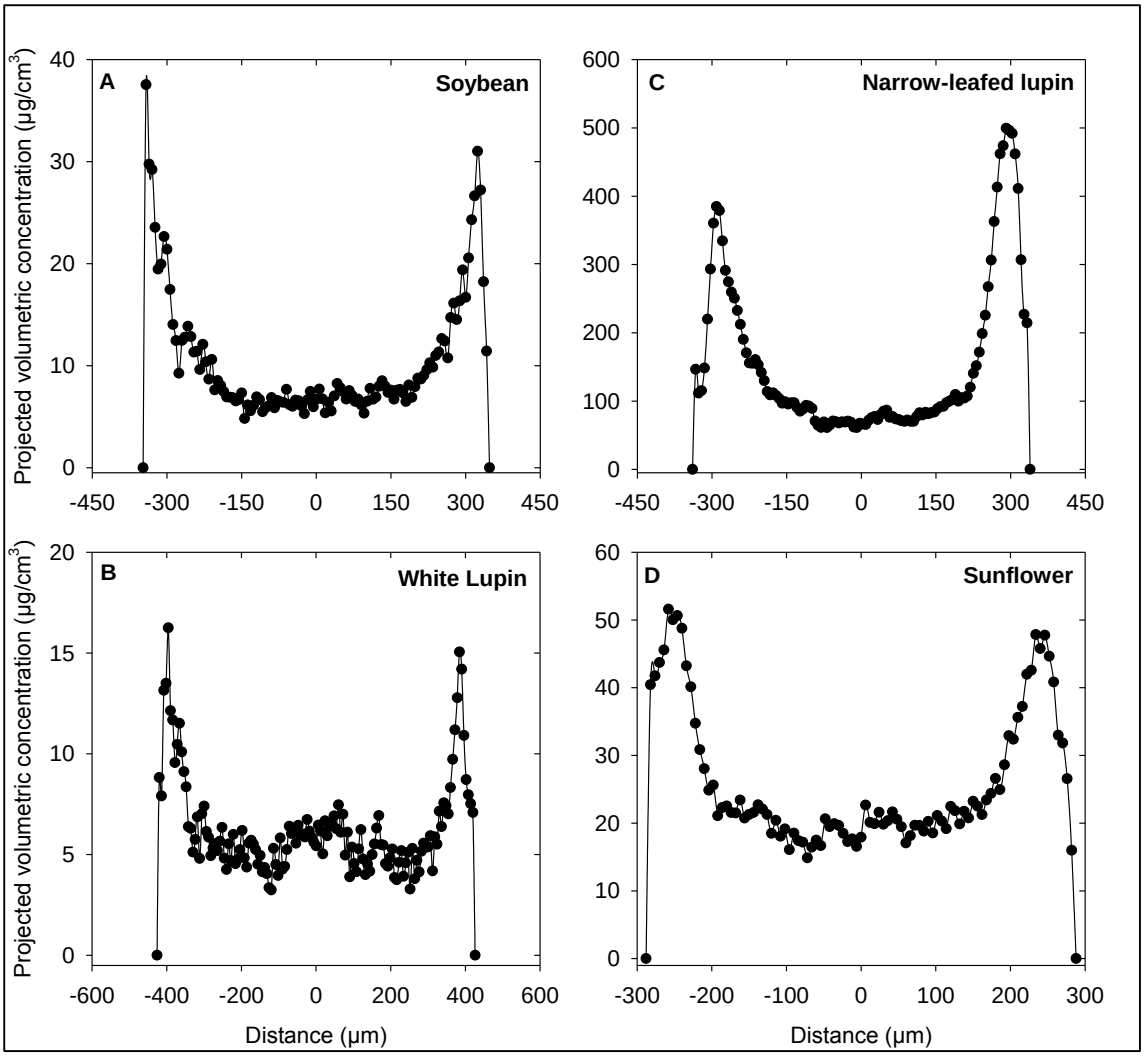

Figure S11. Projected volumetric concentration of Mn across a transect of roots (Supplemental Fig. S10). Given that the µ-XRF image (Supplemental Fig. S10) is a two-dimensional representation of roots of variable thickness, the volumetric concentration was calculated from the areal Mn concentration based on the root diameter. A, Soybean. B, White lupin. C, Narrow-leafed lupin. D, Sunflower.

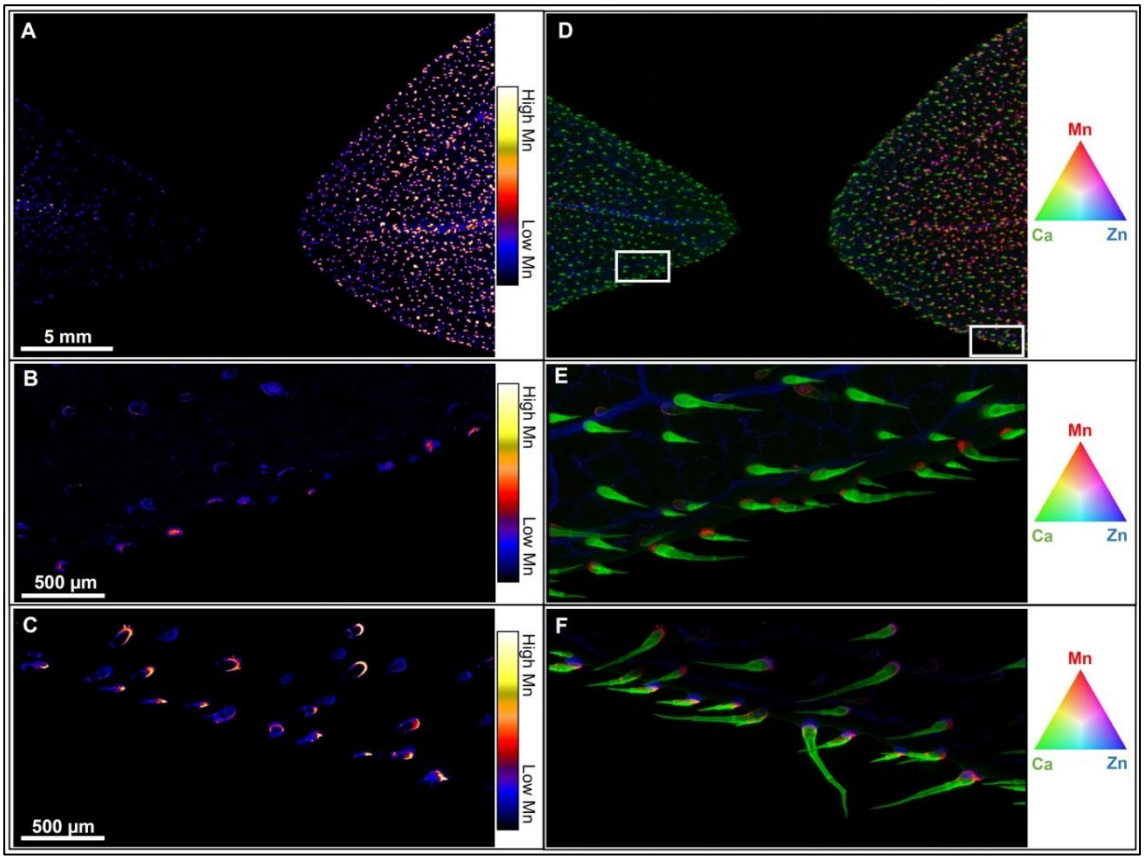

Figure S12. µ-XRF images illustrating the distributions of Mn, Ca, and Zn in non-glandular trichomes of sunflower at 0.5 µM Mn and after 1 d at 100 µM Mn. A, Mn distribution at 0.5 µM Mn (left) and 1 day after transfer from 0.5 to 100 µM Mn (right). B, Mn distribution of part of the leaf at 0.5 µM Mn identified by the white rectangle (left) in D. C, Mn distribution of part of the leaf after 1 d at 100 µM Mn identified by the white rectangle (right) in D. D, E, and F, Distributions of Ca (green), Mn (red), and Zn (blue) in the same parts of leaves in A, B, and C, respectively.

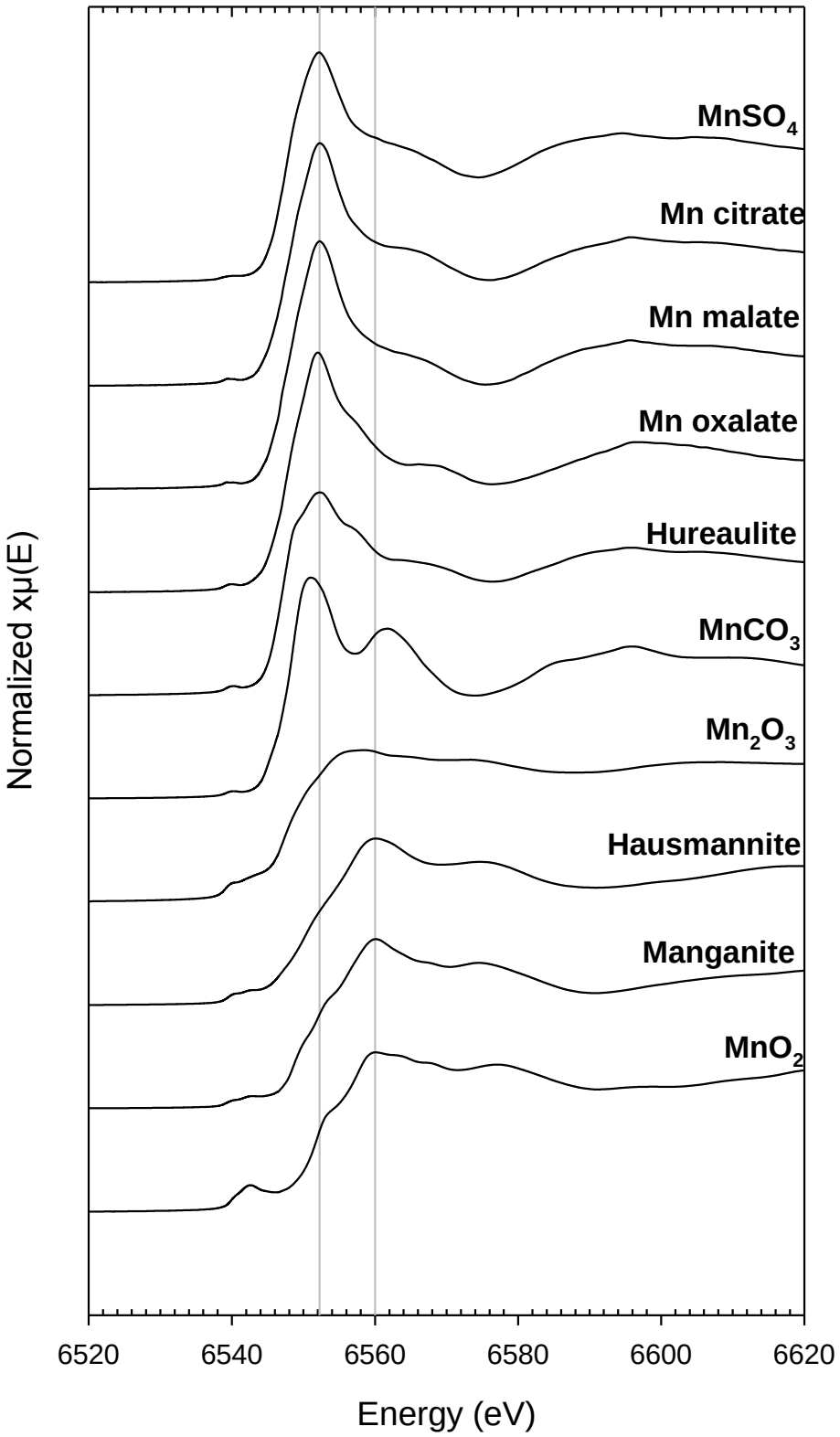

Figure S13. Normalized K-edge XANES spectra of standard Mn compounds as Mn(II) ($MnSO_4$, Mn citrate, Mn malate, Mn oxalate, Hureaulite, and $MnCO_3$), Mn(II) and Mn(III) (Hausmannite), Mn(III) (Mn_2O_3 and Manganite), and Mn(IV) (MnO_2). To allow comparison, the vertical lines correspond approximately to the white-line peaks of Mn(II) (6,552 eV) and Mn(III) and Mn(IV) (6,560 eV).

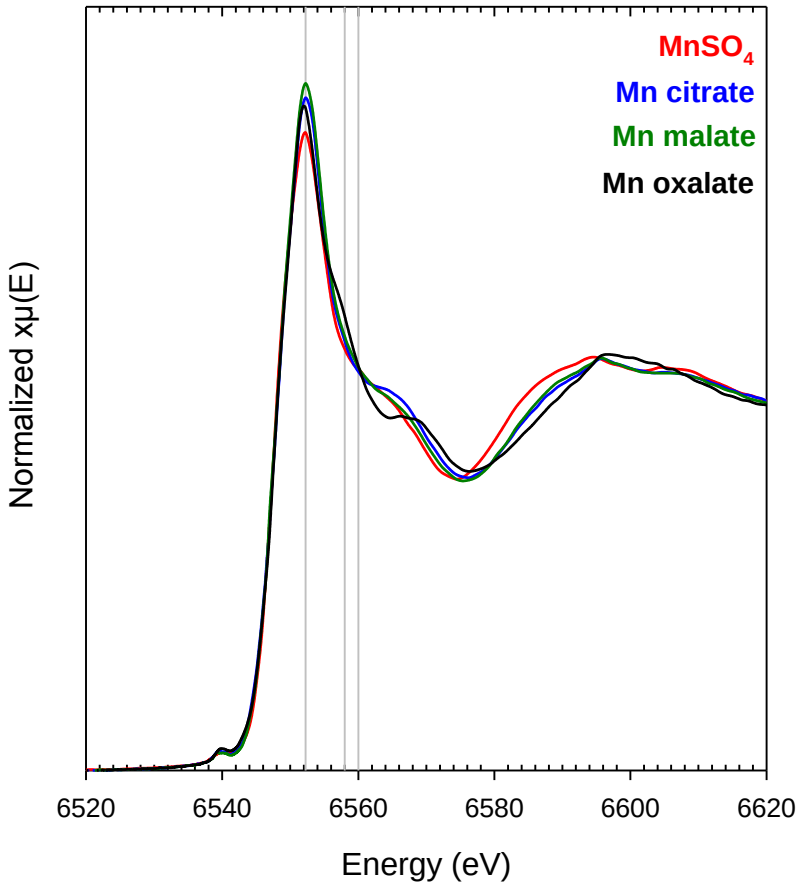

Figure S14. Normalized K-edge XANES spectra of four standard Mn(II) compounds. Note the similarity of the spectra for Mn citrate and Mn malate, precluding the differentiation of these two compounds using linear combination fitting (LCF). There are, however, minor differences in the aqueous Mn ($MnSO_4$) and Mn oxalate spectra.

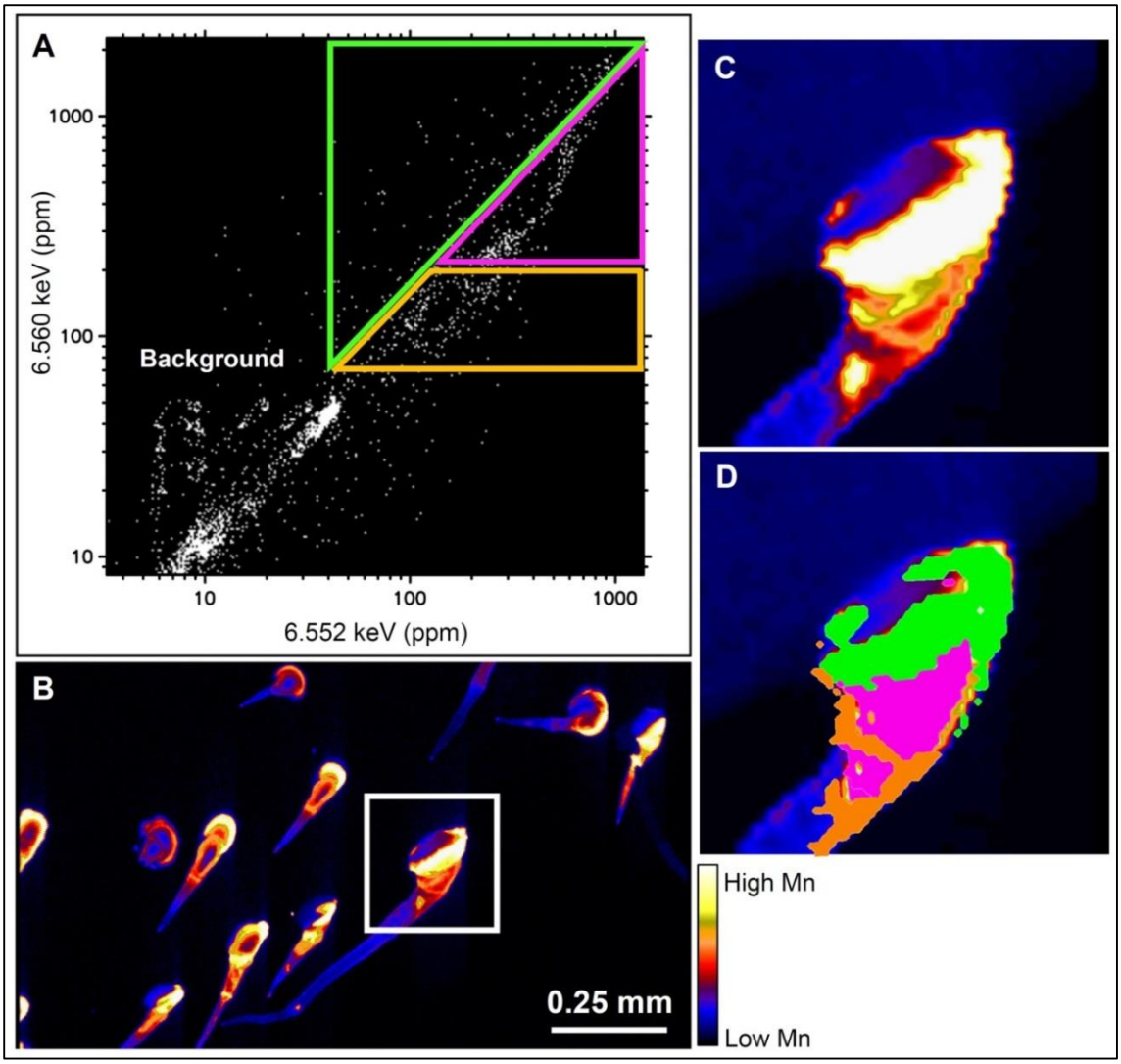

Figure S15. Illustration of the procedure used to identify the distribution of Mn populations using XANES imaging and the 'energy association' module in GeoPIXE. A, Relative energies of pixels at 6.552 and 6.560 keV, corresponding approximately to Mn(II) and Mn(III). This plot identified three populations bordered by (i) green as having higher concentrations of Mn(III) than Mn(II), and (ii) violet and orange as having higher concentrations of Mn(II) than Mn(III). Absolute concentrations of Mn(III) are higher in regions shaded violet than in those shaded orange. The pixels low in Mn were classified as being no different to the background. B, The sunflower NGT in the white box was used in this procedure. C, Higher magnification of the base of the sunflower NGT identified by the white box in B. D, Location of the three Mn populations identified in A.

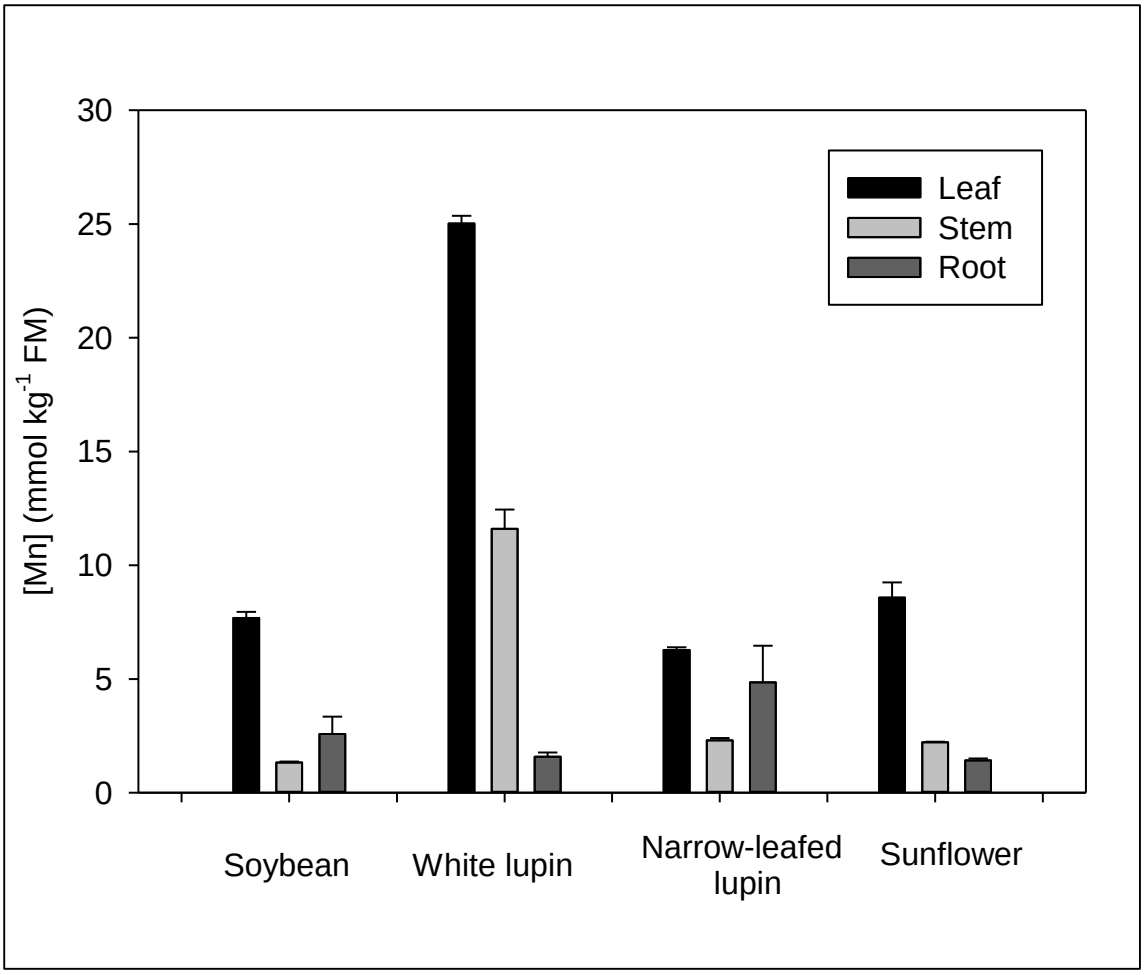

Figure S16. Concentration of Mn in freeze-dried leaves, stems, and roots of soybean, white lupin, narrow-leafed lupin, and sunflower grown for 13 d at 100 µM prior to XAS analyses. The data have been converted to a fresh mass (FM) basis using the percentage dry mass values in Supplemental Table S3.

Table S1. Dry mass of soybean, white lupin, narrow-leafed lupin, and sunflower leaf, stem plus petiole, and root tissues grown for 16 d after imposing treatments of 0.5, 30, and 100 µM Mn in solution. Data are means ± SE (n = 3).

	Dry mass of plant parts (g plant^{-1})				
Plant tissue	**[Mn] (µM)**	**Soybean**	**White Lupin**	**Narrow-leafed Lupin**	**Sunflower**
Leaf	0.05	1.28±0.41	0.78±0.16	0.47±0.04	0.98±0.09
	30	0.91±0.33	0.73±0.13	0.46±0.06	0.95±0.02
	100	0.67±0.24	0.76±0.14	0.52±0.09	0.66±0.13
Stem	0.05	0.75±0.27	0.35±0.01	0.27±0.05	0.53±0.10
	30	0.56±0.20	0.34±0.01	0.26±0.03	0.60±0.02
	100	0.45±0.13	0.35±0.01	0.31±0.06	0.47±0.08
Root	0.05	0.62±0.11	0.53±0.01	0.36±0.09	0.50±0.16
	30	0.41±0.14	0.54±0.05	0.38±0.05	0.49±0.10
	100	0.31±0.16	0.54±0.05	0.42±0.08	0.35±0.02

Table S2. Concentrations of citrate and malate in leaves, stems, and roots of soybean, white lupin, narrow-leafed lupin, and sunflower grown in nutrient solutions containing 100 µM Mn as determined by liquid chromatography mass spectrometry.

Species	**Tissue**	**Organic ligand concentration (mg kg^{-1} DM)**	
		Citrate	**Malate**
Soybean	Leaf	18.1	14.5
White lupin	Leaf	3.3	32.4
Narrow-leafed lupin	Leaf	7.0	16.2
Sunflower	Leaf	2.7	2.1
Soybean	Stem	6.1	5.1
White lupin	Stem	2.8	25.0
Narrow-leafed lupin	Stem	2.2	8.1
Sunflower	Stem	2.4	7.6
Soybean	Root	5.9	4.6
White lupin	Root	12.2	1.5
Narrow-leafed lupin	Root	15.6	1.7
Sunflower	Root	1.9	1.3

Table S3. The percentage Mn speciation in roots, stems, and leaves of four plant species grown for 13 d at 100 µM Mn as calculated using linear combination fitting of the K-edge XANES spectra.

Tissue	Species	Mn(II) malate or citrate[1]	Manganite [Mn(III)]	Mn(III) oxide	R-factor[2]
Leaf	Soybean	34	66		0.0012
	White lupin	84	16		0.0064
	Narrow-leafed lupin	85	15		0.0068
	Sunflower	57	43		0.0045
Stem	Soybean	74		26	0.0015
	White lupin	81	19		0.0099
	Narrow-leafed lupin	85	15		0.0078
	Sunflower	78	22		0.0053
Root	Soybean	87	13		0.0042
	White lupin	87	13		0.0060
	Narrow-leafed lupin	90	10		0.0086
	Sunflower	91	9		0.0063

[1]*Given the similarity in the spectra for Mn(II) malate and Mn(II) citrate, we did not distinguish between these two compounds during LCF.*

[2]*The goodness of fit is indicated by the R-factor.*

R factor $= \sum i(\text{experimental} - \text{fit})^2 / \sum i(\text{experimental})^2$*, where the sums are over the data points in the fitting region.*

Table S4. Mean dry mass percentage of soybean, white lupin, narrow-leafed lupin, and sunflower leaf, stem, and root grown for 13 d at 100 µM Mn in solution culture. Data are the means ± SE (n = 2).

Species	**Dry mass (%)**		
	Leaf	**Stem**	**Root**
Soybean	24.2 ± 1.0	14.8 ± 0.6	5.9 ± 0.4
White lupin	11.1 ± 0.3	11.0 ± 1.7	4.4 ± 0.1
Narrow-leafed lupin	12.0 ± 2.0	13.5 ± 1.4	4.4 ± 0.4
Sunflower	15.6 ± 0.8	8.5 ± 0.1	5.6 ± 0.1