Electronic Supplementary Information (ESI) for:

Benzotriazole (BT) and BT Plant Metabolites in Crops Irrigated with Recycled Water

Gregory H. LeFevre,[a,b,c] *Alicia Lipsky,*[b,c,d,e] *Katherine C. Hyland,*[b,f,g] *Andrea C. Blaine,*[b,f] *Christopher P. Higgins,*[b,f] *Richard G. Luthy*[b,c]*

[a]Department of Civil & Environmental Engineering, University of Iowa, Iowa City IA, United States; [b]ReNUWIt Engineering Research Center; [c]Department of Civil & Environmental Engineering, Stanford University, Stanford CA, United States; [d]Department of Environmental Systems Science, ETH Zürich, Switzerland; [e]ARCADIS, Zürich, Switzerland; [f]Department of Civil & Environmental Engineering, Colorado School of Mines, Golden CO, United States; [g]SCIEX, Redwood City CA, United States

***Corresponding Author:**

email: luthy@stanford.edu; Phone: 650-721-2615; Fax: 650-725-8662; 473 Via Ortega, Stanford University, Stanford, CA 94305, United States

ESI Contains:

Method Details:

Supporting Figures:

Supporting Tables:

Total Pages: 18 (including all)

Supporting Methods:

Method Detail 1:

Arabidopsis Seed Sterilization Procedure. Adopted from refs 1–3 as cited. All procedures for seed sterilization were conducted over a flame and the bench and gloves were sterilized with a 70% ethanol to create a sterile working environment. Approximately 50 µL of seeds and 1 mL of seed sterilization solution were added to a 1.5 ml autoclaved tube. The seed sterilization solution consisted of 0.8 ml autoclaved deionized water, 0.2 ml bleach (8.25% sodium hypochlorite, Clorox brand) and 10 µL Tween 20 surfactant (Polyoxyethylene sorbitan monolaurate, BioRad Laboratories Inc.). The tube was vortexed briefly and slowly inverted for 5 minutes on a rocking table. The supernatant was removed using an autoclaved pipet. 1 mL of sterile water was added to wash the sterilization solution from the seeds and again the supernatant was removed. The washing step needed to be repeated for a total of four times. The seeds were then stored at 4°C overnight to stratify.

Method Detail 2:

Arabidopsis Hydroponic Growth. Adopted from refs 1–3 below. Sterilized seeds were added to autoclaved Magenta boxes (Magenta Corp., n = 30 seeds per box) with 25 mL of filter sterilized (0.22 µm PES, Corning) Murashige and Skoog (MS) basal medium. The MS medium contained (per 1L): Milli-Q water, 4.43g MS basal medium with vitamins (PhytoTechnology Laboratories; M519), 0.5 g of MES hydrate (PhytoTechnology Laboratories, CAS: 14522-94-8), 5.0 g of sucrose, and was adjusted to pH = 5.7 using 1N KOH. Magenta box edges were wrapped with breathable microporous tape (3M) and placed into a growth chamber (Percival) under fluorescent growth lights with a 16 h light/8 h dark period at 22 °C and a relative humidity of 50%. Plant seedlings were grown for 14 days prior to any BT exposure and were visually checked for any

signs of microbial contamination. Observed germination rates were typically high (>90%) in the boxes. Any contaminated or poorly germinating plant boxes were discarded and not included for use in the experiments. All boxes were treated identically as biological replicates prior to BT exposure.

Method Detail 3:

1D-Gel Electrophoresis (based on ref 4). From each sample, 25 µL of extracted protein were transferred into a new 1.5mL Eppendorf vial and mixed with 25µL of a Laemmli buffer[5] that contained a blue dye (bromphenol blue). The new samples were heated for 5 min at 95 °C and placed on wet ice for 2 min. Concurrently, the gel electrophoresis was prepared. The gel (Mini-Protean, Tris Tricine Gel, Bio-Rad) was rinsed with deionized water and placed in a Mini Protean Tetra System (Bio Rad). The Tetra cell was half-filled with a sodium dodecyl sulfate (SDS) buffer solution (Bio-Rad) and samples were loaded into lanes. A voltage of 50V was applied for 5 min followed by 100V for 10 min. The gel was removed from the Tetra cell and placed in a box and stained with Coomassie Brilliant Blue (Bio-Rad) for approximately 8 hours. The samples were excised, de-stained, and reduced using dithiothreitol (DTT) and alykylated with propionamide, and finally digested with trypsin/LysC (Promega). Digestion with trypsin/LysC cleaves C-terminal to the basic amino acids lysine and arginine creating peptide products. The peptides were extracted from the gel after overnight digestion and dried in a speed-vac.

Method Detail 4:

Description and Analysis of Benzotriazole (BT) and benzotriazole plant metabolites (BTM). Benzotriazoles were quantified in using liquid chromatography – electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS; Applied Biosystems API 3000) with Shimadzu SCL-10A

VP system controller and Analyst 1.5.2 software (AB SCIEX). The chromatography column was a Higgins Analytical Sprite Targa C18 (40 x 2.1 mm, 5µm). The mobile phases were 0.4% formic acid in water (A) and in methanol (B) at a flow rate of 0.2 mL/min. The mobile phase gradient (as percent B) BT and each BT plant transformation product is depicted in Figure S.6. Injection volume was 10 µL. A 6 min equilibration time was set between each sample run. Specific mass spec parameters are described in Table S.3. The MS/MS was set in multiple reaction monitoring mode (MRM). Two MRM transitions were used for each compound for quality control. A six-point BT (or greater) internal standard normalized external calibration curve was used to account for surrogate recovery and matrix effects during ionization. The instrument response was linear throughout the calibration range. The instrumental method detection limit[8] for BT without enrichment was 16 ng/L.

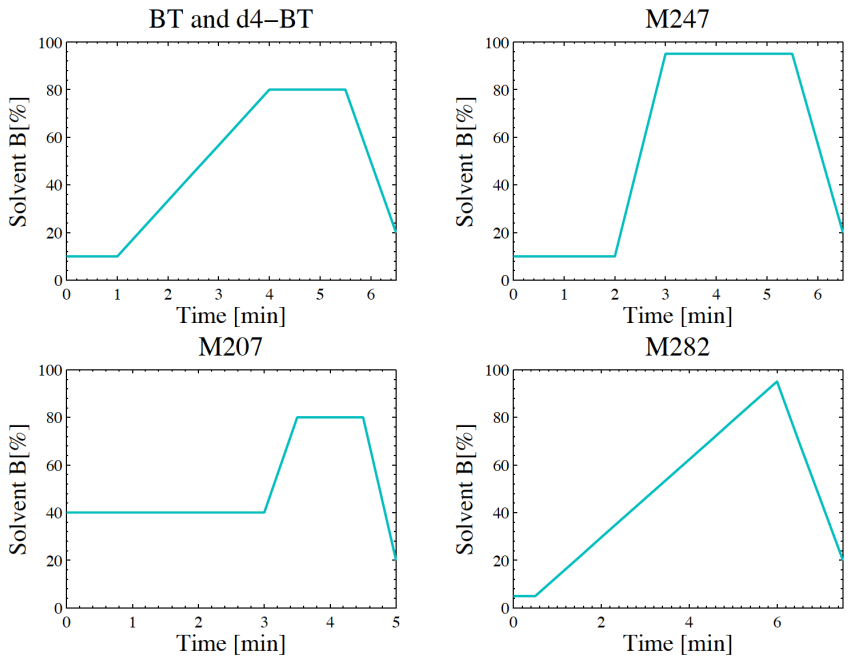


Figure S.1: Mobile phase gradient for liquid chromatography used in the analysis of BT and the various BT plant transformation products studied.

Table S.1: Details including chemical structures of the three benzotriazole plant metabolites quantified in the manuscript. Details are adapted from LeFevre et al.[1] All BT plant transformation products were confirmed Level 1 standard as per the Schymanski et al.[9] framework (Standard confirmation [^{1}H, ^{13}C NMR], HR-MS, MS/MS, RT confirmed) using synthesized standards. The benzotriazole component is highlighted in blue.

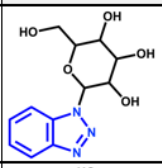
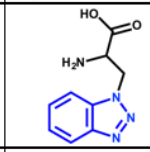
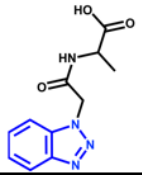
Benzotriazole Plant Transformation Products							Fragment Ions			
BT Transformation Product			Proposed Structure	Formula	ESI Mode (+/-)	Accurate Mass (m/z)	Fragment ions (nominal mass; m/z)	Accurate mass (m/z)	Accurate Mass Deviation (ppm)	Proposed Molecular Formula
Glucose Conjugates	M282	Glycosylated Benzotriazole		$C_{12}H_{15}N_3O_5$	+H	282.10690	120	120.055327	2.49	$C_6H_5N_3$
Amino Acid Conjugates	M207	Benzotriazole Alanine ("BT-tryptophan")		$C_9H_{10}N_4O_2$	+H	207.08746	120	120.05533	2.49	$C_6H_5N_3$
							88	88.03934	-0.35	$C_3H_5NO_2$
							146	146.05905	-2.43	$C_7H_5N_4$
							164	164.06984	-3.52	$C_7H_7N_4O$
	M247	Benzotriazole Acetyl-Alanine		$C_{11}H_{12}N_4O_3$	-H	247.08246	118	118.04087	1.69	$C_6H_5N_3$
							160	160.03924	-1.15	$C_7H_5N_4O$
							132	132.0445	-2.66	$C_6H_5N_4$
							146	146.03593	0.39	$C_7H_5N_3O$
							104	104.0483	-3.87	$C_4H_5O_3$

Table S.2: Chemical structure comparison between the natural plant compound tryptophan and the benzotriazole (BT) substituted analogue targeted in the present study. The BT component is shown in blue for visual distinction.

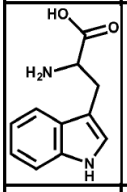
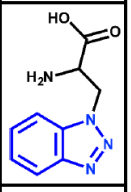
Natural Plant Compound	Benzotriazole Substituted Analogue
	
Tryptophan; Amino Acid; Intermediate in Biosynthesis	**Benzotriazole Alanine**; Tryptophan structural analogue

Table S.3: Mass spectrometer optimized parameters used for quantifying benzotriazole and the BT plant transformation products described in the manuscript. Mass spec analysis was conducted in multiple reaction monitoring (MRM) mode with two transition ions; one for quantification, one for confirmation. Details are adapted from LeFevre et al.[1]

MRM Mass Transition	Q1 Mass (Da)	Q3 Mass (Da)	Declustering Potential (V)	Focusing Potential (V)	Entrance Potential (V)	Collision Energy (V)	Collision Cell Exit Potential (V)
Benzotriazole-1	119.951	65.110	51	240	10	32	4.0
Benzotriazole-2	119.951	92.127	51	240	10	25	6.0
d4-Benzotriazole-1	123.972	69.100	41	170	10	35	4.0
d4-Benzotriazole-2	123.972	96.000	41	170	10	27	6.0
M282-1	281.900	119.900	23	200	10	25	10
M282-2	281.900	85.000	23	200	10	35	10
M207-1	207.088	120.000	36	170	10	22	8.0
M207-2	207.088	179.100	36	170	10	13	12
M247-1	247.080	118.000	-36	-150	-10	-24	-9.0
M247-2	247.080	157.900	-36	-150	-10	-24	-11

Method Detail 5:

Determination of Pollutant-Plant Relative Compartmental Affinity. The "relative compartmental affinity "(RCA) of the BT in the plant part was based on the method of Hyland et al.[11] Because the data were most closely described by a log-normal distribution (See Figure S.2), the geometric mean for each plant part were used for determining the relative compartment affinity (rather than the mean). The relative compartment affinity was calculated by:

$$RCA\ (unitless\ proportion) = \frac{Normalized\ BT\ concentration\ in\ given\ plant\ component\ (\frac{ngBT}{g\ dry\ plant})}{\Sigma(normalized\ BT\ concentration\ in\ each\ plant\ component, \frac{ngBT}{gDryPlant})}$$

Example for the Relative Compartment Affinity for BT in strawberry fruits:

$$RCA_{Fruit} = \frac{[BT_{Fruit}]}{[BT_{Fruit}]+[BT_{Shoot}]+[BT_{Roots}]} \frac{(\frac{ngBT}{gDryPlantTissue})}{(\frac{ngBT}{gDryPlantTissue})}$$

Method Detail 6:

Estimation of Daily Benzotriazole Daily Intake. An estimate was made for the approximate per capita BT consumption through food and water based on the results of this study. Although this should be considered a rough estimate based on many assumptions, the value in communicating this estimate is to provide a rough order of magnitude based on literature assumptions. The assumptions made in the estimation are described below:

Assuming that BT levels encountered in the different strawberry parts and in lettuce from this study represent the average BT levels present in fruits, stem vegetables, root and bulb vegetables and leafy greens we calculated a rough estimation of the daily BT intake of an average adult human. According to the WHO Global Environmental Monitoring System (GEMS) database, the average American/European adult consumes 0.622 kg fruits (including berries, citrus-, pome-, stone fruits, nuts, cereals and other fruiting vegetables and mushrooms), 0.196 kg root vegetables (including tubers and bulb vegetables), 0.070 kg leafy greens and 3.4 g stalk and stem vegetables (all weight specifications refer to fresh, moist weight of food plants)[12]. Using these estimates of dietary intakes, an average water ingestion volume[13] of 2 L containing BT at concentration of 9.3 ng/L (average of the two tap water measurements from the study) and assuming an average vegetable moisture of 90% (BT content in plants and water were not double-counted; moisture content was only used to total mass purposes), we calculated that an average adult ingests approximately 2.2 µg BT on a daily basis. Because the US (*i.e.*, BT is not an EPA regulated drinking water contaminant) and the WHO do not provide any guideline values for BT, it is difficult to estimate if the calculated daily BT intake may pose a risk for human health. The Austrian federal ministry of health calculated an acceptable daily intake (ADI) value of BT of 1.5 µg per kg of body weight,[14] based on the Swiss guidelines for proper handling of non-controlled

foreign substances.[15] Comparison of the calculated daily intakes with the suggested ADI of BT indicates that the risk to human health from BT ingestion is probably low; these values also assume that the BT consumed from plants is fully bioavailable. These assumptions do not consider any possible exposure from animal products or possible biomagnification of BT from plants through consumption of animal products (*e.g.*, meat, eggs, dairy).

Supporting Results:

Table S.4: Summary data table of results of benzotriazole and BT-plant transformation products (M282 and M247; no M207 was detected). Samples are described by growth conditions / location (i.e., greenhouse or field grown), plant type (i.e., strawberry or lettuce plant), and plant part (roots, shoots, fruits [lettuce are whole plants]). "Control" was irrigated with tap water; the greenhouse doings levels are described in Table S.5 ('low'=27 ng/L, 'med'=92 ng/L, 'high'=279 ng/L). "Recycled" indicated that the fields had been reported irrigated with recycled water whereas "well" indicates that the fields were reported irrigated with well water. Values of "0" were non-detect.

Sample Description			Compound Concentration (ng/g dry plant biomass)		
			Benzotriazole	M282 [glycosylated BT]	M247 [BT-acetylalanine]
Greenhouse Strawberries	Fruits	Control 1	14.7	0	0
		Control 2	29.4	0	0
		Low Dose	11.0	0	0
		Med Dose	14.3	0	0
		High Dose	17.9	0	0
		High Dose 2	21.1	0	0
	Shoots	Control 1	11.2	19.8	0
		Control 2	7.5	17.7	0
		Control 3	7.1	8.8	0
		Control 4	0.6	54.8	0
		Low Dose 1	3.8	16.0	0
		Low Dose 2	6.2	18.2	0
		Low Dose 3	5.6	8.0	0
		Low Dose 4	0.9	37.7	0
		Med. Dose 1	0.9	21.4	0
		Med. Dose 2	0.6	18.6	0
		Med. Dose 3	13.3	30.7	0
		Med. Dose 4	5.5	42.3	0
		High Dose 1	3.9	41.5	0
		High Dose 2	12.6	22.5	0
		High Dose 3	10.8	23.5	0
		High Dose 4	18.1	71.7	0
	Roots	Control 1	6.8	5.7	0
		Control 2	12.8	65.6	0
		Control 3	6.5	6.6	0
		Low Dose 1	14.8	33.5	19.4
		Low Dose 2	23.5	9.8	0
		Low Dose 3	15.5	14.7	0
		Med. Dose 1	48.6	236	0
		Med. Dose 2	20.3	28.4	0
		Med. Dose 3	32.2	16.0	0
		High Dose 1	6.5	33.8	0
		High Dose 2	16.9	21.5	0
		High Dose 3	11.6	23.1	0
Field Samples	Strawberry	Shoot, Recycled	16.6	7.1	0
		Shoot, Well	9.8	0.4	0
		Root, Recycled	61.9	26.2	40.9
		Root, Well	23.9	33.8	0
		Fruit, Well 1	16.1	0	0
		Fruit, Well 2	23.7	0	0
		Fruit, Well 3	44.0	0	0
		Fruit, Recycled 1	23.5	0	0
		Fruit, Recycled 2	11.9	0	0
		Fruit, Recycled 3	17.0	0	0
	Lettuce	Leaves, Recycled	62.2	0	0
		Leaves, Recycled	88.7	0	0
		Leaves, Recycled	10.2	0	0
		Leaves, Well 1	6.1	0	0
		Leaves, Well 2	153	0	0
		Leaves, Well 3	73.4	0	0

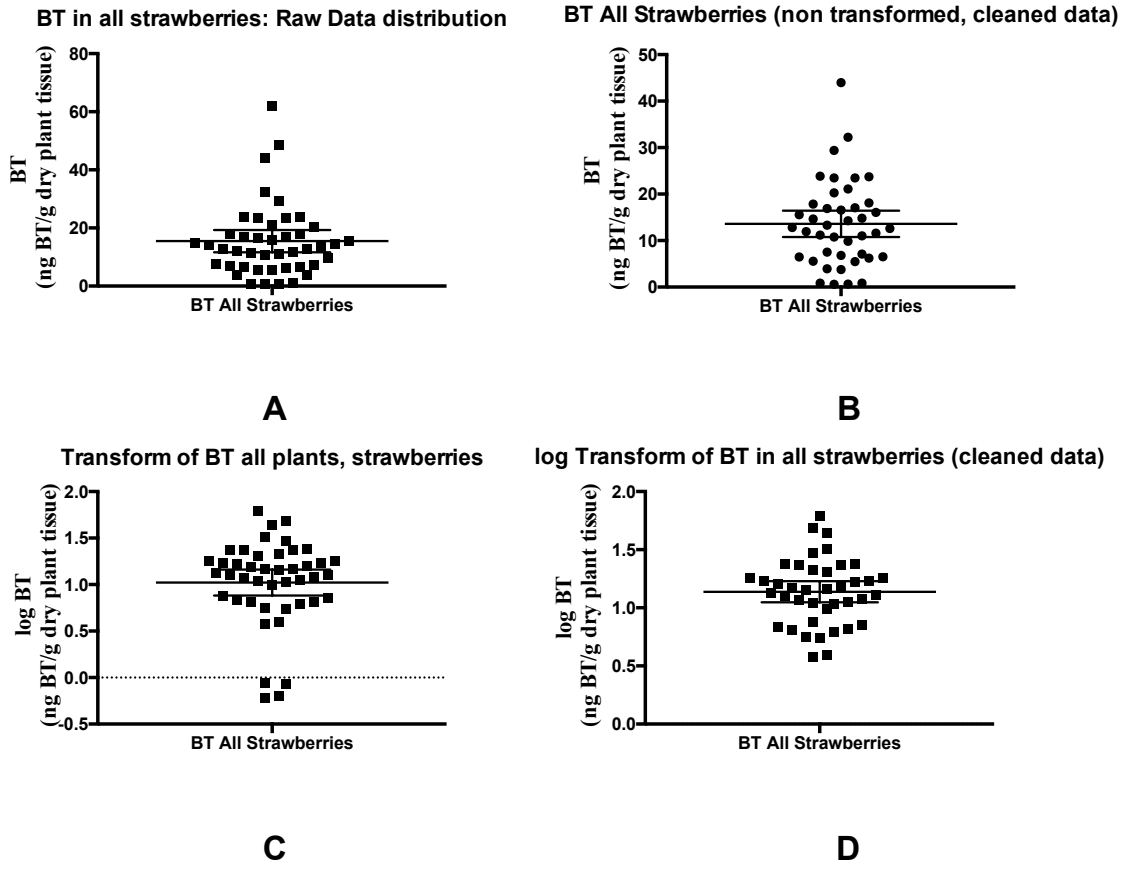


Figure S.2: Distribution of BT residual measured in strawberry plant tissues reported in Table S.4. The mean value and 95% confidence intervals of the data are shown. Clockwise from upper left: (A) Raw data distribution of all data, (B) Distribution of data with statistical outliers removed, (C) log-transformed data, all included, (D) log transformed data with statistical outliers excluded. Outliers were determined at the 1% allowable false discovery rate using the ROUT approach, as described in the manuscript.

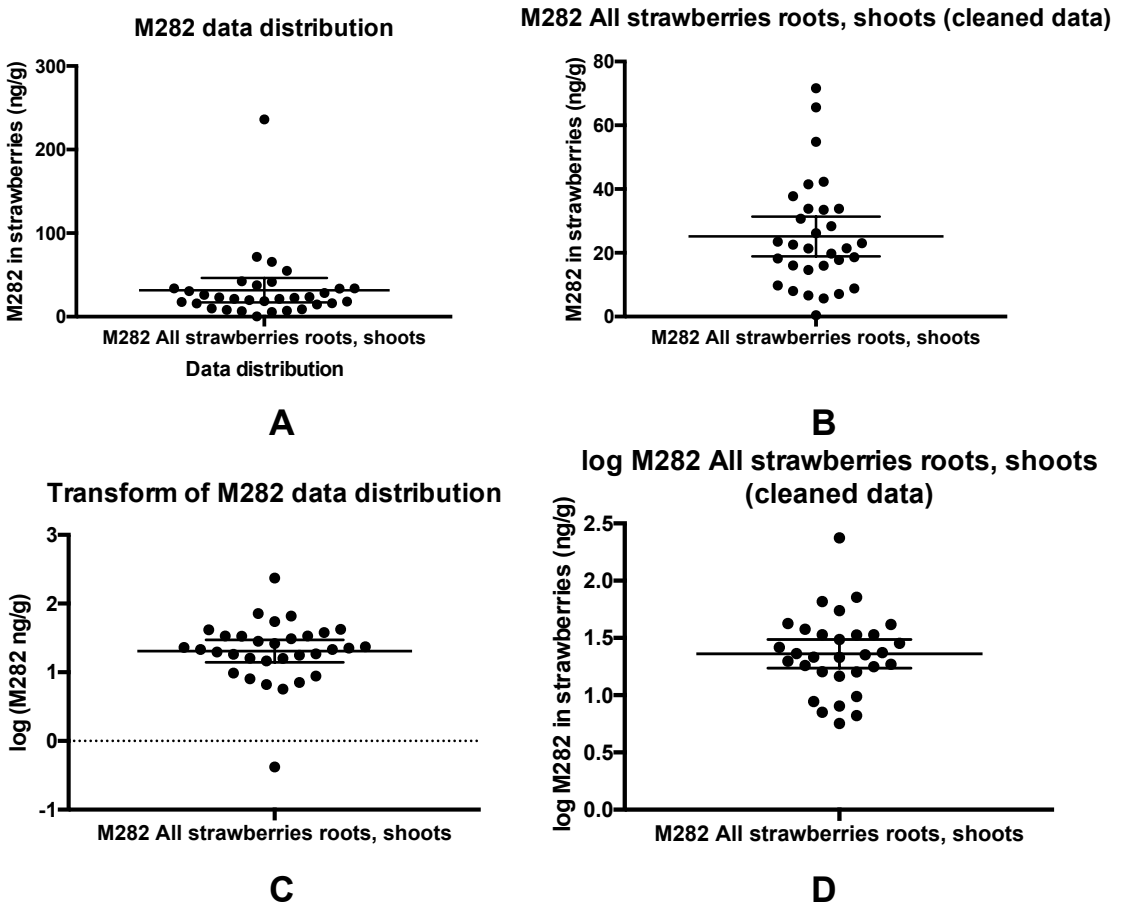


Figure S.3: Distribution of M282 (glycosylated BT) residual measured in strawberry plant tissues (roots and shoots only) reported in Table S.4. The mean value and 95% confidence intervals of the data are shown. Clockwise from upper left: (A) Raw data distribution of all data, (B) Distribution of data with statistical outliers removed, (C) log-transformed data, all included, (D) log transformed data with statistical outliers excluded. Outliers were determined at the 1% allowable false discovery rate using the ROUT approach, as described in the manuscript.

Table S.5: (Below) Full descriptive statistics of the data distributions for BT and M282 in strawberries (data from Table S.4). The cleaned (statistical outliers removed as described in the manuscript) log-transformed data were used in analysis because these data distribution was not significantly different from a Gaussian distribution (using both the D'Agostino & Pearson omnibus normality test and Shapiro-Wilk normality test), which allowed use of parametric-based statistics.

Descriptive Statistics of Data Distribution	BT All Strawberries, Data Distribution				M282 All Strawberries (roots/shoots), Data Distribution			
	non-transformed		log-transformed		non-transformed		log-transformed	
	raw data	cleaned data	raw data	cleaned data	raw data	cleaned data	raw data	cleaned data
Number of values n=	44	42	44	40	32	31	32	31
Minimum	0.5959	0.5959	-0.2248	0.5755	0.4197	0.4197	-0.3771	0.754
25% Percentile	6.599	6.521	0.8194	0.9055	14.99	14.67	1.175	1.203
Median	13.07	12.71	1.116	1.16	22	21.45	1.342	1.353
75% Percentile	19.74	17.92	1.295	1.32	33.81	33.81	1.529	1.529
Maximum	61.92	43.96	1.792	1.792	236	71.66	2.373	2.373
Mean	15.49	13.59	1.022	1.138	31.73	25.14	1.307	1.362
Std. Deviation	12.59	9.12	0.4591	0.2835	40.83	16.94	0.4545	0.3403
Std. Error of Mean	1.897	1.407	0.06922	0.04482	7.218	3.043	0.0803	0.06112
Lower 95% CI of mean	11.66	10.75	0.8828	1.048	17.01	18.93	1.144	1.237
Upper 95% CI of mean	19.31	16.43	1.162	1.229	46.46	31.36	1.471	1.487
95% CI of median								
Actual confidence level	95.12%	95.64%	95.12%	96.15%	97.99%	97.06%	97.99%	97.06%
Lower confidence limit	10.79	9.843	1.033	1.049	16.04	16.04	1.205	1.249
Upper confidence limit	16.56	16.06	1.219	1.232	33.53	30.69	1.525	1.525
D'Agostino & Pearson omnibus normality test								
K2	25.94	10.64	14.13	0.07328	61.21	8.602	19.19	4.31
P value	< 0.0001	0.0049	0.0009	0.964	< 0.0001	0.0136	< 0.0001	0.1159
Passed normality test (alpha=0.05)?	**No**	**No**	**No**	**Yes**	**No**	**No**	**No**	**Yes**
P value summary	****	**	***	ns	****	*	****	ns
Shapiro-Wilk normality test								
W	0.844	0.9374	0.8797	0.9823	0.5266	0.914	0.8824	0.9588
P value	< 0.0001	0.0231	0.0003	0.7752	< 0.0001	0.0164	0.0023	0.2709
Passed normality test (alpha=0.05)?	**No**	**No**	**No**	**Yes**	**No**	**No**	**No**	**Yes**
P value summary	****	*	***	ns	****	*	**	ns
Coefficient of variation	81.27%	67.09%	44.91%	24.90%	128.68%	67.38%	34.76%	24.99%
Geometric mean	10.53	9.733	NA	1.102	20.29	18.75	NA	1.32
Lower 95% CI of geo. mean	7.634	7.091		1.012	13.92	13.18		1.202
Upper 95% CI of geo. mean	14.52	13.36		1.199	29.6	26.68		1.45
Skewness	1.793	1.015	-1.276	0.09342	4.296	1.134	-1.318	0.5534
Kurtosis	4.157	1.786	1.813	-0.1073	21.32	1.283	5.691	1.432
Number of outliers (ROUT method Q=1%)	NA	2	NA	4	NA	1	NA	1

Table S.6: Summary of Results from 2-Way Analysis of Variance (ANOVA) test examining effects of plant part and BT exposure on BT residual in the strawberry tissues grown in the greenhouse (largest sample pool available for analysis). A Tukey's multiple comparisons post-test was conducted (alpha value = 0.05) between plant-part comparisons. A narrative description of the 2-way AVOVA results interpretation is included.

Two-way ANOVA	**2-way BT straw GH, exposure vs. plant part**				
	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	Significant?		
Interaction	16.95	0.2543	No		
BT Exposure	1.839	0.8205	No		
Plant Part	33.84	0.0019	Yes		
ANOVA table	Sum of Squares	DF	Mean Square	F (DFn, DFd)	P value
Interaction	1.241	6	0.2068	F (6, 22) = 1.412	P = 0.2543
BT Exposure	0.1346	3	0.04486	F (3, 22) = 0.3063	P = 0.8205
Plant Part	2.477	2	1.239	F (2, 22) = 8.459	P = 0.0019
Residual	3.221	22	0.1464		

Compare column means (main column effect)		
Number of families	1	
Number of comparisons per family	3	
Alpha	0.05	
Tukey's multiple comparisons test	Adjusted P Value	Significant?
Fruit vs. Shoot	0.0096	Yes
Fruit vs. Root	0.9478	No
Shoot vs. Root	0.0036	Yes

NARRATIVE RESULTS of 2-Way ANOVA, description of test results
Data analyzed: 2-way BT strawberry GH, exposure vs. plant part

Does Plant Part have the same effect at all values of BT Exposure?

Interaction accounts for approximately 16.95% of the total variance.
F = 1.41. DFn=6 DFd=22
The P value = 0.2543
If there is no interaction overall, there is a 25% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered not significant.

Does Plant Part affect the result?

Plant Part accounts for approximately 33.84% of the total variance.
F = 8.46. DFn=2 DFd=22
The P value = 0.0019
If Plant Part has no effect overall, there is a 0.19% chance of randomly observing an effect this big (or bigger) in an experiment of this size. **The effect is considered significant.**

Does BT Exposure affect the result?

BT Exposure accounts for approximately 1.84% of the total variance.
F = 0.31. DFn=3 DFd=22
The P value = 0.8205
If BT Exposure has no effect overall, there is a 82% chance of randomly observing an this big (or bigger) in an experiment of this size. The effect is considered not significant.

Table S.7: Summary of "hits" found during the proteomics investigation. Negative samples were Arabidopsis seedlings NOT exposed to benzotriazole. Samples 1 and 2 were biological replicates of a composite sample of 30 seedlings each. The reported summary table shows instances where a variable mass of +1.990498 Da on tryptophan (trp) residues to allow for the detection of benzotriazole substituted-trp molecules. Over 10,000 peptides were examined for a nominal 2 Da mass shift where benzotriazole would substitute for the indole ring in tryptophan to form BT-alanine. We observed only five peptides where this phenomenon occurred in the BT treated plants, and three occurrences also in the no-BT exposure negative control; both highly infrequent events are likely false positives and are equally distributed between the treatment and control. Therefore, we conclude that BT is not systematically incorporated into higher plant proteins through the tryptophan biosynthesis pathway. Data analyzed by the Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford University Mass Spectrometry (http://mass-spec.stanford.edu).

		Prot. Rank	Pos.	Sequence	PEP 2D	Score	z	Obs. m/z	Obs. MH	Calc. MH	ppm err.	Delta Score	Delta Mod. Score	Protein Name	Scan Time	PID	Mods (variable)
Negative Samples	1	53	53	K.HGAPDTW[+1.99050]TLIK.A	0.001	366.7	2	620.8306	1240.654	1240.6433	8.63	216.9	216.9	>sp|O04499|PMG1_ARATH 2,3-bisphosphoglycerate-independent phosphoglycerate mutase 1 OS=Arabidopsis thaliana GN=PGM1 PE=2 SV=3	58.39	1 | 440012	W7(BenzotriazoleA / 1.9905)
		56	285	K.IVPATAIPDGW[+1.99050]MGLDIGPDSIK.T	2.20E-05	353.5	2	1134.58	2268.153	2268.1692	-7.16	209	209	>tr|Q9SAJ4|Q9SAJ4_ARATH Phosphoglycerate kinase OS=Arabidopsis thaliana GN=T8K14.3 PE=1 SV=1	76.94	2 | 668609	W11(BenzotriazoleA / 1.9905)
		56	285	K.IVPATAIPDGW[+1.99050]MGLDIGPDSIK.T	0.0011	268.9	2	1134.58	2268.153	2268.1692	-7.16	150.8	150.8	>tr|Q9SAJ4|Q9SAJ4_ARATH Phosphoglycerate kinase OS=Arabidopsis thaliana GN=T8K14.3 PE=1 SV=1	76.92	3 | 668489	W11(BenzotriazoleA / 1.9905)
	2	40	352	R.N[+0.98402]HITTEW[+1.99050]DTPRPSAR.L	0.0023	224.2	3	595.2882	1783.8501	1783.847	1.71	167.5	167.5	>sp|Q9LRR9|GLO1_ARATH Peroxisomal (S)-2-hydroxy-acid oxidase GLO1 OS=Arabidopsis thaliana GN=GLO1 PE=1 SV=1	48.8	1 | 555791	N1(Deamidated / 0.984); W7(BenzotriazoleA / 1.9905)
BT-Exposed Samples	1	50	285	K.IVPATAIPDGW[+1.99050]MGLDIGPDSIK.T	4.90E-05	332.4	2	1134.579	2268.1515	2268.1692	-7.81	220.2	220.2	>tr|Q9SAJ4|Q9SAJ4_ARATH Phosphoglycerate kinase OS=Arabidopsis thaliana GN=T8K14.3 PE=1 SV=1	77.47	1 | 644316	W11(BenzotriazoleA / 1.9905)
		50	285	K.IVPATAIPDGW[+1.99050]MGLDIGPDSIK.T	0.18	196.4	2	1134.579	2268.1515	2268.1692	-7.81	19.4	19.4	>tr|Q9SAJ4|Q9SAJ4_ARATH Phosphoglycerate kinase OS=Arabidopsis thaliana GN=T8K14.3 PE=1 SV=1	77.45	2 | 644238	W11(BenzotriazoleA / 1.9905)
		54	53	K.HGAPDTW[+1.99050]TLIK.A	0.0012	374.8	2	620.8304	1240.6536	1240.6433	8.34	202.7	202.7	>sp|O04499|PMG1_ARATH 2,3-bisphosphoglycerate-independent phosphoglycerate mutase 1 OS=Arabidopsis thaliana GN=PGM1 PE=2 SV=3	60.46	3 | 430886	W7(BenzotriazoleA / 1.9905)
		54	53	K.HGAPDTW[+1.99050]TLIK.A	0.0035	343.5	2	620.8304	1240.6536	1240.6433	8.34	170.9	170.9	>sp|O04499|PMG1_ARATH 2,3-bisphosphoglycerate-independent phosphoglycerate mutase 1 OS=Arabidopsis thaliana GN=PGM1 PE=2 SV=3	60.45	4 | 430766	W7(BenzotriazoleA / 1.9905)
		569	13	K.KW[+1.99050]GGGLMGSK.S	0.0042	332.2	2	511.7687	1022.5301	1022.52	9.88	122.9	122.9	>tr|Q42245|Q42245_ARATH Ribosomal protein (Fragment) OS=Arabidopsis thaliana PE=2 SV=1	50.11	5 | 286313	W2(BenzotriazoleA / 1.9905)
		14	294	R.ALDFILGW[+1.99050]HLDTTTFGDYPQIMK.D	0.0024	297.5	2	1342.67	2684.3327	2684.3177	5.6	197	197	>sp|Q9SR37|BGL23_ARATH Beta-glucosidase 23 OS=Arabidopsis thaliana GN=BGLU23 PE=1 SV=1	81.59	1 | 671967	W8(BenzotriazoleA / 1.9905)
	2	34	352	R.N[+0.98402]HITTEW[+1.99050]DTPRPSAR.L	0.017	215.4	3	595.288	1783.8495	1783.847	1.4	152.7	152.7	>sp|Q9LRR9|GLO1_ARATH Peroxisomal (S)-2-hydroxy-acid oxidase GLO1 OS=Arabidopsis thaliana GN=GLO1 PE=1 SV=1	50.05	2 | 546923	N1(Deamidated / 0.984); W7(BenzotriazoleA / 1.9905)
		34	352	R.N[+0.98402]HITTEW[+1.99050]DTPRPSAR.L	0.024	104.4	3	595.288	1783.8495	1783.847	1.4	45.5	45.5	>sp|Q9LRR9|GLO1_ARATH Peroxisomal (S)-2-hydroxy-acid oxidase GLO1 OS=Arabidopsis thaliana GN=GLO1 PE=1 SV=1	50.05	3 | 546843	N1(Deamidated / 0.984); W7(BenzotriazoleA / 1.9905)
		104	362	K.PSW[+1.99050]K.Q	0.055	126.8	1	519.2674	519.2674	519.2674	-0.01	0.5	0.5	>sp|Q9C525|BGL21_ARATH Beta-glucosidase 21 OS=Arabidopsis thaliana GN=BGLU21 PE=1 SV=1	62.28	4 | 73602	W3(BenzotriazoleA / 1.9905)
		943	70	R.LSDTGKNW[+1.99050]R.H	0.11	222	2	539.7723	1078.5374	1078.5388	-1.31	8.4	8.4	>sp|Q67YI9|EPN2_ARATH Clathrin interactor EPSIN 2 OS=Arabidopsis thaliana GN=EPSIN2 PE=1 SV=1	42.86	5 | 339544	W8(BenzotriazoleA / 1.9905)

NOTES (for all samples above): Fragment types=high-energy collision-induced dissociation; Cleavage type=Specific.

Table S.8: Summary statistics of peptides examined on each proteomics run from which the above data were collected. Over 10,000 peptides were examined for each tissue sample to examine systematic substitution of benzotriazole for indole in tryptophan containing proteins. Data analyzed by the Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford University Mass Spectrometry (http://mass-spec.stanford.edu).

		Summary Statistics			
		Negative		BT Exposed	
		1	2	1	2
Reporting Proteins	forward:	1112	1137	1072	1124
	reverse:	20	20	20	20
Unique forward peptides:		5193	5126	4804	4960
Spectra matched to forward peptides:		12068	11825	10769	11478
Estimated spectrum-level FDR on true proteins is:		0.3%	0.4%	0.4%	0.4%

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