Supporting information

Spatial localization of monoterpenoid indole alkaloids in *Rauvolfia tetraphylla* by high resolution mass spectrometry imaging

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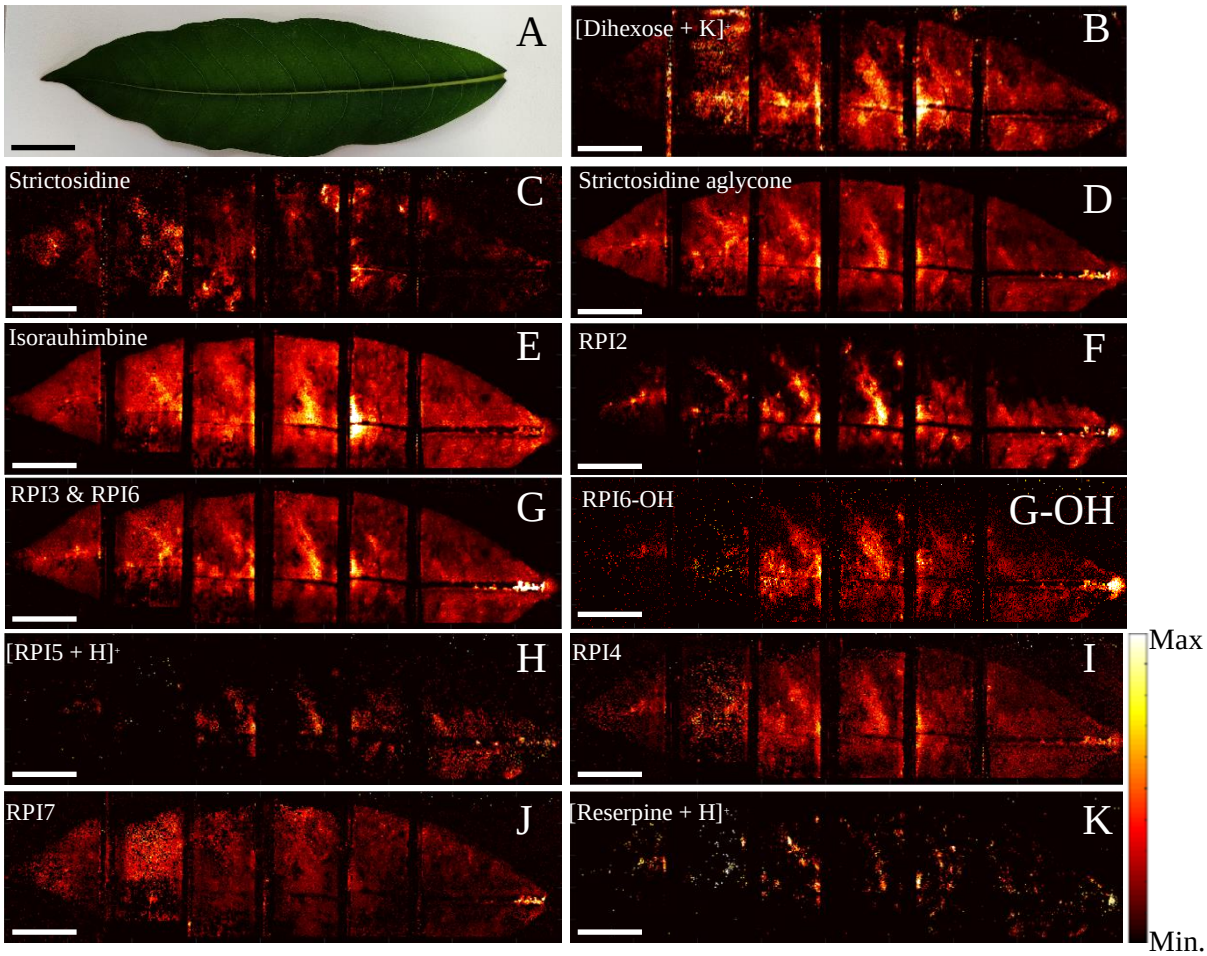
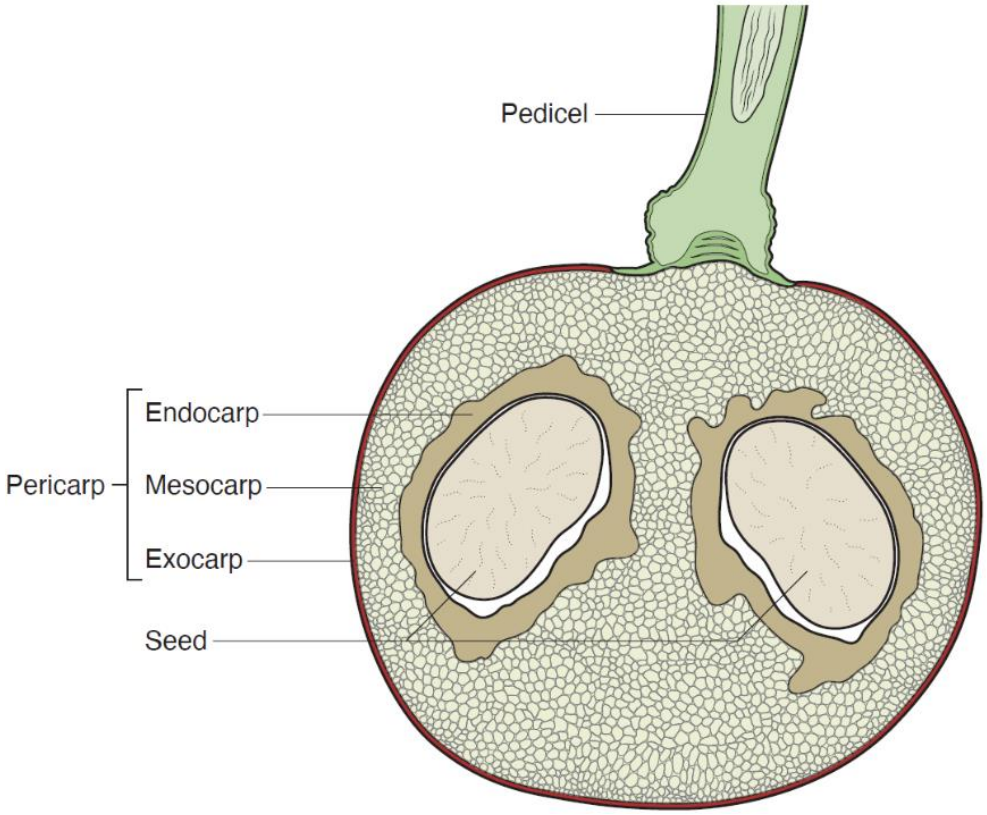
S1: DESI-MS images of old leaf Rauvolfia tetraphylla imprinted on PTFE.

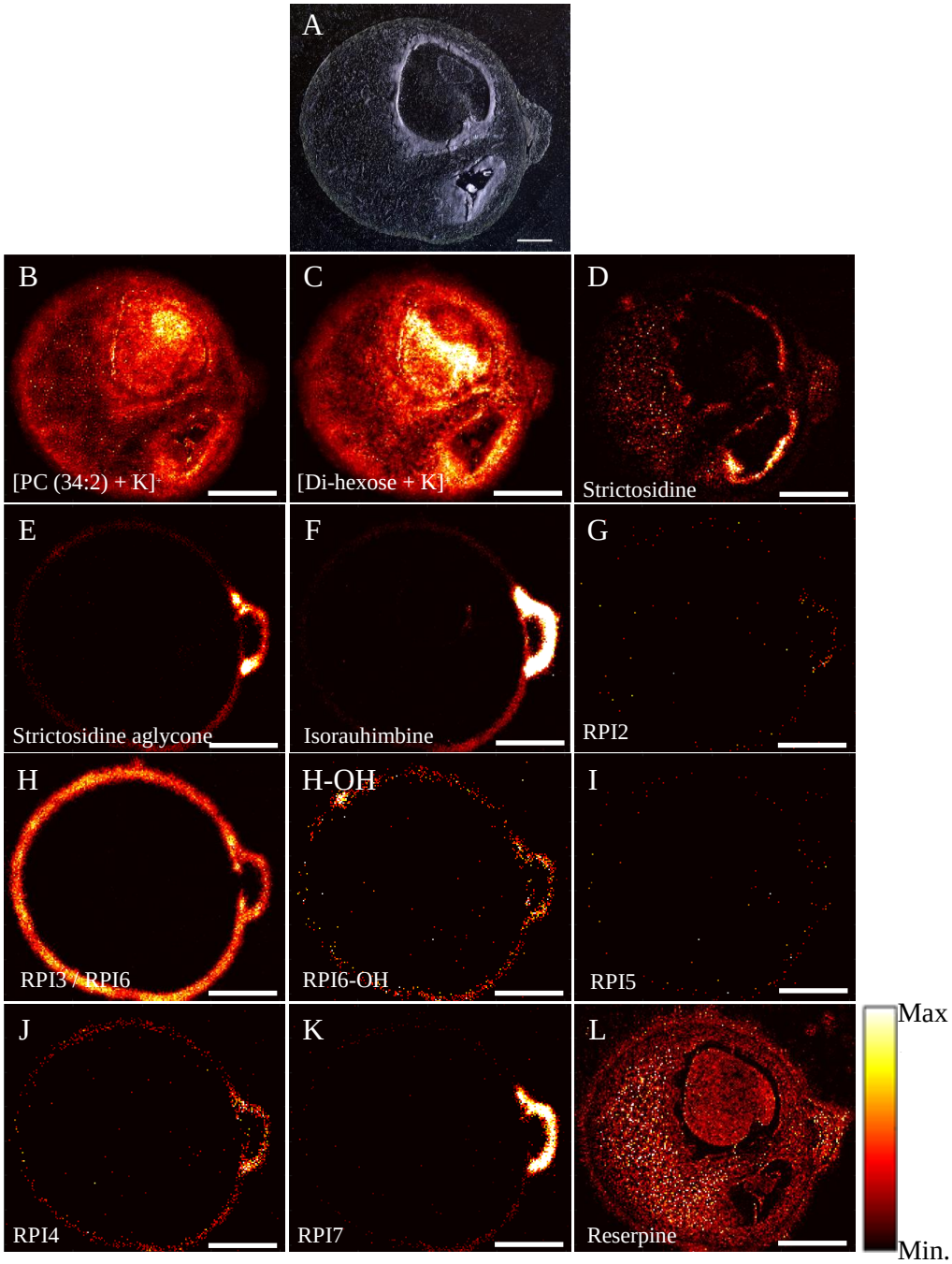


(A) Photo of leaf. **(B)** Di-hexose. **(C)** Strictosidine. **(D)** Strictosidine aglycone. **(E)** Isorauhimbine. **(F)** RPI2. **(G)** RPI3 & RPI6. **(G-OH)** RPI6-OH. **(H)** RPI5. **(I)** RPI4. **(J)** RPI7. **(K)** Reserpine. The pixel size is 300 µm. Scale bars: 20 mm.

S2: Illustration of fruit tissue of *Rauwolfia tetraphylla*.

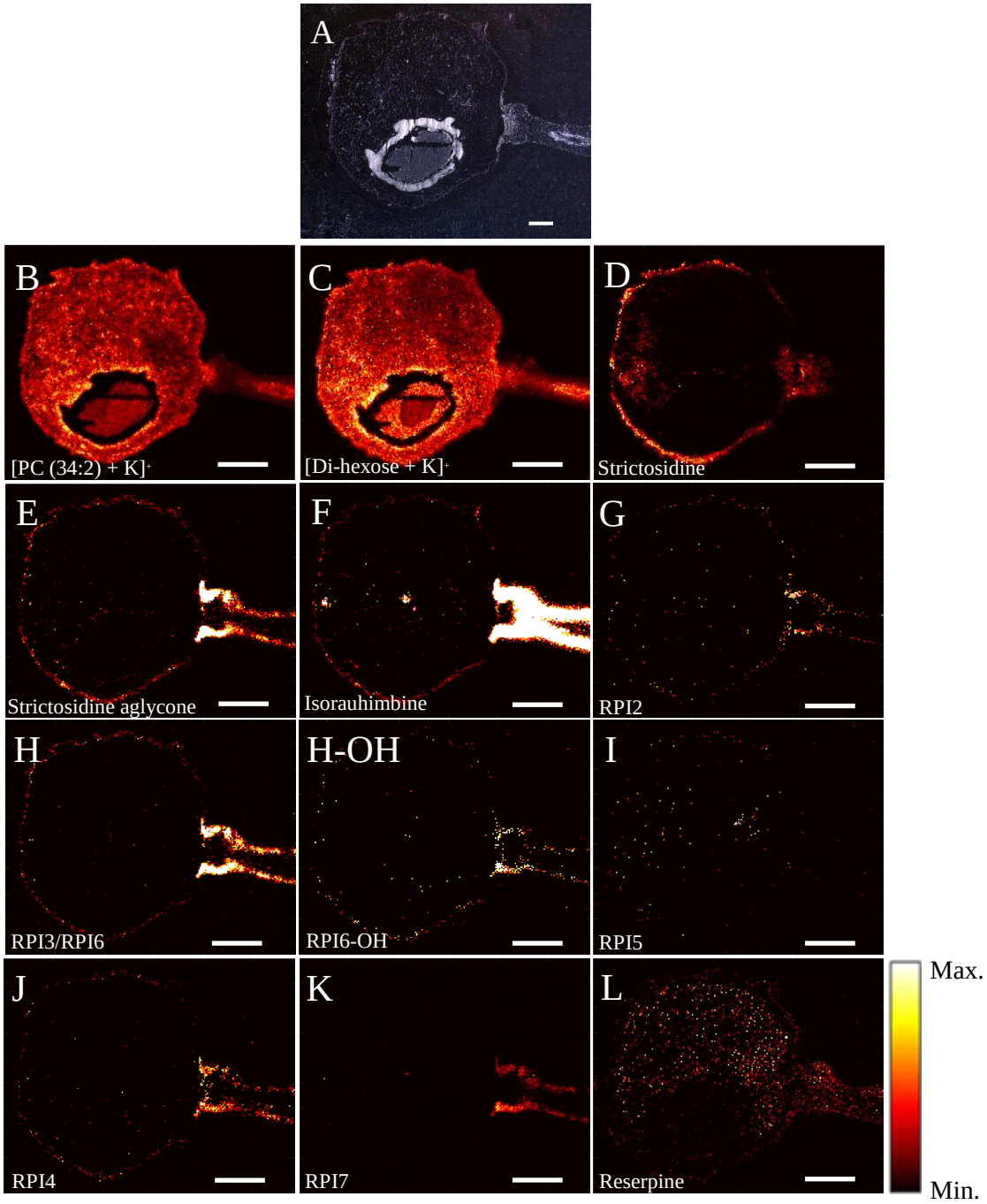


S3: MALDI-MS images of immature fruit tissue



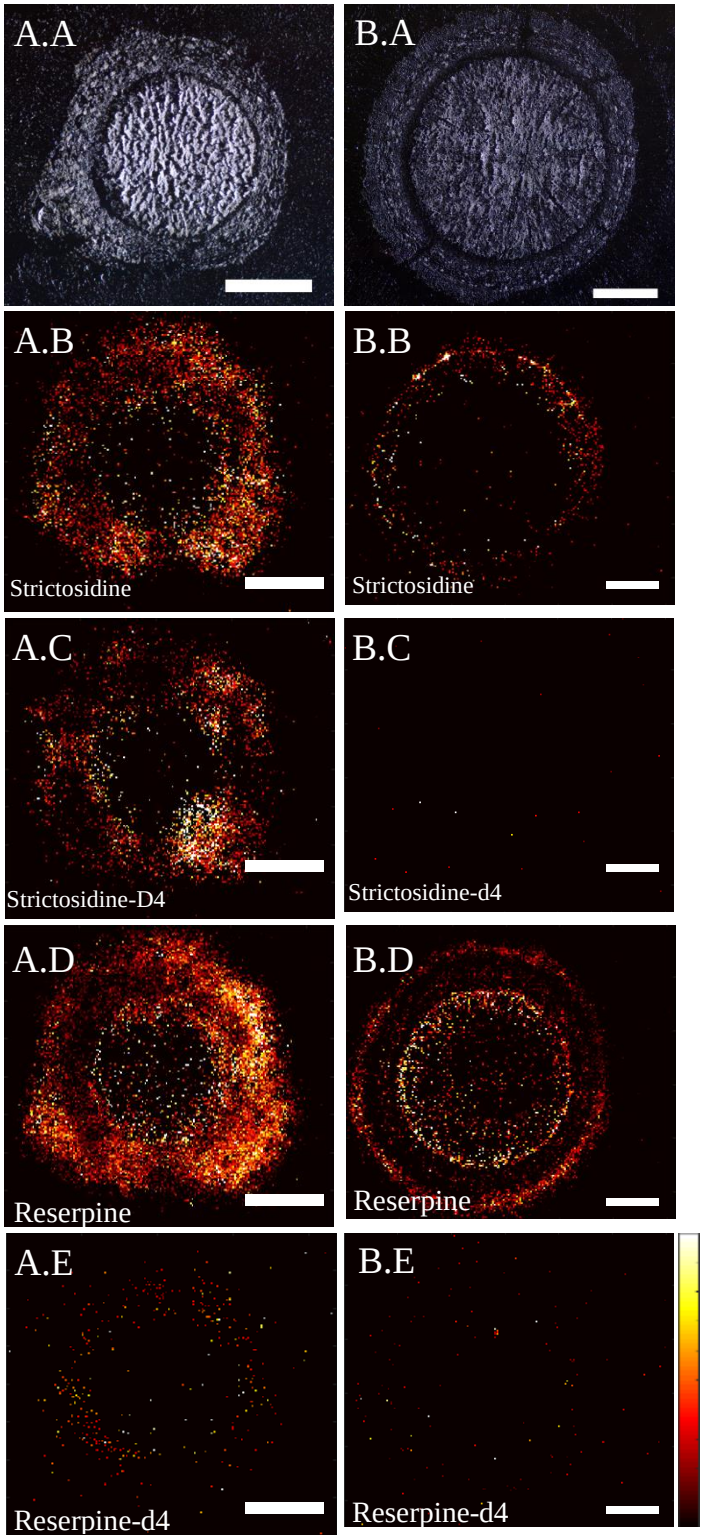
(A) Microscope image, scale bar = 1 mm. **(B)** PC (34:2). **(C)** Di-hexose. **(D)** Strictosidine. **(E)** Strictosidine aglycone. **(F)** Isorauhimbine. **(G)** RPI2. **(H)** RPI3/RPI6. **(H-OH)** RPI6-OH. **(I)** RPI5. **(J)** RPI4. **(K)** RPI7. **(L)** Reserpine. The pixel size is 40 µm. Scale bars: 2 mm.

S4: MALDI-MS images of mature fruit tissue



(A) Microscope image, scale bar = 1 mm. **(B)** PC (34:2). **(C)** Di-hexose. **(D)** Strictosidine. **(E)** Strictosidine aglycone. **(F)** Isorauhimbine. **(G)** RPI2. **(H)** RPI3/RPI6. **(H-OH)** RPI6-OH. **(I)** RPI5. **(J)** RPI4. **(K)** RPI7. **(L)** Reserpine. The pixel size is 50 µm. Scale bars: 2 mm.

S5: MALDI MS-images of root tissue treated with tryptamine-d4 and untreated root tissue.



In this figure, two MSI experiments are depicted. The MS images of a tryptamine-d4 administered root are marked with A.A to A.E. The root that have not been administered 4D-tryptamine is marked with B.A to B.E. Each MS-image contains a scale bar, which corresponds to 1 cm.

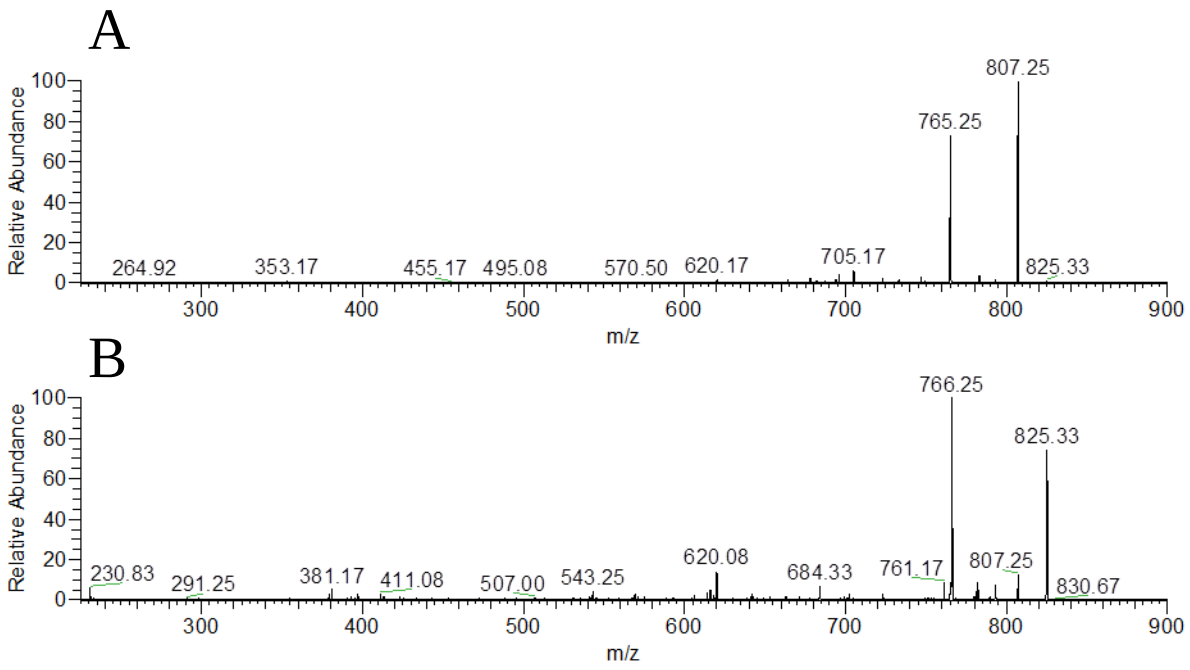
(**A.A**) Microscope picture, scale bar 1000 µm. (**A.B**) Strictosidine. (**A.C**) strictosidine-d4. (**A.D**) Reserpine. (**A.E**) Reserpine-d4.

(**B.A**) Microscope picture, Bar 1000 µm. (**B.B**) Strictosidine. (**B.C**) strictosidine-d4. (**B.D**) Reserpine. (**B.E**) Reserpine-d4.

The presented MS images represent for each compound the sum of the $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions, generated from the *m/z* values 531.23371, 553.21565 and 569.18959 for strictosidine, 535.25881, 557.24075 and 573.21469 for strictosidine-d4, 609.28066, 631.26260 and 647.23654 for reserpine and 613.30576, 635.28770 and 651.26164 for reserpine-d4.

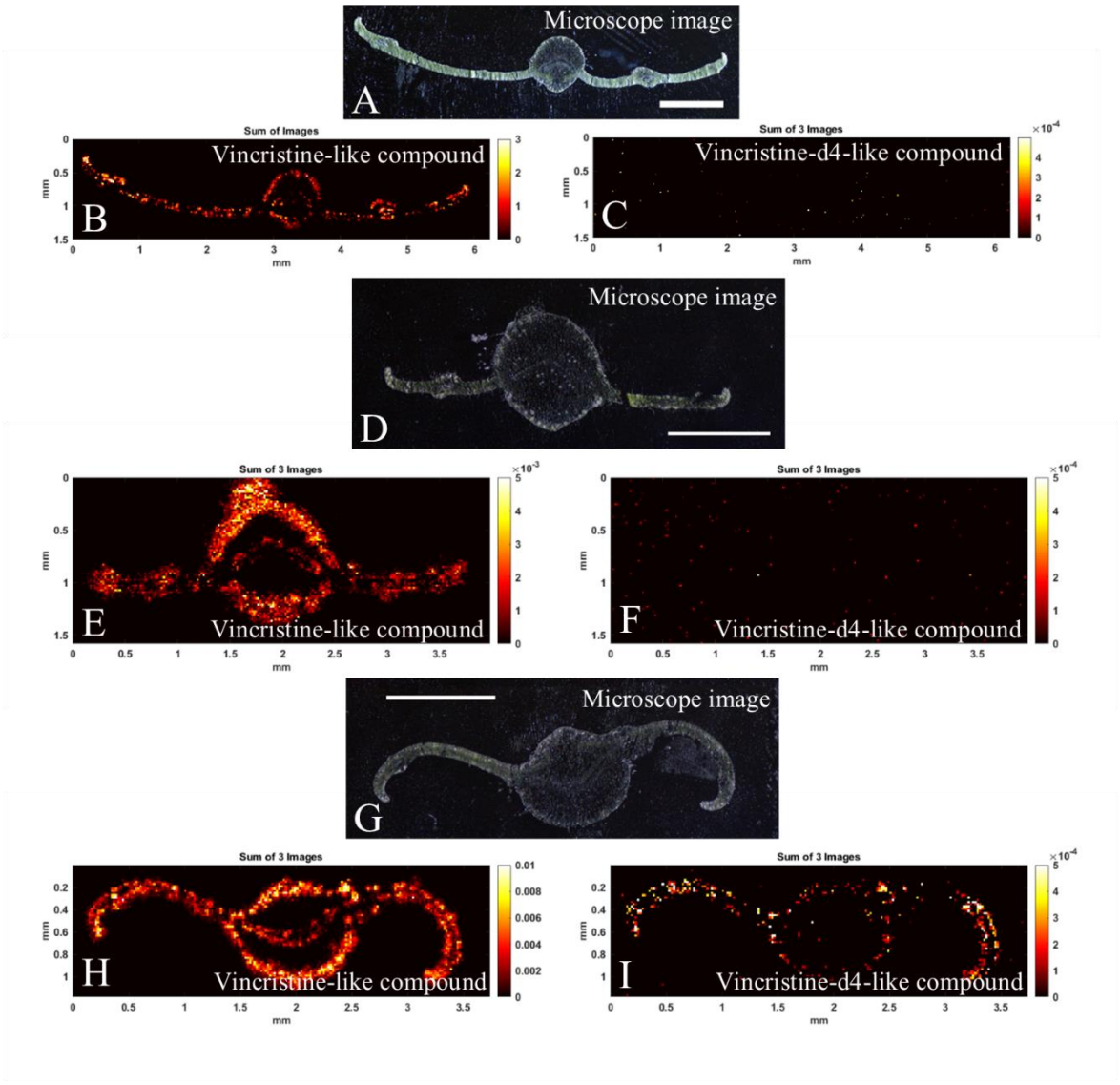
Each MS-image was made with a bin width of 5 ppm. MS-images The pixel size in A.A to A.E is 20 µm, and the pixel size in B.A to B.E is 30 µm.

S6: MS/MS spectra of vincristine standard and the unknown compound with same exact mass.



Mass spectra A shows the fragmentation pattern of vincristine from a vincristine standard. Mass spectra B shows the fragmentation pattern of the unknown compound with the same exact mass as vincristine. Both compounds produce a fragment *m/z* 807.25, however this fragment is much higher abundant in vincristine than the unknown compound. Vincristine also produces a fragment of *m/z* 765, which the unknown compounds does not produce. It does however produce a fragment of *m/z* 766.

S7: Unknown vincristine-like compound ($C_{46}H_{56}N_4O_{10}$) in three different leaves.



(A) Microscope picture of young untreated leaf (bar = 1mm). (B) MS image of ions corresponding to the unknown compound in an untreated leaf. (C) MS image of ions corresponding to the d4-labelled unknown compound in an untreated leaf.

(D) Microscope picture of young leaf treated with tryptamine (bar = 1mm). (E) MS image of ions corresponding to the unknown compound in a leaf treated with tryptamine. (F) MS image of ions corresponding to the d4-labelled unknown compound in a leaf treated with tryptamine.

(G) Microscope picture of young leaf treated with tryptamine-d4 (bar = 1mm). (H) MS image of ions corresponding to the unknown compound in a leaf treated with tryptamine-d4. (I) MS image of ions corresponding to the d4-labelled unknown compound in a leaf treated with tryptamine-d4.

All images are normalized by the total ion current (TIC) and generated from the sum of the ion intensities of [M+H]+, $[M+Na]^+$ and $[M+K]^+$ (*m/z* values are found in Table 1).