

# Supporting Information

## Source Supported Suspect Screening (4S) of Phytotoxins in Terrestrial and Aquatic Environment – a Field Study of *Lupinus angustifolius* L. (Blue Lupin)

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Summary: 13 pages, 2 tables, 5 figures.

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## S-1. Overview of the Sampling Campaign

Table S1. Sampling campaign. Numbers below Plant, Soil and Water are samples collected, including replicates.

Date (m/d/y)	Adjusted calendar day	Plant			Soil (0-20 cm)	Water		Note
		Root	Leave	Stem		Drainage	Surface	
4/15/2019	0				3*	2		Manure application
5/17/2019	31	1	1**		3	3		Growing season
6/11/2019	56	3	3	3	3	3	3	
6/28/2019	73	***	3	3	3	3		
7/03/2019	78	3	3	3	3	3		
7/13/2019	88	3	3	3	2	3	3	
7/23/2019	98	3	3**		3	7	3	
8/10/2019	116				3	3		Post-harvest
8/20/2019	126				3	3		
	Sum	41			26	39		

\*Soil-manure mixture

\*\* Sample collected as shoot (combined leave and stem)

\*\*\* Sample cannot be obtained due to extremely dry soil.

## S-2. Chemicals

Water was purified in a Millipore Milli Q-Plus system (Bedford, USA). Methanol (MeOH), acetonitrile (ACN), formic acid (FA), ammonium formate (AF) and ammonia (AM) were of LC-MS grade and purchased from Sigma-Aldrich (Germany). AF was dissolved in millipore water at a concentration of 5 M for preparing mobile phase solutions. Authentic phytotoxin standards of analytical grade were obtained from different suppliers. Convallatoxin (CTX) was purchased from MP Biomedicals (Irvine, CA, USA). Strophanthidin (STR), digoxin (DGX), bufalin (BUF), withanolide A (WTH), caffeine (CAF), genistein (GEI), genistin (GTI), gramine (GRA), (-)-lupinine (LUP), (+)-sparteine (SPA), cytisine (CYT) and artemisinin (ART) were purchased from Sigma-Aldrich (Hamburg, Germany). Formononetin (FOR), daidzein (DAI) and chelidonine (CHE) were purchased from PhytoLab (Vestenbergsgreuth, Germany). Lycopsamine-D7 (LYC-D7) and lycopsamine N-oxide-D7 (LYCNO-D7) were purchased from Toronto Research Chemicals (North York, Ontario, Canada). All stock solutions were prepared in MeOH at 0.5 mg/mL and stored at -20 °C until use.

### S-3. Optimization of MS<sup>E</sup> Scan Time

The effect of scan time using MS<sup>E</sup> mode was investigated at scan times of 0.3, 0.5, 0.7 and 1 sec. Evaluation was primarily based on peak heights of eight PT standards (i.e. CTX, BUF, GRA, LUP, SPA, RTR, SEN and ART) prepared in 50% aqueous MeOH. As shown in Fig. S1, longer scan time yielded higher peak heights and this tendency leveled out at 0.7 sec and above. The number of mass spectra acquired for a chromatographic peak was counted, which were 18, 11, 5 and 5 for scan time of 0.3, 0.5, 0.7 and 1 sec respectively. Since a reasonable chromatographic peak representation typically requires 10-30 data points per peak, the optimal scan time should be decided by a trade-off between desired peak representation and peak height. We decided to set the scan time at 0.5 sec as it gave sufficient data points across a chromatographic peak (i.e. 11 points) meanwhile the peak height was reduced by approximately a factor of 2 in comparison to 0.7 sec and 1.0 sec.

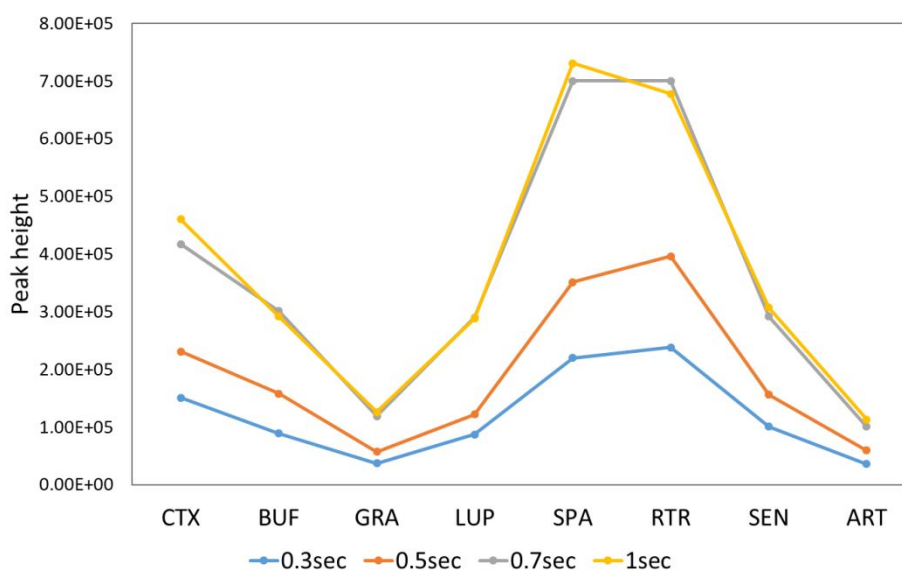


Figure. S1. Signal intensities (determined as peak heights) of reference PTs at different scan time settings. CTX, convallatoxin; BUF, bufalin; GRA, gramine; LUP, lupinine; SPA, sparteine; RTR, retrosine; SEN, senecotine; ART, artemisinin.

## S-4. Quality Assurance

The following quality control procedures were applied to the study in order to ensure the reliability of the results. Two isotopically labelled alkaloids, i.e. LYC-D7 and LYCNO-D7, were spiked to all samples as internal standards (iSTDs) before extraction (plant and soil) or concentration (water). Procedural blanks (i.e. sPLE cells packed with packing materials (glass beads), mSPE cartridges loaded with millipore water) were analyzed to confirm there was no interferences from the laboratory procedures. For LC-MS analysis, samples were divided into four batches according to matrices, i.e. plant (split into two batches), soil and water. In each batch, samples were randomized. In-between batches, the ion source was cleaned. A mixture of thirteen analytical standards covering different PT classes and diverse physiochemical properties (i.e. CTX, STR, DGX, BUF, WTH, CAF, GEI, GTI, GRA, LUP, SPA, CYT and ART) was prepared in composite plant and soil extracts respectively and used as matrix-matched quality control (M-QC), or in 50% MeOH solvent as solvent-matched quality control (S-QC). The M-QCs were assorted with the corresponding batch and injected five times at the beginning of the sequence to matrix-equilibrate the system (which serves to minimize drift in the subsequent runs). While running the sequence, one S-QC and two M-QCs were injected after every five samples and in the end of the sequence. The instrument was calibrated both externally with sodium formate clusters and internally using leucine enkephalin. Prior to data processing, the signal intensities of analytes were corrected by the averaged signal intensities of iSTDs. The S-QCs and the M-QCs were used to evaluate the drift within and between batches. Furthermore, the QCs were used to evaluate the matrix effects in plant and soil, and thus in the concentration estimates for plant and soil, matrix effects have been compensated for.

## S-5. Analytical Information of PTs Identified at Level 1

Table S-2. Analytical information of PTs identified at level 1. Detection limit (DL) and limit of quantification (LOQ) were estimated based on signal-to-noise ratio (S/N) of background-subtracted spiked sample ( $S/N_{\text{Spiked}} - S/N_{\text{Unspiked}}$ ), which were extrapolated to  $S/N = 3$  and 10 respectively. Matrix effect (ME) was determined by comparing signal intensities (peak heights) of spiked sample, unspiked sample and spiked solvent, calculation detailed previously.<sup>1</sup>

Compound	Plant		
	DL (μg/g)	LOQ (μg/g)	ME (%)
GTN	1.29	4.29	-52
GEN	0.611	2.04	-31
GRA	0.117	0.389	-22
SPA	$7.30 \times 10^{-3}$	$2.43 \times 10^{-2}$	-16

Compound	Soil		
	DL (ng/g)	LOQ (ng/g)	ME (%)
GTN	8.62	28.7	-66
GEN	5.14	17.1	-37
GRA	0.586	1.95	-12
SPA	$6.57 \times 10^{-2}$	0.219	3
CAF	1.15	3.85	5
FOR	0.581	1.94	-5
DAI	2.59	8.62	-12

Compound	Water*	
	DL (ng/L)	LOQ (ng/L)
GTN	1.37	4.55
GEN	0.684	2.28
GRA	0.361	1.20
SPA	$3.27 \times 10^{-2}$	0.109
CAF	0.108	0.359
FOR	0.259	0.863
DAI	0.468	1.57
SEN	0.199	0.663
RETNO	0.115	0.384
CHE	0.102	0.339

\*ME not determined.

S-6. Heat map of detected PTs in root, leaf and stem along the growing season

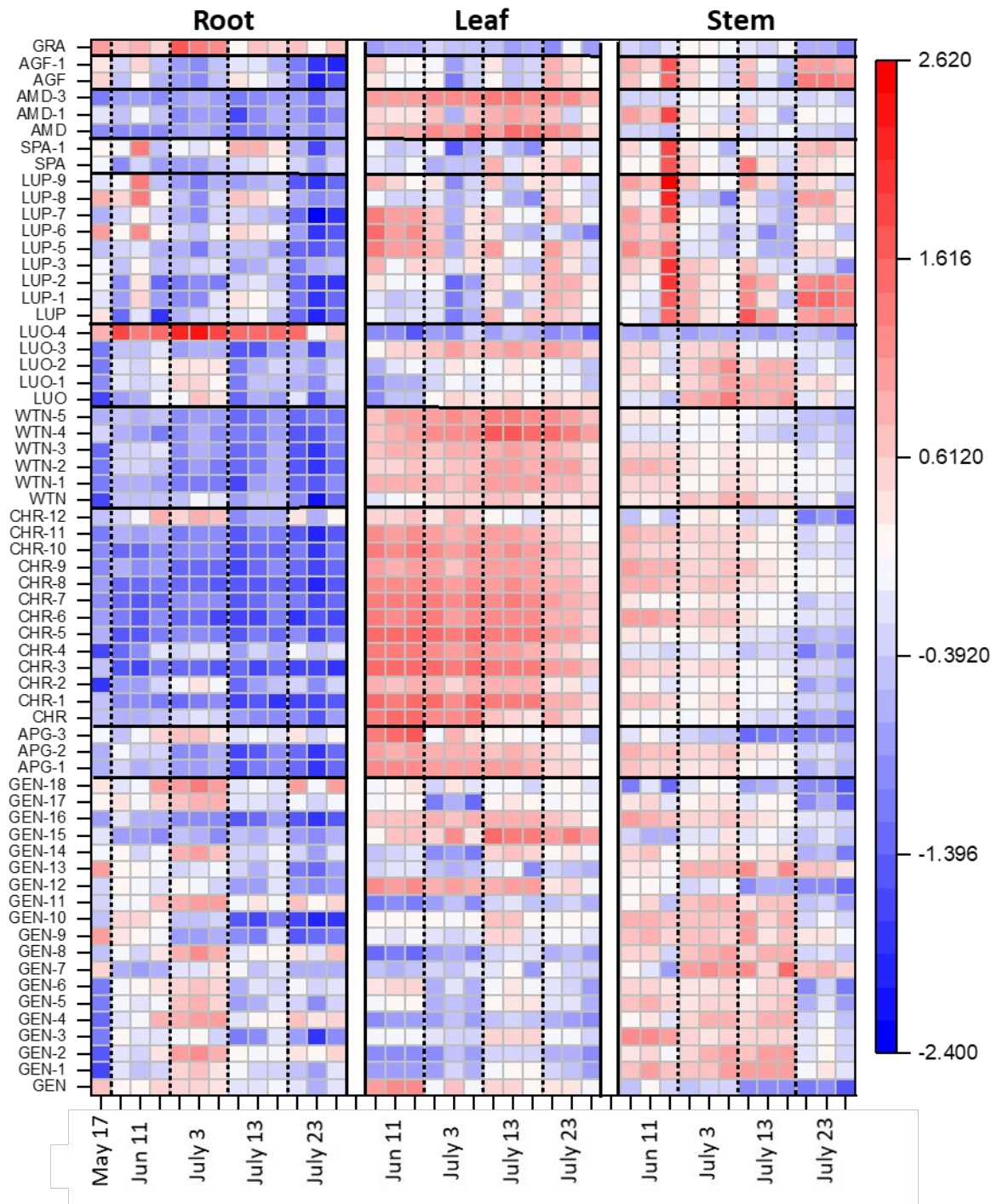




Figure. S2. Heatmap of the PTs (for full names of abbreviations, see Table 2) in *L. angustifolius* root, leaf and stem. The abundance (chromatographic peak height) of each compound was logarithmic-transformed and Pareto-scaled. RT, root; LV, leaf; ST, stem.

S-7. Overview of precipitation, plant biomass and plant water content along the growing season

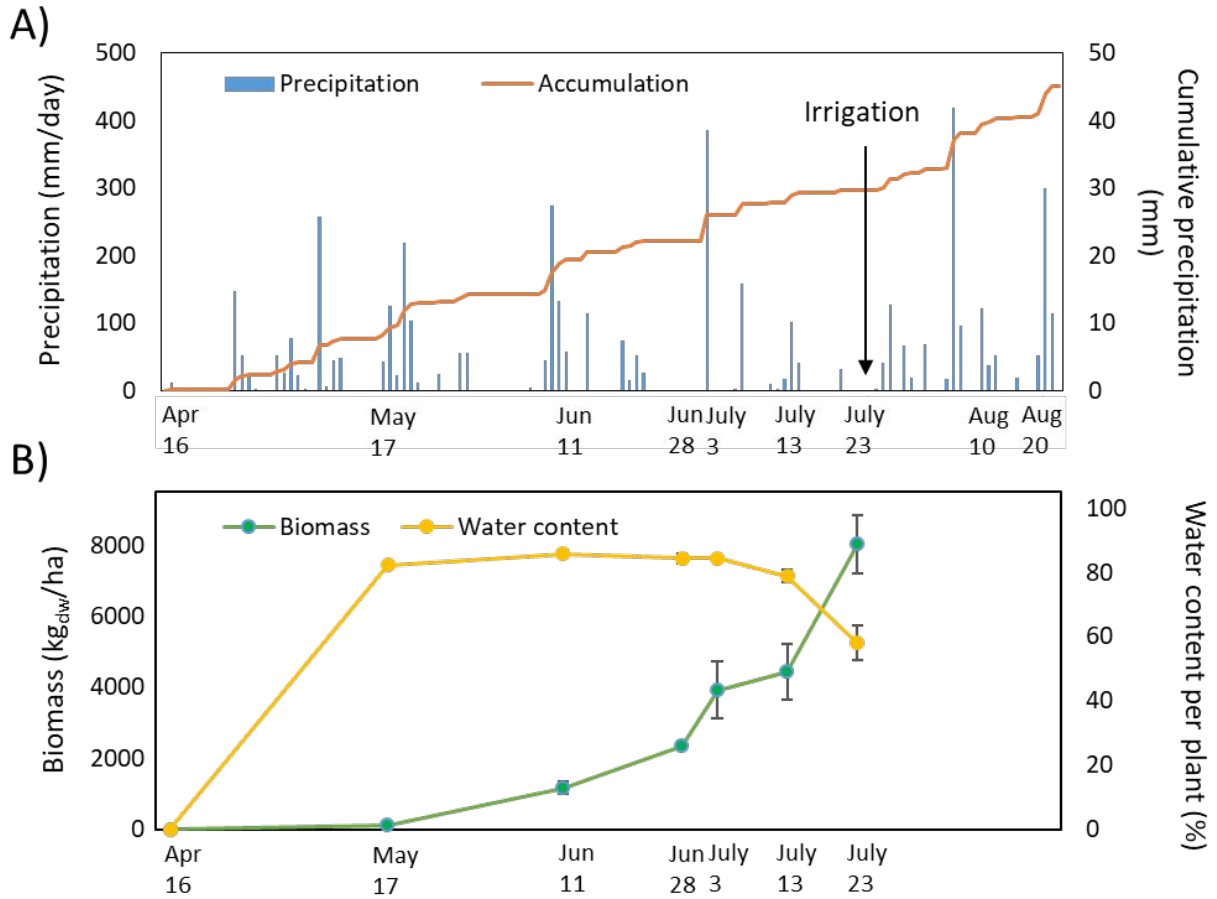


Figure S3. (A) Precipitation and cumulative precipitation monitored at the field site through Apr 15<sup>th</sup> to Aug 20<sup>th</sup> in 2019. (B) Plant biomass and water content. Note Aug 10<sup>th</sup> and Aug 20<sup>th</sup> were post-harvest.

S-8. Principal component analysis (PCA) of PTs in plant, soil and water.

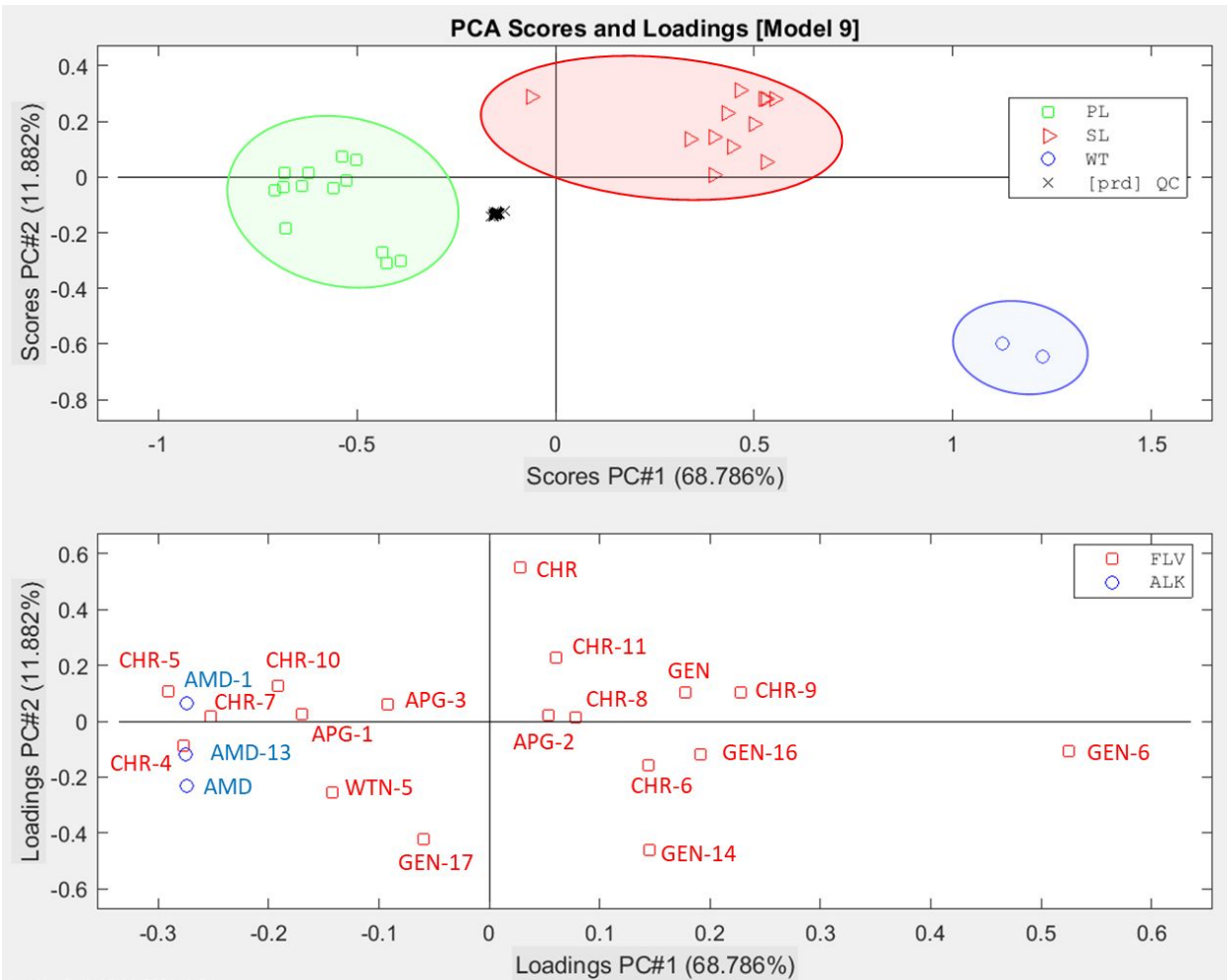


Figure. S4. PCA of flavonoids (FLVs) and alkaloids (ALKs) in plant (PL), soil (SL) and water (WT). Data were normalized (2-Norm), logarithmic-transformed and Pareto-scaled.

## S-9. Pulses of PTs in drainage water upon irrigation on July 23<sup>rd</sup> (day 98).

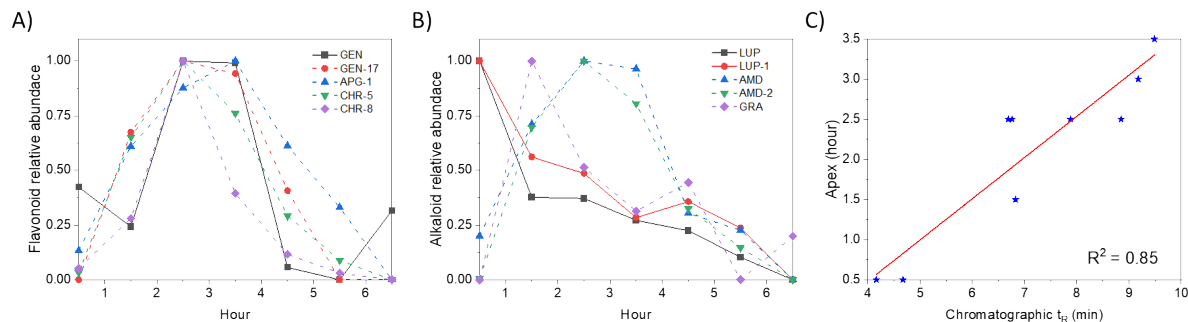


Figure S5. Pulses of flavonoids (A) and alkaloids (B) in drainage water upon irrigation. Five example PTs were given for each compound class. The relative abundances were determined by range-scaled PT peak heights. (C) The correlation of chromatographic  $t_R$  values and the pulse apexes of the example PTs. Note in (C) GEN was excluded from the plot as it had no obvious pulse apex, meanwhile the apexes of LUP-1 and LUP were manually input as 0.5 h (start sampling time).

124    **Reference:**

- 125    1.        Liang, X.; Nielsen, N. J.; Christensen, J. H., Selective pressurized liquid extraction of plant  
126    secondary metabolites: *Convallaria majalis* L. as a case. *Analytica Chimica Acta: X* **2020**, 100040.

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