Supporting Information for

Metabolism of the Fusarium mycotoxins T-2 toxin and HT-2 toxin in wheat

Alexis V. Nathanail,[†,‖] Elisabeth Varga,[‡,‖] Jacqueline Meng-Reiterer,[‡,§] Christoph Bueschl,[‡] Herbert Michlmayr,[#] Alexandra Malachova,[‡] Philipp Fruhmann,[⊥] Marika Jestoi,[△] Kimmo Peltonen,[∂] Gerhard Adam,[#] Marc Lemmens,[§] Rainer Schuhmacher,[‡] Franz Berthiller*[,‡]

[†] Chemistry and Toxicology Unit, Research and Laboratory Department, Finnish Food Safety Authority (Evira), Mustialankatu 3, 00790 Helsinki, Finland

[‡] Christian Doppler Laboratory for Mycotoxin Metabolism and Center for Analytical Chemistry, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Konrad Lorenz Str. 20, 3430 Tulln, Austria

[§] Institute for Biotechnology in Plant Production, IFA-Tulln, BOKU, Konrad Lorenz Str. 20, 3430 Tulln, Austria

[#] Department of Applied Genetics and Cell Biology, BOKU, Konrad Lorenz Str. 24, 3430 Tulln, Austria

[⊥] Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9/163, 1060 Vienna, Austria

[△] Product Safety Unit, Control Department, Finnish Food Safety Authority (Evira), Mustialankatu 3, 00790 Helsinki, Finland

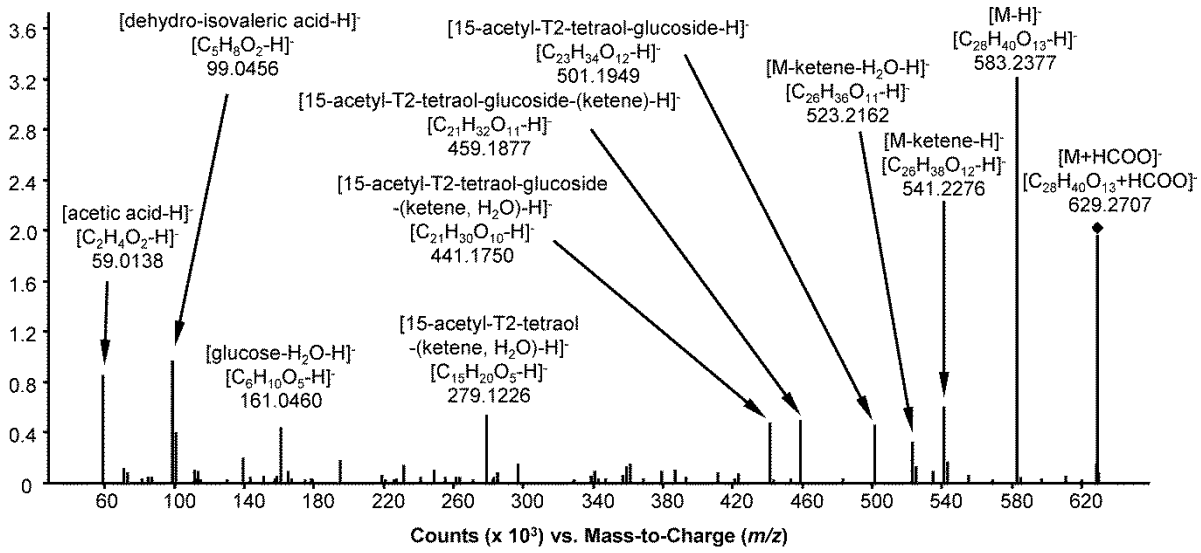
[∂] Finnish Safety and Chemicals Agency (Tukes), Opastinsilta 12 B, 00521 Helsinki, Finland

[‖]A.V.N. and E.V. contributed equally to the work.

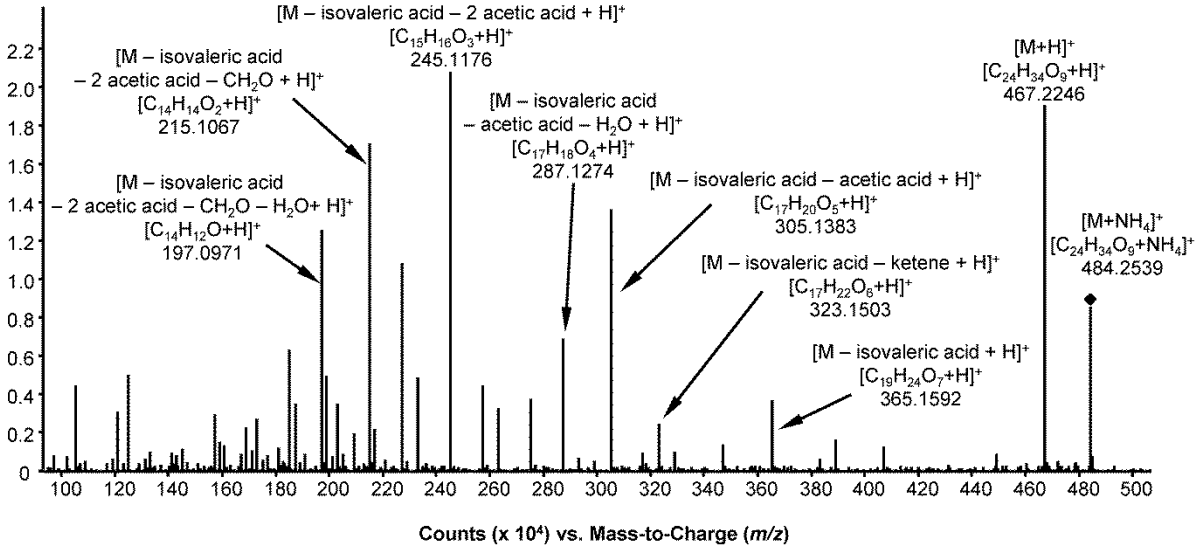
Corresponding author

* (F.B.) E-mail: franz.berthiller@boku.ac.at.

Phone: +43 2272/66280-413. Fax: +43 2272/66280-403.



Supplemental Figure S1: LC-HRMS/MS-spectrum of dehydro-HT2-glucoside, an *in planta* metabolite of HT-2 toxin and T-2 toxin. The chosen precursor (formate adduct) is marked with a diamond and the spectrum was acquired at a collision energy of 20 eV.



Supplemental Figure S2: LC-HRMS/MS-spectrum of 3-acetyl-HT-2 toxin, an *in planta* metabolite of HT-2 toxin. The chosen precursor (ammonium adduct) is marked with a diamond and the spectrum was acquired at a collision energy of 10 eV.

Supplemental Table S1. Results from the method validation (mean values (n= 3) ± relative standard deviation). Biological triplicates of the mock samples (variety Remus) of two treatment points (1 day and full ripening) were spiked on one level before extraction to obtain the apparent recovery (R_A). Additionally, different dilutions of blank extracts of these biological triplicates were spiked on one level to obtain the values for signal suppression and enhancement (SSE) of the respective dilution. The extraction recovery (R_E) was calculated from both values and for the relative standard deviation the propagation of uncertainty was considered.

HT-2 toxin	R_A	SSE (UD)	SSE (1:10)	SSE (1:50)	R_E
1 day	132 ± 2.4	146 ± 3.0	117 ± 1.5	109 ± 0.6	91 ± 4.2
full ripening	124 ± 13	114 ± 13	137 ± 6.4	113 ± 3.6	109 ± 17
HT-2 toxin 3-*O*-*β*-glucoside	R_A	SSE (UD)	SSE (1:10)	SSE (1:50)	R_E
1 day	129 ± 2.1	130 ± 4.0	111 ± 0.7	106 ± 0.3	99 ± 4.6
full ripening	121 ± 4.3	112 ± 8.0	133 ± 4.5	112 ± 1.3	108 ± 8.4
T-2 toxin	R_A	SSE (UD)	SSE (1:10)	SSE (1:50)	R_E
1 day	96 ± 3.5	99 ± 3.5	105 ± 1.6	104 ± 1.3	97 ± 5.1
full ripening	65 ± 30	59 ± 34	122 ± 6.3	108 ± 3 0	112 ± 41
3-acetyl-T-2 toxin	R_A	SSE (UD)	SSE (1:10)	SSE (1:50)	R_E
1 day	74 ± 3.0	79 ± 3.5	97 ± 0.5	105 ± 0.4	94 ± 4.9
full ripening	56 ± 31	52 ± 34	97 ± 2.3	104 ± 1.7	108 ± 42

UD – “undiluted” sample = 1000 µg/kg in wheat corresponds to 200 µg/L in the extract, 1:10 (v/v), 1:50 (v/v) – different dilutions of the raw extract in acetonitrile-water (50:50, v/v)