Determination of the Plant Uptake Factor (PUF): pH Buffering in Hydroponics and the Example of Atrazine with Sugar Beet and Wheat Plants

Gourlay V.[1], Frenzel C.[1,2], Fent G.[1], Kubiak R.[1]

[1] RLP AgroScience GmbH, Breitenweg 71, 67435 Neustadt an der Weinstraße, Germany // [2] Universität Koblenz-Landau, Landau, Germany

INTRODUCTION

Experimental background

- Guideline: "EUregPUF Workshop report", York, UK [Hingston et al. 2013]
- Round robin test: "Development of a new test design for quantifying plant uptake as input parameter for regulatory fate modelling" [Lamshoeft et al. (2015)]
- Protocol: Draft version (08.2016) of "Study design to determine uptake of chemicals by plant roots", ECPA/IVA Working Group "Plant Uptake Factor", Frankfurt/Main, Germany, 26th May 2017

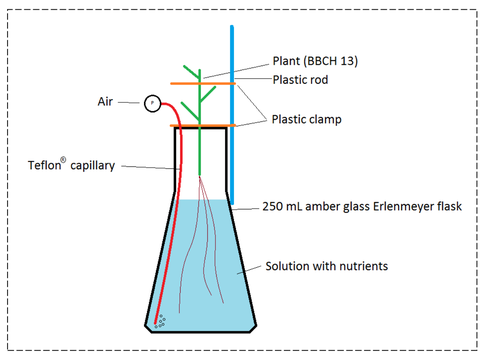

Figure 1: Schematic of the test system

Quantification of the plant uptake: PUF & TSCF

$$PUF = \frac{\ln\left(\frac{m_{sol}}{m_0}\right)}{\ln\left(\frac{V_{sol}}{V_0}\right)} = \frac{\ln\left(\frac{m_o - m_{uptake}}{m_0}\right)}{\ln\left(\frac{V_o - V_{uptake}}{V_0}\right)} \quad (1)$$

[Sweeney et al. (2013)]

$$TSCF = \frac{\ln\left(1 - \frac{m_{shoots}}{m_{shoots} + m_{sol}}\right)}{\ln\left(\frac{V_{sol}}{V_0}\right)} \quad (2)$$

[Reitz et al. (2016)]

m_0	Initial mass of test item in solution	V_{uptake}	Volume of test solution taken up
V_0	Initial volume of test solution	m_{shoots}	Mass of test item taken up
m_{uptake}	Mass of test item taken up		

Purpose of the study

- Further **investigation** of the proposed test protocol (Fig.1) with different crop types (Experiment I)
- Determination of the adapted concentration of **pH buffer** (Experiment I)
- Determination of the **Plant Uptake Factor** (PUF) for Atrazine as model compound (Experiment II)

MATERIAL AND METHODS

Area of investigation

- Crop types (Fig. 2)
  - Tomato (I),
  - Oilseed rape (II),
  - Wheat (III),
  - Maize (IV),
  - Red radish (V),
  - Sugar beet (VI)
- Test compound
  - **^{14}C-atrazine** (1)
  - 20 µg·L^{-1}
- Greenhouse conditions settings
  - Day/Night variation (16:8)
  - Temperature: 20°C ± 5°C
  - Air humidity: > 50 % of saturation
  - Illuminance: Sodium lights
- Plant cultivation according to ECPA
  - 5-7 days pre-incubation
  - Initial BBCH 12-21

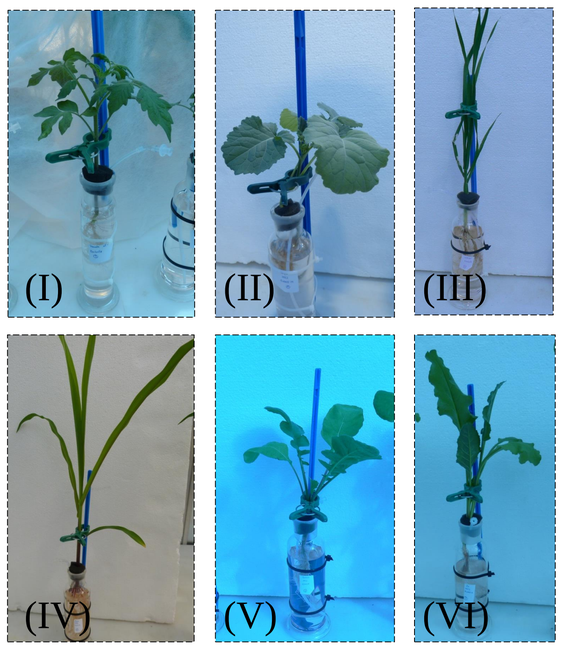

Figure 2: Investigated crops

Used buffers

- MES (2) [pH 6.0-6.5]
- Tris (3) [pH 7.3-7.8]
- Without buffer (WB)

Experiment I: pH buffer test variants

Test solution	MES		Tris		WB
Buffer concentration	0.005 M	0.010 M	0.005 M	0.010 M	-
With plants	3×	3×	3×	3×	3×
Without plant	1×	1×	1×	1×	1×

Experiment II: Atrazine test variants

Test solution (Buffer 0.005 M)	MES		Tris		WB	
Treatment	Y	N	Y	N	Y	N
Wheat	4×	2×	4×	2×	4×	3×
Sugar beet	4×	2×	4×	2×	4×	3×
Without plant	1×	-	1×	-	1×	4×

Control systems

- Phytotoxicity: buffer // atrazine
- Evaporation, stability

Results validation

- Intermediate **samplings**: LSC, mass
- **Purity** in solution: Radio-HPLC
- Equivalent on **root surface**
  - Acetonitrile/Water, 4:1 (v/v), 150 mL
  - 3 min
- Equivalents in **plant material**:
  - Combustion + LSC
  - Roots / Beet / Shoots

RESULTS AND DISCUSSION

Experiment I: pH buffer

pH level in solution

- Without buffer (WB):
  - Natural variation, specific nutrient needs
- MES buffer:
  - No strong variations between concentrations or crops
- Tris buffer:
  - No strong variations between concentrations
  - Stronger pH variations between crops

Test plants

- Leaf **senescence** vs. leaf / roots **development**
- **Wheat & Sugar beet**: Chosen for PUF investigation
- Buffer: **0.005 M**, chosen for Experiment II

Experiment II: PUF for Atrazine

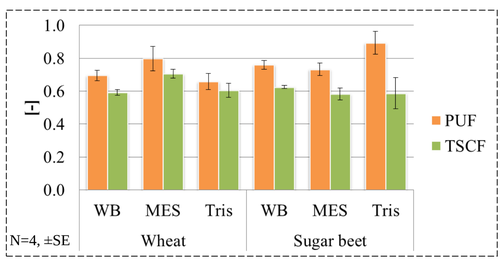

Figure 3: PUF / TSCF

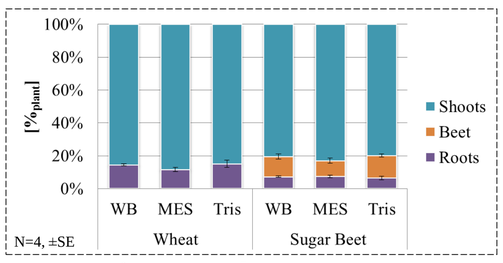

Figure 4: Plant content

Main results

W: Wheat, SB, Sugar beet

		Min – Max	Mean	SD	CV (%)
Initial biomass [g]	W	3.3 – 7.4	5.3	1.4	27.2
	SB	2.2 – 9.8	4.8	2.6	54.5
Biomass increase ($\%_{initial}$)	W	17.3 – 115.0	51.3	23.7	46.2
	SB	26.3 – 200	125.4	49.0	39.1
VUP ($\%_{initial}$)	W	7.3 – 30.3	19.9	6.1	30.8
	SB	10.4 – 46-9	24.5	11.2	45.8
Radioactive mass balance ($\%_{AR}$)	W	95.8 – 104.6	100.0	2.9	2.9
	SB	95.2 – 103.6	99.4	2.7	2.7
Translocation to shoots ($\%_{plant}$)	W	82.9 – 89.9	86.0	2.3	2.6
	SB	75.6 – 85.6	80.9	3.2	3.9
PUF [-]	**W**	**0.56 – 0.94**	**0.72**	**0.12**	**16.4**
	SB	**0.64 – 1.03**	**0.80**	**0.10**	**14.4**
TSCF [-]	**W**	**0.48 – 0.78**	**0.63**	**0.08**	**12.2**
	SB	**0.40 – 0.85**	**0.60**	**0.11**	**18.0**

- Lamshoeft et al. (2015) [ring test] → PUF = 0.48 to 0.94 (N = 40)

CONCLUSION AND FURTHER INVESTIGATION

- ✓ **Good adaptation** of the test system proposed by Lamshoeft et al. (2015) to **root vegetable**
- ✓ No strong influence of the **pH level or crop type** on the plant uptake
- ✓ **Significant plant uptake** (always > 0.5) were determined for both crops and all pH levels

- ○ Need for investigation of sugar beet with other compounds
- ○ Need for investigation of sugar beet at different BBCH stage (e.g. developed tuber)

REFERENCES

[1] Lamshoeft M, Resseler H, Reinken G, Schriever C, Schubert S, Webb J, Webb S, Zillgens B, Keenan D, Doucette W, Fent G, Gourlay V, Malekani K, Letourneau M, Martin J, Rooney P, vanBeinum W, Rieder B, Traub M, Swales S, Weinfurtner K, Derz K, Kemmerling K (2015): **First results with a new test design for the determination of a substance specific Plant Uptake Factor (PUF) for use in regulatory fate modeling.** Proc. XV Symposium in Pesticide Chemistry. Environmental Risk Assessment and Management, p. 19-20, Piacenza, Italy. Edited by Cigolini M, Luzzani G, Sacchettini G: Mattioli1885, Fidenza, Italy, 2015. (ISBN 978-88-6261-508-2)

[2] Reitz MU, Lamshoeft M, McCall S, Resseler H, Sur R, Schriever C, Webb S & Zilligens B. (2016): **Effect of plant species and chemcial substance properties on plant uptake.** SETAC EU Conference, Nantes, France, poster session

[3] Sweeney P, Harvey B, Webb J, Webb S, Humphrey J, Marchant E (2013): **A Simple Method for Measuring the Uptake of Chemicals from Solution into Plants**, submitted for publication.

(1) $\log K_{ow}$ = 2.7 (pH 7, 20°C), pK_a = 1.7 (25°C), (2) MES : 2-(N-morpholino)ethanesulfonic acid, (3) Tris•HCl : Tris(hydroxymethyl)aminomethane hydrochloride , (4) 1 g·L^{-1} *9 Hydro*, NPK (Mg) =15 + 7 + 22 (+ 6), (5) Acetonitrile/Water, 4:1 (v/v), 150 mL (roots fully submerged), 3 min