Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Tracing the metabolism of HT-2 toxin and T-2 toxin in barley by isotope assisted untargeted screening and quantitative LC-HRMS analysis

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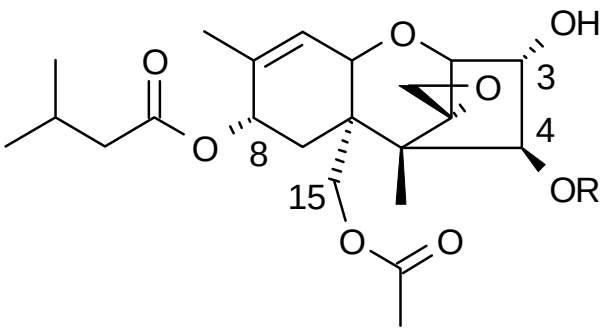

Fig. S1 Structural formula of HT-2 toxin (R = H) and T-2 toxin (R = acetyl). Most relevant positions for this plant metabolism study are highlighted.

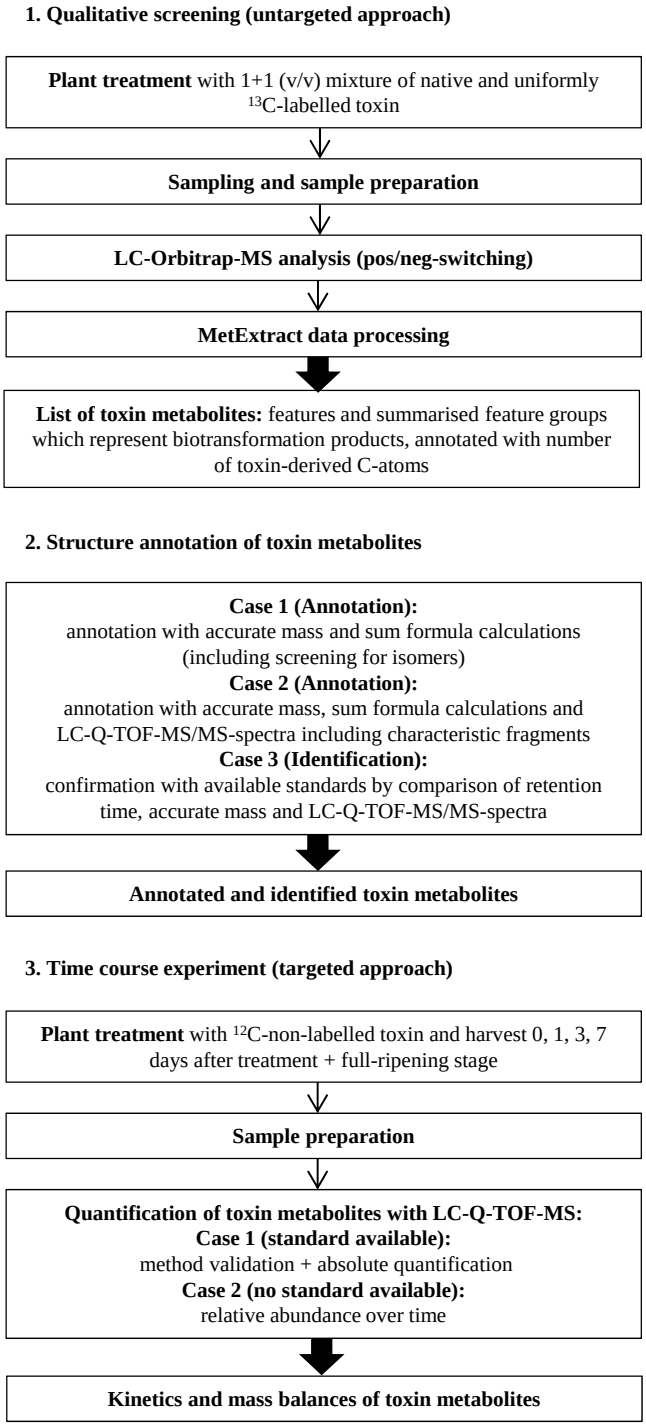

Fig. S2 Workflow for the study of the metabolism of HT-2 toxin and T-2 toxin in barley. Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS(/MS)) was used for qualitative screening, structure annotation and (relative) quantification.

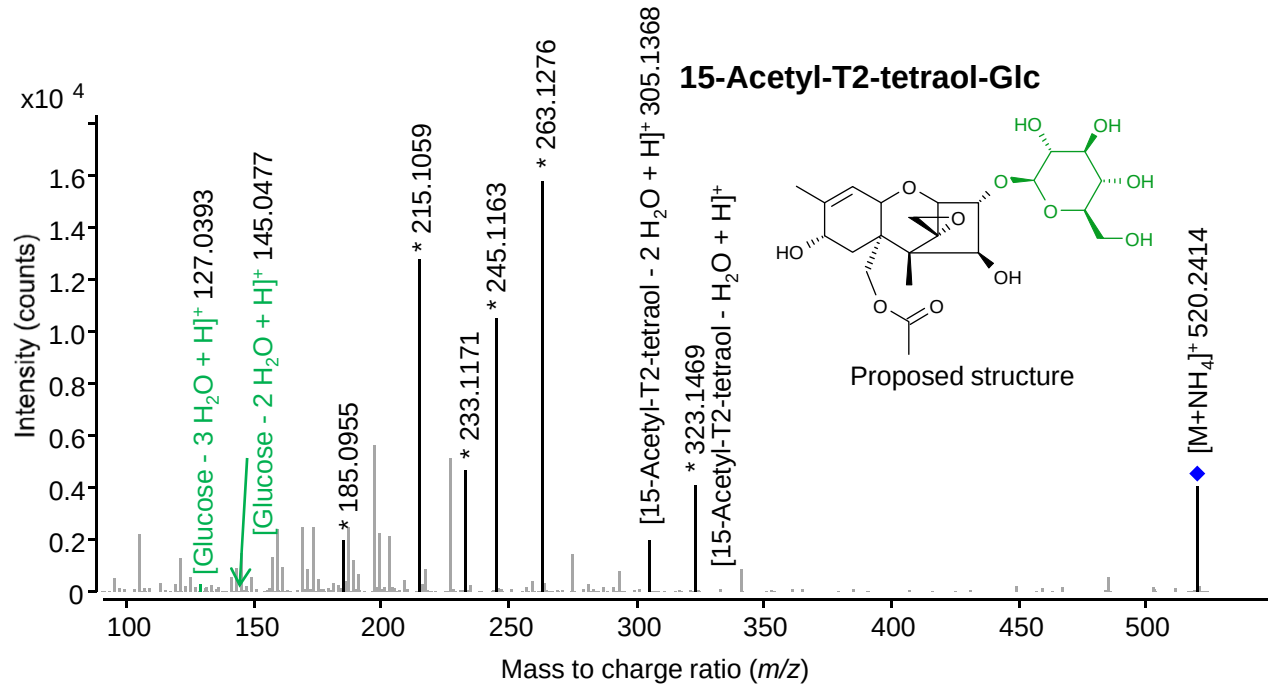

Fig. S3 LC-HRMS/MS-spectrum of 15-acetyl-T2-tetraol-Glc, an *in planta* metabolite of HT-2 toxin and T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 10 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted, those fragments originating from the conjugate glucose are displayed in green and characteristic HT-2 toxin fragments are marked with an asterisk (*). T2 (T-2 toxin), Glc (glucoside)

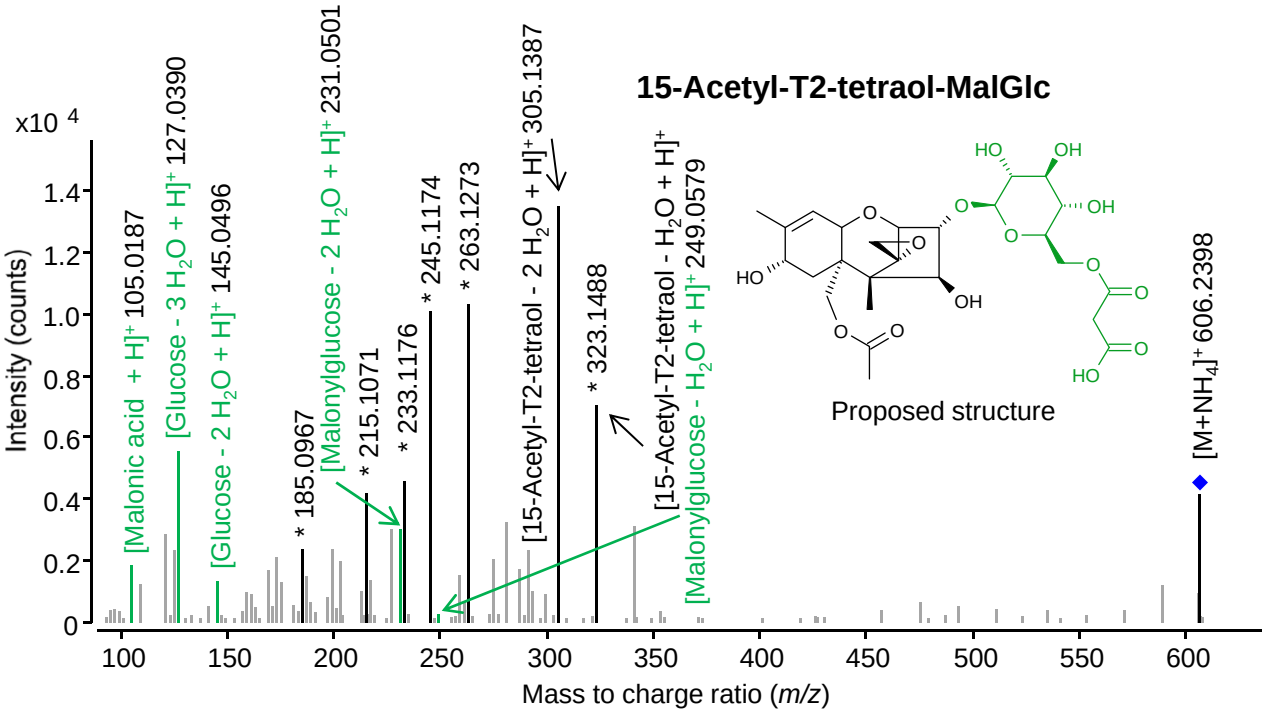

Fig. S4 LC-HRMS/MS-spectrum of 15-acetyl-T2-tetraol-MalGlc, an *in planta* metabolite of HT-2 toxin and T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 16 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted, those fragments originating from the conjugate malonylglucose are displayed in green and characteristic HT-2 toxin fragments are marked with an asterisk (*). T2 (T-2 toxin), MalGlc (malonylglucoside)

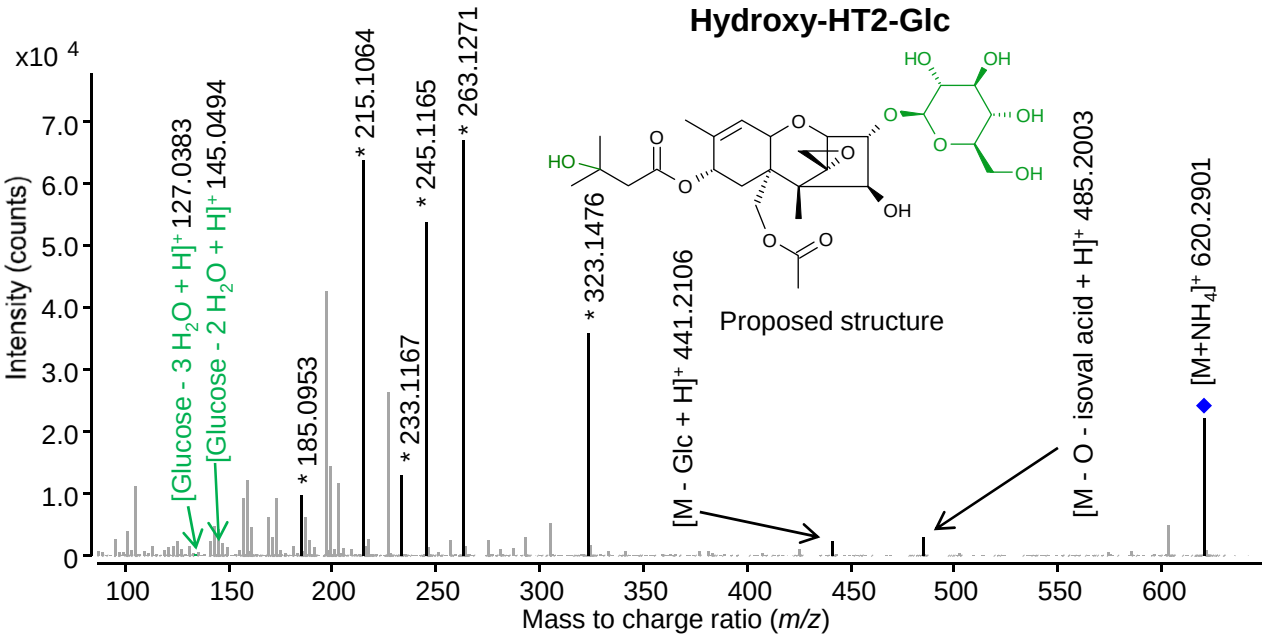

Fig. S5 LC-HRMS/MS-spectrum of hydroxy-HT2-Glc, an *in planta* metabolite of HT-2 toxin and T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 10 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted, those fragments originating from the conjugate glucose are displayed in green and characteristic HT-2 toxin fragments are marked with an asterisk (*). HT2 (HT-2 toxin), Glc (glucoside), isoval acid (isovaleric acid)

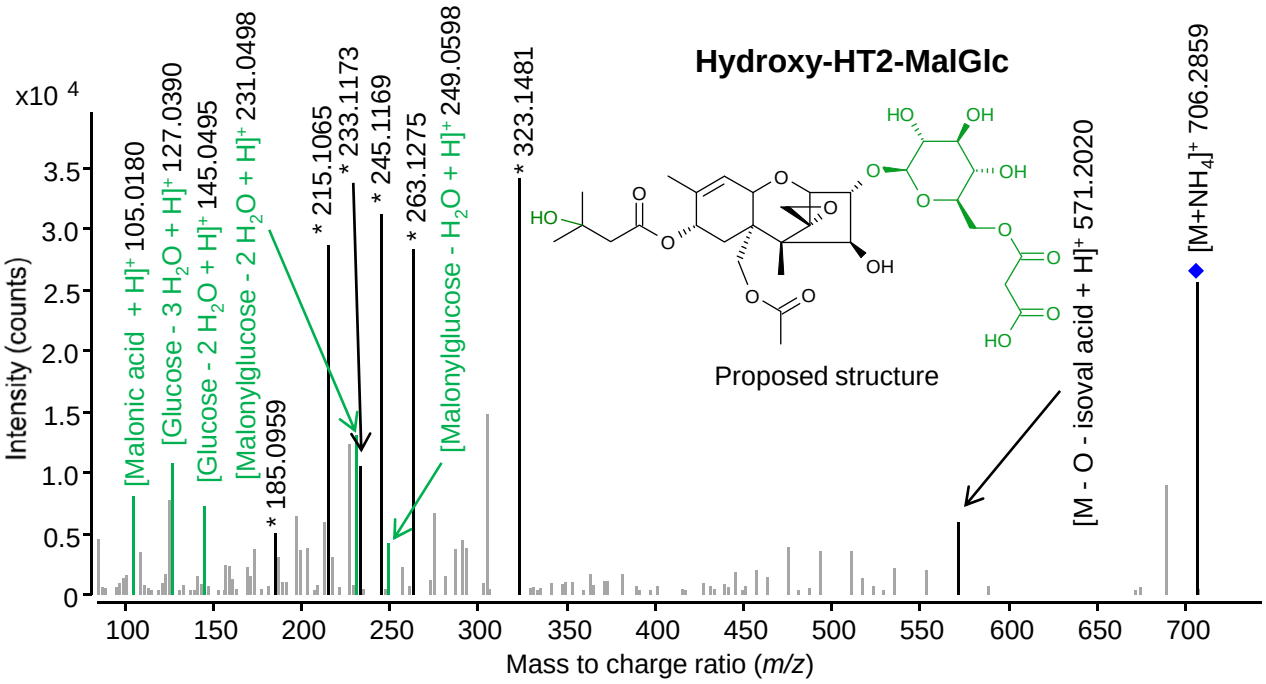

Fig. S6 LC-HRMS/MS-spectrum of hydroxy-HT2-MalGlc, an *in planta* metabolite of HT-2 toxin and T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 16 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted, those fragments originating from the conjugate malonylglucose are displayed in green and characteristic HT-2 toxin fragments are marked with an asterisk (*). HT2 (HT-2 toxin), MalGlc (malonylglucoside), isoval acid (isovaleric acid)

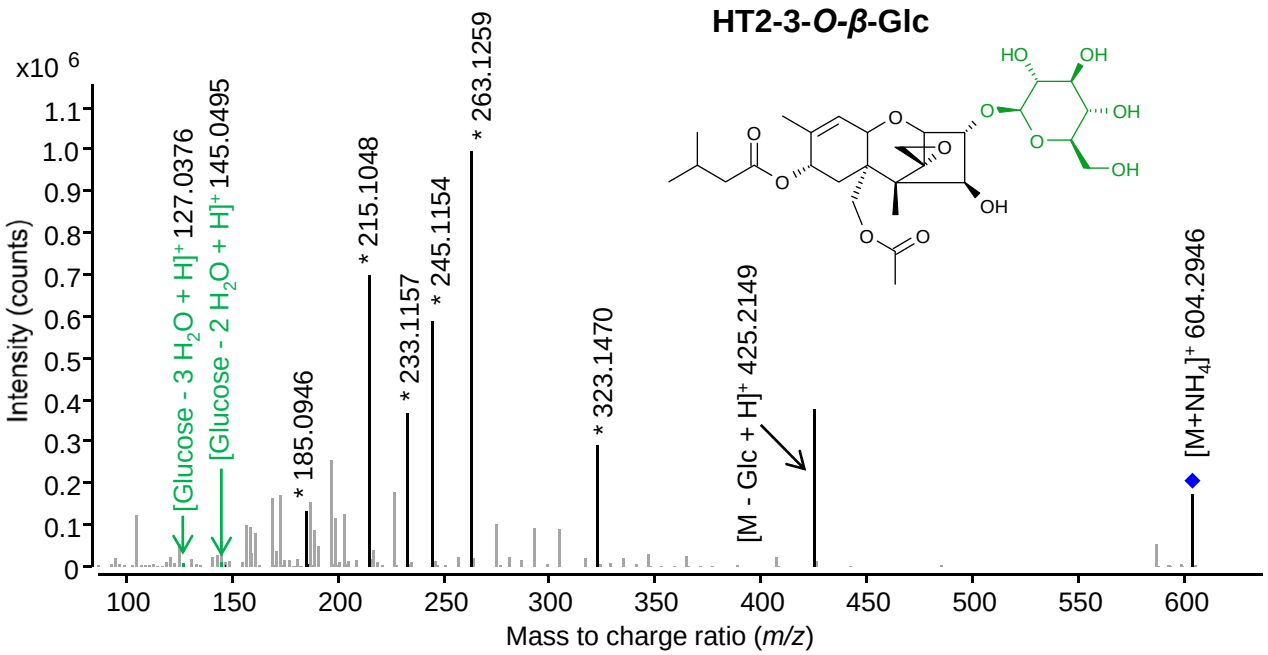

Fig. S7 LC-HRMS/MS-spectrum of HT2-3-*O*-*β*-Glc, an *in planta* metabolite of HT-2 toxin and T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 10 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted, those fragments originating from the conjugate glucose are displayed in green and characteristic HT-2 toxin fragments are marked with an asterisk (*). HT2 (HT-2 toxin), Glc (glucoside)

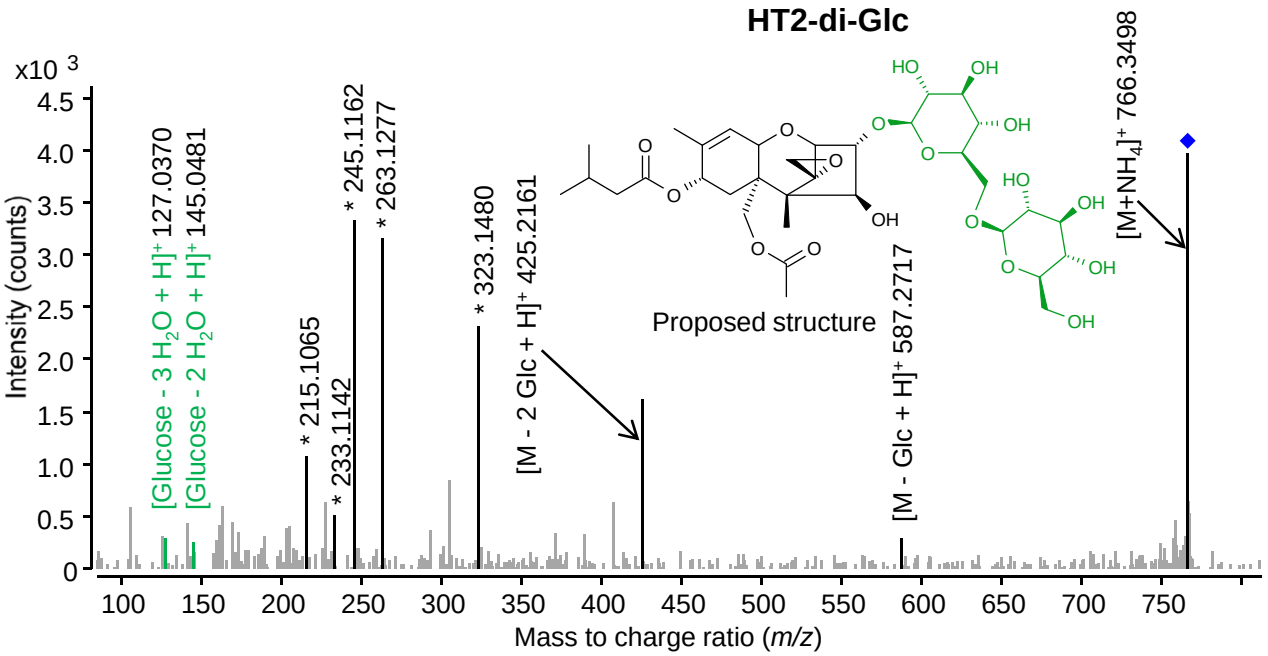

Fig. S8 LC-HRMS/MS-spectrum of HT2-di-Glc, an *in planta* metabolite of HT-2 toxin and T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 10 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted, those fragments originating from the conjugate glucose are displayed in green and characteristic HT-2 toxin fragments are marked with an asterisk (*). HT2 (HT-2 toxin), Glc (glucoside)

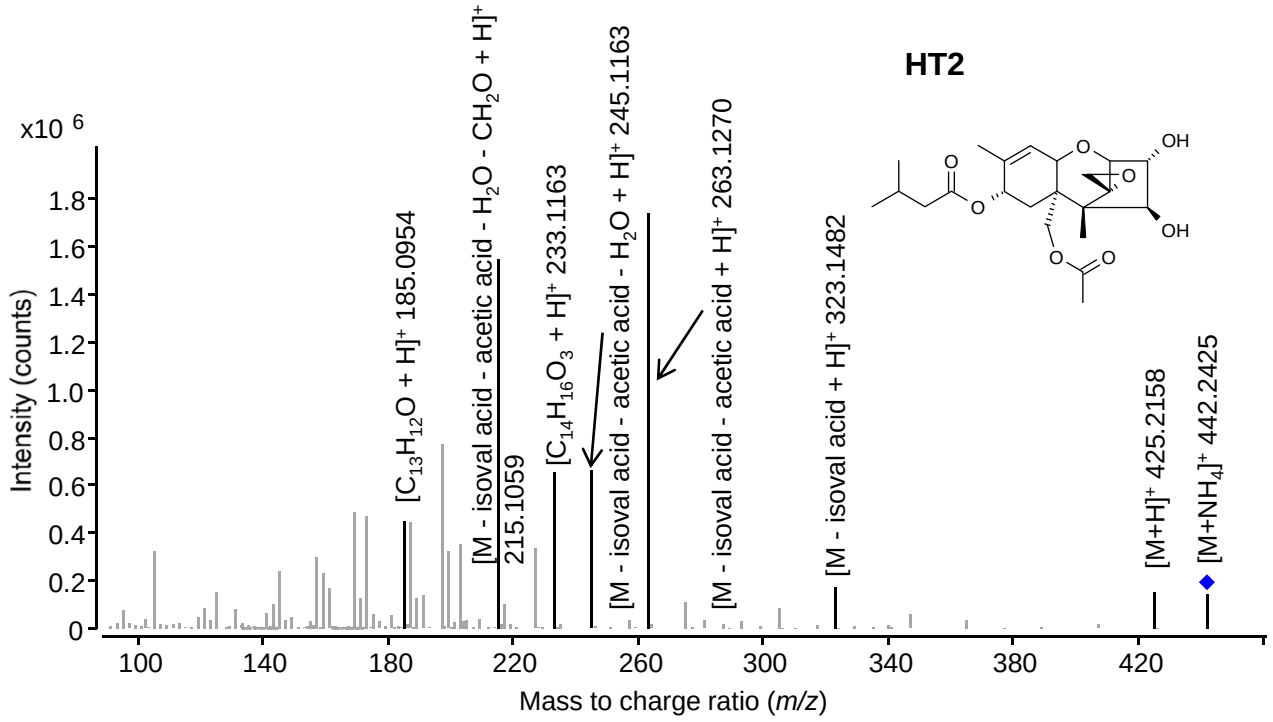

Fig. S9 LC-HRMS/MS-spectrum of HT-2 toxin, an *in planta* metabolite of T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 10 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted. HT2 (HT-2 toxin), isoval acid (isovaleric acid)

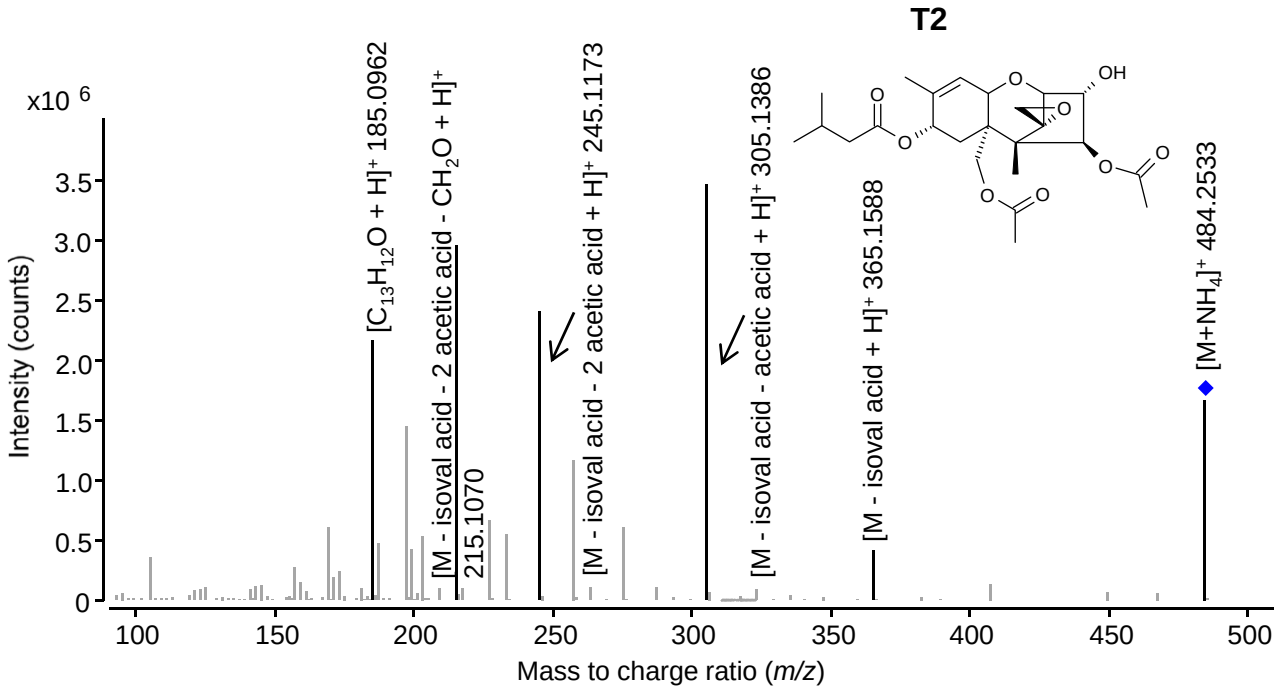

Fig. S10 LC-HRMS/MS-spectrum of T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 10 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted. T2 (T-2 toxin), isoval acid (isovaleric acid)

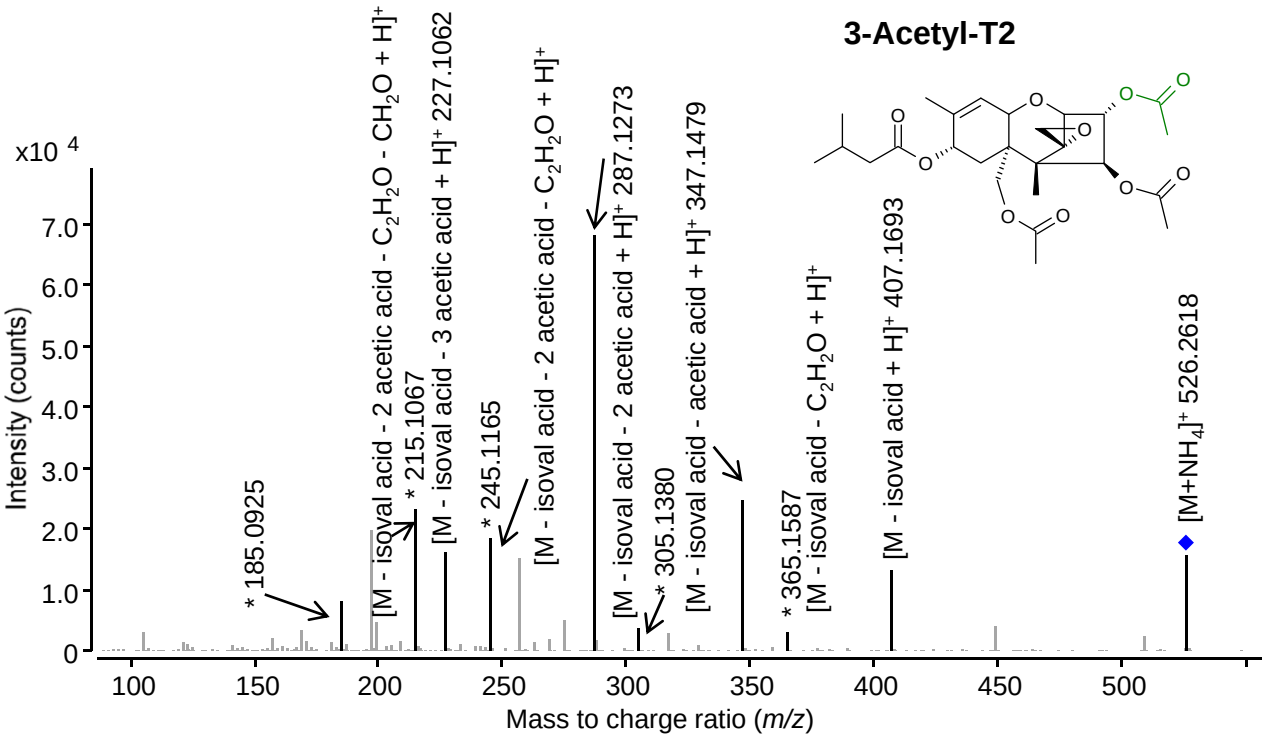

Fig. S11 LC-HRMS/MS-spectrum of 3-acetyl-T2, an *in planta* metabolite of T-2 toxin. Analysis was performed with a 6550 iFunnel Q-TOF LC/MS system in positive electrospray ionisation mode with a collision energy of 10 V. The ammonium adduct was chosen as precursor (marked with a diamond). Characteristic fragments used for annotation are highlighted and characteristic T-2 toxin fragments are marked with an asterisk (*). T2 (T-2 toxin), isoval acid (isovaleric acid)

Table S12 Signal suppression or enhancement (%) of different dilutions of 1 day and ripen barley extraction solution determined for HT2, T2, HT2-3-*O*-*β*-Glc, T2-Glc and 3-acetyl-T2.

Analyte	**Undiluted**		**1+9**		**1+49**	
	1 day	**ripen**	**1 day**	**ripen**	**1 day**	**ripen**
HT2	137±4	144±3	107±2	114±1	107±1	108±1
T2	116±5	106±3	102±3	102±1	104±1	103±1
HT2-3-*O*-*β*-Glc	138±3	142±2	106±1	112±3	107±1	106±1
T2-Glc	131±3	134±1	105±3	109±1	104±1	105±0.3
3-Acetyl-T2	89±3	88±3	98±4	98±1	104±1	102±1

HT2 (HT-2 toxin), T2 (T-2 toxin), Glc (glucoside). Mean value of spiked biological triplicate ± relative standard deviation is stated.