Supporting Information

Article Title: Transpiration and metabolisation of TCE by willow plants – a pot experiment

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Authors: Philipp Schöftner, Andrea Watzinger*, Philipp Holz knecht, Bernhard Wimmer, Thomas G. Reichenauer

Affiliation: AIT Austrian Institute of Technology GmbH, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria

Corresponding author: andrea.watzinger@ait.ac.at

Detailed procedure of TCE, TCOH, DCAA and TCAA extractions from leaves and bark

2 g moist weight was ground manually using a mortar and a pestle filled with liquid nitrogen. Ground samples were put in 20 ml glass vials and closed with Teflon-sealed septa. This step was followed by analyte-specific extraction procedures:

TCE extraction

TCE was extracted according to (Newman et al. 1997) with minor adaptations. After grinding, 2 ml of a 0.5 M H₂SO₄ solution (instead of a 1 M solution) that was made out of a 10 % NaCl solution were added and the vials were shaken in a horizontal shaker (300 spm) for 5 min (instead of vortexing). Afterwards 10 ml of MTBE (methyl tert-butyl ether) was added and the vial was shaken again for 3 min at 300 spm (instead of vortexing). Hereafter the mixture was centrifuged (3000 rpm) for 5 min, 7 ml of the supernatant were withdrawn and put in 20 ml glass vials that were sealed with Teflon septa. Finally the extract was dehydrated with 2 g Na₂SO₄ for 2 h.

TCOH extraction

TCOH was extracted according to (Shang et al. 2001) with minor adaptations. After grinding, 7 ml of acetone were added and the vials were shaken for 5 min (300 spm). Supernatants were withdrawn and samples were

extracted a second time with 7 ml acetone. Both extracts were filled through a Whatman Nr.1 (11 μ m) filter into 20 ml vials that were sealed with Teflon septa. Afterwards acetone was evaporated with a rotary evaporator at 25 °C and 130 mbar. Hereafter 0.5 ml of Millipore water were added to the residues and the residues transferred into 2 ml Eppendorf vials. These were centrifuged for 5 min at 12000 rpm and the supernatant was divided into two 200 μ l aliquots. One aliquot was combined with 200 μ l of a δ -glucosidase solution; the other was combined with 200 μ l of a BSA-solution (bovine serum albumin). Both enzymes were solved in a 0.1 M KH_2PO_4 / K_2HPO_4 buffer solution at a concentration of 1 mg ml⁻¹. The solution was separated in to two aliquots to determine glycolized TCOH (δ -glucosidase) and free TCOH (BSA). The aliquots were incubated at 37 °C for 2 h and transferred into 20 ml glass vials that were sealed with Teflon septa. Into these vials, 200 μ l of a 0.5 M H_2SO_4 (instead of 1 N) solution that was made out of a 10 % NaCl solution were added. These solutions were vortexed for 1 min. Then 7 ml of MTBE were added (instead of 10 ml), vortexed for another minute and centrifuged for 5 min (2500 rpm) (instead of 6000 rpm). Finally MTBE supernatant was withdrawn and dehydrated with 2 g Na_2SO_4 for 1 h.

DCAA and TCAA extraction

TCOH was extracted according to (Newman et al. 1997) and methylated according to (James et al. 2009). After grinding, D/TCAA was extracted two times with 7 ml NaOH (0.5 M) (instead of 0.25 N) and shaken for 5 min on a horizontal shaker (300 spm) (instead of vortexing) followed by centrifugation (3000 rpm). Both extracts were combined and acidified below pH-value 1 by adding 1 ml H_2SO_4 (50 %). The supernatant was transferred into 20 ml glass vials that were sealed with Teflon septa and dehydrated with 2 g Na_2SO_4 . 2.5 ml MTBE (instead of 3 ml) were added and the mixture was shaken on a horizontal shaker (300 spm) and (instead of vortexing) centrifuged for 10 min (2500 rpm). Hereafter 1 ml of the supernatant was methylated. Then 2 ml methanol and 0.1 ml H_2SO_4 (50 %) were added into a 20 ml glass vial that was sealed with Teflon septa. The mixture was incubated at 50 °C for 1 h. Hereafter 5 ml of a Na_2SO_4 solution (10 %) and 1 ml MTBE were added and vortexed for 2 min. Finally MTBE supernatant was withdrawn; the vials were put aside for 10 min until the MTBE phase was separated.

Detailed analytical procedure of TCE, TCOH, DCAA and TCAA measurement from extractions from leaves and bark

One or two μl of extracts were injected splitless at 200 °C. Analytes were separated with an Agilent Technologies (USA) DB-VRX capillary column (60m, I.D. 0.32 mm, widebore film 1.8 μm) with He (1.5 ml min^{-1} in 2010 and 2 ml min^{-1} in 2011) as carrier gas. Initial temperature was 45 °C for 1 min followed by ramp (4 °C min^{-1}) to 100 °C hold for 2 min, ramp (10 °C min^{-1}) to 165 °C (0 min) and ramp (30 °C min^{-1}) to 240 °C (10 min). For MSD measurements (in 2010), samples were ionized by electron impact (EI) ionization at 70 mA. Retention time of substances and most appropriate m/z peaks were determined by running the MSD in SCAN-mode. The subsequent sample measurements were conducted in SIM-mode, whereby solvent delay was activated for the first 10 min of analysis to avoid detector overload by solvent. The ECD detector (in 2011) was operated at 300 °C, whereby N_2 was used as make-up gas (60 ml min^{-1}).

Chemicals and Materials

Trichloroethene (Sigma Aldrich ACS Reagent $\geq 99.5\%$), Trichlorethene- $^{13}\text{C}_2$, 99 atom%, 97% CP (Sigma-Aldrich) 1,2-dichloroethene (Sigma Aldrich 98 %), trichloroacetic acid (Merck $\geq 99.5\%$), dichloroacetic acid (Merck $\geq 98\%$) and 2,2,2 trichloroethanol (Merck $\geq 97\%$) were used for standard preparation. Extractions were conducted using methyl-tert-butylether (Sigma Aldrich Chromasolv for HPLC), sodium hydroxide (Merck pro analysis), acetone (Sigma Aldrich Chromasolv plus for HPLC 99.9 %), sodium chloride (Merck pro analysis), water-free sodium sulfate (Sigma Aldrich puris ACS), sulfuric acid (Sigma Aldrich 95 - 98 %), methanol (Merck LiChrosolv), δ -glucosidase from almonds (Sigma Aldrich 7.8U mg^{-1}), bovine serum albumin (Sigma Aldrich) and potassium dihydrogen orthophosphate (Merck pro analysis). Laboratory apparatus used were SM-30-control horizontal shaker (Edmund Bühler GmbH, Echingen, Germany), 5415R centrifuge (Eppendorf AG, Hamburg, Germany), RC5CPlus centrifuge (Sorvall, Thermo Fisher), Laborota 4000 - rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) with a PC 2001 Vario diaphragm pump (Vacuubrand GmbH & Co. KG, Wertheim, Germany).

Fig. S1 Comparison of measured and computed TCE concentrations of experiments in 2010 and 2011. Pots with nominal TCE concentrations of 144 mg l⁻¹ (◇), 288 mg l⁻¹ (○) and 721 mg l⁻¹ (Δ), and unplanted pots with nominal concentrations of 721 mg l⁻¹ (□). Concentrations stated in the legend refer to initial measurements of TCE concentrations.

Fig. S2 Concentrations of TCE and its metabolites TCOH (trichloroethanol), DCAA (dichloroacetic acid) and TCAA (trichloroacetic acid) in (a) leaves and (b) barks of willows from experiments in 2010 and 2011. Concentrations represent nmol g⁻¹ fresh weight. n.d. not detected, below the detection limit. n.m. not measured/extracted due to lack of samples.

References:

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