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Quantification of *p*-Hydroxyphenylpyruvate Dioxygenase and Phosphinothricin Acetyltransferase in Event SYHT0H2 Soybean Tissues

Final Report

DATA REQUIREMENT(S): Not applicable

AUTHOR: [redacted]

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LABORATORY PROJECT ID: Report Number: TK0050018
Task Number: TK0050018

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No claim of confidentiality is made for any information contained in this report on the basis of its falling within the scope of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 10 (d) (1) (A), (B), or (C).

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Manager, Regulatory Affairs

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This study was conducted in compliance with the relevant provisions of Good Laboratory Practice Standards (GLPS) (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) with the following exceptions:

- Test- and control-substance characterizations were not conducted according to GLPS.
- Weather-data collection, soil characterization, application of maintenance chemicals, and irrigation practices were not conducted according to GLPS.
- Field history records were not generated or maintained according to GLPS.
- Liberty Herbicide was not labelled in accordance with GLPS.

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QUALITY ASSURANCE STATEMENT

Study Title: Quantification of *p*-Hydroxyphenylpyruvate Dioxygenase and Phosphinothricin Acetyltransferase in Event SYTH0H2 Soybean Tissues

Study Director: [redacted]

Study Number: TK0050018

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Dates
Audit Protocol	13-OCT-2010 - 13-OCT-2010	13-OCT-2010
CRO Inspect Planting	13-DEC-2010 - 13-DEC-2010	28-DEC-2010
CRO Inspect Planting	14-DEC-2010 - 14-DEC-2010	28-DEC-2010
CRO Inspect Planting	14-DEC-2010 - 14-DEC-2010	28-DEC-2010
CRO Inspect Planting	21-DEC-2010 - 21-DEC-2010	28-DEC-2010
CRO Inspect Application and or Calibration	11-JAN-2011 - 11-JAN-2011	11-FEB-2011
CRO Inspect Sampling or Harvest	01-FEB-2011 - 01-FEB-2011	11-FEB-2011
CRO Inspect Sampling or Harvest	03-FEB-2011 - 03-FEB-2011	11-FEB-2011
CRO Inspect Sample Prep	08-FEB-2011 - 08-FEB-2011	15-FEB-2011
CRO Inspect Sampling or Harvest	02-MAR-2011 - 02-MAR-2011	17-MAR-2011
CRO Inspect Sampling or Harvest	06-APR-2011 - 06-APR-2011	15-APR-2011
CRO Inspect Sample Prep	06-APR-2011 - 08-APR-2011	15-APR-2011
CRO Inspect Sampling or Harvest	07-APR-2011 - 07-APR-2011	15-APR-2011
CRO Inspect Sampling or Harvest	07-APR-2011 - 07-APR-2011	15-APR-2011
CRO Inspect Sampling or Harvest	10-APR-2011 - 10-APR-2011	15-APR-2011
CRO - Inspect Biology	10-APR-2011 - 10-APR-2011	15-APR-2011
CRO Inspect Sampling or Harvest	12-APR-2011 - 12-APR-2011	15-APR-2011
CRO - Inspect Data	20-JUN-2011 - 20-JUN-2011	06-JUL-2011
CRO - Inspect Data	20-JUN-2011 - 20-JUN-2011	06-JUL-2011
CRO - Inspect Data	21-JUN-2011 - 21-JUN-2011	06-JUL-2011

QUALITY ASSURANCE STATEMENT *continued*

Inspection/Audit Type	Inspection/Audit Dates	Reporting Dates
CRO - Inspect Data	21-JUN-2011 - 21-JUN-2011	06-JUL-2011
CRO - Inspect Data	22-JUN-2011 - 22-JUN-2011	06-JUL-2011
Audit Study Data	05-JUL-2011 - 06-JUL-2011	14-JUL-2011
Inspect Analytical	14-DEC-2011 - 14-DEC-2011	19-DEC-2011
Audit Study Data	09-JAN-2012 - 11-JAN-2012	12-JAN-2012
Audit Study Data	16-JAN-2012 - 23-JAN-2012	23-JAN-2012
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Prepared By: [redacted] Date: March 27, 2012

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GENERAL INFORMATION

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Study dates

Study initiation date:	October 20, 2010
Experimental start date:	December 13, 2010
Experimental termination date:	December 16, 2011

Records Retention

Raw data, the original copy of this report, and other relevant records are archived at Syngenta, 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA.

Additional Test Sites

The field trials for this study were conducted by:

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For the list of contributors at IBCA, see the Field Trial Summary in Appendix B

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LIST OF ACRONYMS AND ABBREVIATIONS

ai	active ingredient
avhppd-03	*p*-hydroxyphenylpyruvate dioxygenase gene derived from oat
AvHPPD-03	*p*-hydroxyphenylpyruvate dioxygenase enzyme encoded by *avhppd-03*
DW	dry weight
ELISA	enzyme-linked immunosorbent assay
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FW	fresh weight
g	gram
GLPS	Good Laboratory Practice Standards
ha	hectare
HPPD	*p*-hydroxyphenylpyruvate dioxygenase enzyme
IBCA	Investigaciones Biotecnológicas en el Campo Argentino
kg	kilogram
LOD	limit of detection
LOQ	limit of quantitation
ml	milliliter
ng	nanogram
PAT	phosphinothricin acetyltransferase
SD	standard deviation
US EPA	United States Environmental Protection Agency
µg	microgram

Abbreviations for Soybean Growth Stages (Pederson 2009)

Vegetative:

V3	Three fully developed trifoliate leaf nodes
V4	Four fully developed trifoliate leaf nodes
V8	Eight fully developed trifoliate leaf nodes
V10	Ten fully developed trifoliate leaf nodes

Reproductive:

R6	Full seed; pod containing a green seed that fills the pod capacity at one of the four uppermost nodes on the main stem
R8	Full maturity; 95% of the pods have reached their full mature color

1.0 EXECUTIVE SUMMARY

The purpose of this study was to measure the concentrations of the proteins *p*-hydroxyphenylpyruvate dioxygenase (AvHPPD-03) and phosphinothricin acetyltransferase (PAT) in several tissues of soybean plants derived from transformation Event SYHT0H2, grown at several locations.

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the novel genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT). Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of SYHT0H2 soybean.

SYHT0H2 soybean and the corresponding nontransgenic, near-isogenic soybean were grown at four field-trial locations in Argentina during the 2011/2012 growing season. Each field trial was designed to generate tissue samples from field-grown soybean plants cultivated in accordance with common agricultural practices. At each location, two replicate plots were planted with SYHT0H2 soybean and one plot was planted with nontransgenic soybean. One of the replicate SYHT0H2 soybean plots received a single post-emergent spray application of the trait-specific herbicides mesotrione and glufosinate at the V3-V4 growth stage. From each plot, leaves were collected from soybean plants at four different growth stages, roots were collected from plants at two growth stages, and forage and seed were collected from plants at one growth stage per tissue type. Five replicate samples of each tissue type were collected from each plot. Enzyme-linked immunosorbent assays (ELISA) were used to quantify AvHPPD-03 and PAT in each SYHT0H2 soybean tissue sample. Concurrent analysis of tissues from nontransgenic soybean confirmed the absence of plant-matrix effects on the ELISA methods and also confirmed the specificity of the ELISA methods for AvHPPD-03 and PAT.

The results for AvHPPD-03 concentrations are summarized as ranges on a fresh-weight basis across the four locations. In leaves, the concentration of AvHPPD-03 across four growth stages (V4, V8, V10, and R6) ranged from 6.82 to 207.21 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 4.93 to 135.84 µg/g for SYHT0H2 soybean not treated with mesotrione and glufosinate ("untreated"). In roots, the concentration of AvHPPD-03 across two growth stages (V8 and R6) ranged from 1.21 to 50.18 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 0.42 to 45.65 µg/g for untreated SYHT0H2 soybean. In forage, the concentration of AvHPPD-03 at growth stage R6 ranged from 9.88 to 48.79 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 4.31 to 44.32 µg/g for untreated SYHT0H2 soybean. In seed, the concentration of AvHPPD-03 at growth stage R8 ranged from 0.34 to 24.84 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 0.55 to 24.94 µg/g for untreated SYHT0H2 soybean.

The results for PAT concentrations are summarized as ranges on a fresh-weight basis across the four locations. In leaves, the concentration of PAT across four growth stages (V4, V8, V10, and R6) ranged from 0.13 to 47.90 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 0.22 to 41.43 µg/g for untreated SYHT0H2 soybean. In roots, the concentration of PAT across two growth stages (V8 and R6) ranged from less than the limit of detection to 12.53 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 0.07 to 11.98 µg/g for untreated SYHT0H2 soybean. In forage, the concentration of PAT at growth stage R6 ranged from 0.36 to 20.15 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 0.29 to 16.46 µg/g for untreated SYHT0H2 soybean. In seed, the concentration of PAT at growth stage R8 ranged from less than the limit of quantitation to 14.27 µg/g for mesotrione and glufosinate-treated SYHT0H2 soybean and from 0.06 to 13.13 µg/g for untreated SYHT0H2 soybean.

The concentrations of AvHPPD-03 and PAT measured in this study represent the levels of these proteins in SYHT0H2 soybean in various tissue types and developmental stages across four different field environments. Concentrations of AvHPPD-03 and PAT were quantifiable in all SYHT0H2 soybean tissue types analyzed.

2.0 INTRODUCTION

The purpose of this study was to measure the concentrations of proteins *p*-hydroxyphenylpyruvate dioxygenase (AvHPPD-03) and phosphinothricin acetyltransferase (PAT) present in tissues collected from soybean plants derived from transformation Event SYHT0H2 grown at four locations in Argentina, during the 2010/2011 growing season.

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the novel genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide glufosinate, an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of SYHT0H2 soybean.

The data from this study provide a profile of AvHPPD-03 and PAT concentrations present in SYHT0H2 soybean throughout the life of the plant. The concentrations of AvHPPD-03 and PAT in various tissues were quantified by enzyme-linked immunosorbent assay (ELISA). Samples from plants of a corresponding nontransgenic, near-isogenic soybean variety, grown concurrently, were included with analyses as analytical controls.

3.0 MATERIALS AND METHODS

3.1 Test, Control, and Reference Substances

The test substance for this study was SYHT0H2 soybean seed in the genetic background 'Jack' (Nickell *et al.* 1990). The control substance was nontransgenic near-isogenic soybean seed of the same genetic background as the test substance. Table 1 shows the descriptions and material identification codes for the test and control substances.

TABLE 1 Test and control substances

Seed Identification	Material identification
Nontransgenic soybean (control)	10SG025036
SYHT0H2 soybean (test)	10SG025049

Field-grown seed lots of the test and control substances were characterized by real-time polymerase chain reaction testing (Ingham *et al*. 2001) to confirm identity and purity.

A pedigree chart illustrating the production of the test-substance seed shown in Table 1 can be found in Appendix A.

Table 2 shows the protein reference substances used to produce the standard curve for each ELISA.

TABLE 2 Protein reference substances for ELISA analyses

Protein	Reference substance ID	Characterization report
AvHPPD-03	AvHPPD-03-0209	Winslow 2009
PAT	PAT-0109	Seastrum 2009

3.2 Plant Tissue Production and Collection

During the 2011/2012 growing season, soybean plants were grown according to local agronomic practices at four separate field-trial locations in Argentina that are representative of agricultural regions where soybean is commercially cultivated and that are suitable for the cultivation of 'Jack.' These locations are listed in Table 3.

TABLE 3 Field trial locations

City and State	Site Code
Gahan, Provincia de Buenos Aires	R073
Los Angeles, Provincia de Buenos Aires	R074
Inés Indart, Provincia de Buenos Aires	R075
Salto, Provincia de Buenos Aires	R076

At each location, two replicate plots were planted with the test substance and one plot was planted with the control substance. One of the SYHT0H2 replicate plots received a single post-emergent spray application of each of the trait-specific herbicides mesotrione and glufosinate at the labeled rates when the plants reached the V3-V4 growth stage (Pederson 2009). Mesotrione was applied at a nominal rate of 0.105 kg of active ingredient per hectare (ai/ha) and glufosinate was applied at a nominal rate of 0.322 kg of ai/ha. The Field Trial Summary (Appendix B) includes additional information captured during the field phase of this study.

Table 4 shows the plant samples collected for analysis.

TABLE 4 Tissue samples collected for analysis

Growth stage[a]	Tissues collected	No. control samples	No. samples per test plot	Sample description
V4	leaves	2	5	Each sample contained all healthy trifoliate leaves from one plant.
V8	leaves	2	5	Each sample contained all healthy trifoliate leaves from one plant.
	roots	2	5	Each sample contained all root tissue from one plant.
V10	leaves	2	5	Each sample contained all healthy trifoliate leaves from one plant.
R6	leaves	2	5	Each sample contained all healthy trifoliate leaves from one plant.
	roots	2	5	Each sample contained all root tissue from one plant.
	forage	2	5	Each sample contained the entire above-ground portion of one plant.
R8	seed	2	5	Each sample contained all seed from the pods of one plant.

[a] Pederson (2009)

All plant samples were placed on dry ice after collection and transported to Investigaciones Biotecnológicas en el Campo Argentino (IBCA), Salto, Provincia de Buenos Aires, Argentina and then stored at -80°C ± 10°C until they were prepared for protein extraction and analysis.

3.3 Plant Tissue Sample Preparation

Plant tissue samples were ground individually into a fine powder in the presence of dry ice at IBCA and then stored at -80°C ± 10°C until shipment. All ground samples were shipped on dry ice to Syngenta Crop Protection, LLC, Product Safety, Research Triangle Park, NC, USA, where they were stored at -80°C ± 10°C.

A subsample from each homogeneous powdered sample was lyophilized for protein extraction and analysis and stored at -80 °C ± 10°C. The percent dry weight (DW) of each sample was determined from the fresh weight (FW) of the sample before lyophilization and the DW after lyophilization by the following formula:

$$\% \text{ DW} = \left(\frac{\text{DW (g)}}{\text{FW (g)}}\right) \times 100$$

3.4 Protein Extraction and ELISA Analysis

Protein extractions were performed on representative aliquots of the lyophilized samples. ELISA methodology was used to quantify AvHPPD-03 and PAT in each extract. Nontransgenic plant tissue extracts were analyzed concurrently to confirm the absence of plant-matrix effects in each ELISA.

For each ELISA, a standard curve was generated with known amounts of the corresponding reference protein. The mean absorbance for each sample extract was plotted against the appropriate standard curve to obtain the amount of protein as nanograms per milliliter of extract. The concentrations were converted to represent the amount of protein as micrograms per gram of tissue by the following formula:

$$\frac{\text{(ng/ml)} \times \text{(dilution factor)} \times \text{(volume of buffer [ml])}}{\text{(amount of tissue [g])} \times 1000}$$

A description of the AvHPPD-03 and PAT quantification procedures, including validation of ELISA sensitivity and extraction efficiency, can be found in Appendices C and D.

Protein concentrations were converted from a DW basis to a FW basis by the following formula:

$$\mu\text{g/g FW} = \mu\text{g/g DW} \times (\% \text{ DW} \div 100)$$

3.5 Adjustments for Extraction Efficiency

The predetermined extraction efficiencies (provided in Appendices C and D) were used to adjust the AvHPPD-03 and PAT concentrations to the estimated total AvHPPD-03 and PAT concentration in the corresponding tissue sample by the following formula:

$$\left(\frac{\text{amount of protein measured from a single extraction (µg/g)}}{\text{extraction efficiency (\%)}}\right)$$

The AvHPPD-03 concentrations in forage samples were adjusted with the extraction efficiency determined for leaves, which was lower than that for root or seed matrices and therefore provided the most conservative adjustment.

The PAT concentrations in forage samples were adjusted with the extraction efficiency determined for roots, which was lower than that for leaves or seed matrices and therefore provided the most conservative adjustment.

3.6 Control of Bias Statement

Selection of plants was limited to plants at the defined developmental stage and was conducted nonsystematically to avoid bias in sampling. Protein extractions were performed on representative aliquots of homogeneous processed samples, and each extract was analyzed in triplicate. Nontransgenic sample extracts of the corresponding tissue type were included on each assay plate to serve as analytical controls. Any rejected data, and the documented reasons for the rejection of those data, were retained in the study file.

3.7 Statistical Analysis Statement

All calculations, including means and standard deviations (SD), were performed with Microsoft Excel® 2007 spreadsheet software. All decimal places associated with the concentrations determined for each replicate sample were used in calculation of the means, which were then rounded to two decimal places for reporting consistency.

4.0 RESULTS

4.1 Concentrations of AvHPPD-03 in Tissues at Several Developmental Stages

Tables 5 and 6 show the means and ranges of AvHPPD-03 concentrations measured in SYHT0H2 soybean leaves, roots, forage, and seed on a DW and FW basis. The concentrations of AvHPPD-03 in individual samples of the various tissues analyzed are reported in Appendix E. All concentrations reported in this section were adjusted for extraction efficiency as described above.

Analysis of nontransgenic soybean tissue extracts confirmed the absence of performance-inhibiting plant-matrix effects on the AvHPPD-03 ELISA method. In addition, endogenous soybean HPPD was not detected in extracts of the nontransgenic soybean tissue samples, confirming the specificity of the ELISA method for AvHPPD-03.

Variability of AvHPPD-03 concentrations was observed among replicate samples, as indicated by the wide ranges and large standard deviations (Tables 5 and 6). The highly variable concentrations could not be attributed to the study conduct as several levels of bias control were implemented throughout the study.

TABLE 5 Concentrations of AvHPPD-03 in leaves of SYHT0H2 soybean plants of four growth stages grown at four different locations on a dry-weight and a fresh-weight basis

Stage	Site code	Mesotrione + glufosinate applied				No mesotrione + glufosinate applied			
		µg/g DW		µg/g FW		µg/g DW		µg/g FW	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
V4	R073	421.44 ± 294.74	124.83–877.37	88.84 ± 63.08	24.20–185.19	206.91 ± 77.34	77.26–265.96	55.52 ± 21.90	20.96–74.47
	R074	349.99 ± 295.81	141.41–850.49	88.02 ± 69.11	42.06–207.21	257.60 ± 145.17	20.23–383.77	65.28 ± 34.38	6.39–92.94
	R075	331.92 ± 168.14	176.79–545.17	78.28 ± 40.62	40.54–132.40	355.47 ± 185.51	130.72–585.46	80.60 ± 42.85	31.77–135.84
	R076	230.46 ± 234.02	89.23–633.30	55.89 ± 59.30	19.05–157.11	148.00 ± 62.94	50.88–222.83	34.64 ± 15.53	12.45–54.47
	All locations	333.45 ± 243.09	89.23–877.37	77.76 ± 55.83	19.05–207.21	242.00 ± 140.99	20.23–585.46	59.01 ± 32.86	6.39–135.84
V8	R073	169.38 ± 98.03	90.26–307.28	41.23 ± 25.55	22.74–81.37	159.78 ± 117.27	53.77–335.55	37.86 ± 28.09	11.72–79.94
	R074	127.80 ± 52.62	83.08–200.07	33.48 ± 14.50	22.35–56.41	194.76 ± 38.90	136.26–232.47	49.69 ± 8.67	39.16–59.00
	R075	262.51 ± 79.90	160.19–342.45	68.08 ± 18.19	45.72–88.49	262.28 ± 121.81	100.34–386.15	75.95 ± 37.40	29.72–116.85
	R076	235.69 ± 76.03	165.65–358.57	56.69 ± 12.23	44.45–75.64	235.10 ± 108.39	116.06–344.43	63.13 ± 31.25	31.73–98.18
	All locations	198.84 ± 90.26	83.08–358.57	49.87 ± 21.74	22.35–88.49	212.98 ± 102.03	53.77–386.15	56.65 ± 29.95	11.72–116.85
V10	R073	185.71 ± 26.54	157.75–216.85	51.54 ± 7.22	42.72–60.33	164.21 ± 73.37	85.55–281.46	45.85 ± 19.01	24.09–74.46
	R074	204.60 ± 44.39	145.75–244.82	52.20 ± 9.67	38.63–60.99	127.02 ± 22.53	101.16–159.23	33.63 ± 4.90	27.97–40.91
	R075	276.89 ± 145.64	128.49–495.19	68.24 ± 33.00	34.11–117.19	217.94 ± 62.25	148.39–302.90	52.37 ± 14.74	37.60–72.84
	R076	207.47 ± 90.00	108.66–321.28	46.56 ± 19.19	25.07–70.75	151.37 ± 73.87	55.96–263.58	33.66 ± 15.64	12.11–56.03
	All locations	218.67 ± 89.43	108.66–495.19	54.64 ± 20.18	25.07–117.19	165.14 ± 66.11	55.96–302.90	41.38 ± 15.71	12.11–74.46
R6	R073	103.35 ± 60.15	69.91–209.78	33.70 ± 22.43	20.11–73.48	154.99 ± 83.41	61.02–255.30	47.25 ± 25.19	19.35–75.67
	R074	139.50 ± 58.28	76.97–219.09	36.05 ± 15.17	19.11–58.41	89.60 ± 57.54	38.57–173.93	22.53 ± 13.80	10.14–43.20
	R075	63.85 ± 28.03	31.15–94.02	15.58 ± 6.73	7.59–22.31	69.89 ± 41.38	25.96–136.37	18.04 ± 10.96	6.76–35.84
	R076	137.28 ± 87.28	23.61–235.38	39.42 ± 24.89	6.82–65.74	106.80 ± 67.04	16.94–179.22	30.17 ± 19.07	4.93–52.07
	All locations	111.00 ± 65.13	23.61–235.38	31.19 ± 19.60	6.82–73.48	105.32 ± 67.18	16.94–255.30	29.50 ± 20.15	4.93–75.67

N = 5 for all means and ranges except those for "all locations," for which *N* = 20.
The concentrations were adjusted for extraction efficiency.

TABLE 6 Concentrations of AvHPPD-03 in V8 roots, R6 roots, R6 forage, and R8 seed of SYHT0H2 soybean plants grown at four different locations on a dry-weight and a fresh-weight basis

Tissue Type	Site code	Mesotrione + glufosinate applied				No mesotrione + glufosinate applied			
		µg/g DW		µg/g FW		µg/g DW		µg/g FW	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Roots (V8)	R073	49.84 ± 47.68	5.90–124.71	10.99 ± 10.10	1.58–26.47	53.41 ± 25.78	15.43–76.97	12.84 ± 5.95	3.74–18.04
	R074	47.96 ± 14.92	27.52–65.98	10.89 ± 4.10	5.04–16.04	66.53 ± 41.57	16.66–129.13	14.02 ± 8.22	3.71–25.31
	R075	75.73 ± 13.29	56.03–93.44	17.93 ± 3.13	13.33–22.00	104.88 ± 61.03	44.45–201.47	23.32 ± 13.60	10.13–45.65
	R076	109.05 ± 94.96	15.80–266.68	22.25 ± 17.45	5.27–50.18	93.15 ± 49.22	15.44–144.86	20.69 ± 13.63	3.24–39.40
	All locations	70.65 ± 55.73	5.90–266.68	15.51 ± 10.76	1.58–50.18	79.49 ± 47.33	15.43–201.47	17.72 ± 10.96	3.24–45.65
Roots (R6)	R073	42.38 ± 31.77	5.99–75.50	11.81 ± 8.75	1.85–20.17	29.38 ± 27.97	1.50–69.95	7.23 ± 7.11	0.42–18.26
	R074	20.00 ± 9.23	6.42–29.44	5.58 ± 2.46	1.99–7.62	30.36 ± 25.97	2.95–62.44	8.57 ± 7.41	0.87–17.99
	R075	31.13 ± 22.37	5.32–51.84	7.97 ± 5.62	1.55–13.85	15.09 ± 12.51	4.75–32.58	3.82 ± 2.91	1.39–7.89
	R076	31.68 ± 28.07	4.26–63.72	9.00 ± 8.07	1.21–20.03	15.18 ± 13.18	4.46–30.21	3.84 ± 3.26	1.20–7.80
	All locations	31.30 ± 23.82	4.26–75.50	8.59 ± 6.56	1.21–20.17	22.50 ± 20.82	1.50–69.95	5.87 ± 5.55	0.42–18.26
Forage (R6)	R073	122.06 ± 28.69	100.22–171.79	34.69 ± 8.08	28.22–48.79	65.73 ± 35.36	32.36–106.70	18.71 ± 9.96	9.37–31.66
	R074	66.85 ± 23.21	43.77–99.81	17.35 ± 6.03	11.20–25.89	101.62 ± 50.82	48.82–163.10	25.75 ± 12.75	12.47–40.44
	R075	86.90 ± 12.58	76.28–101.57	22.46 ± 3.58	18.74–26.82	58.12 ± 6.08	49.03–63.96	14.52 ± 1.61	12.01–16.22
	R076	96.16 ± 52.17	34.73–165.73	25.15 ± 14.04	9.88–44.00	93.17 ± 62.08	16.76–164.01	24.48 ± 16.90	4.31–44.32
	All locations	92.99 ± 36.16	34.73–171.79	24.91 ± 10.36	9.88–48.79	79.66 ± 44.43	16.76–164.01	20.86 ± 11.72	4.31–44.32
Seed (R8)	R073	8.13 ± 9.42	0.39–20.89	7.17 ± 8.30	0.34–18.48	3.57 ± 3.94	0.62–10.02	3.17 ± 3.50	0.55–8.92
	R074	6.63 ± 5.35	0.60–14.24	5.72 ± 4.61	0.52–12.28	12.12 ± 8.06	2.51–20.61	10.45 ± 6.93	2.19–17.51
	R075	2.82 ± 0.86	1.37–3.48	2.49 ± 0.76	1.21–3.05	5.59 ± 5.40	0.75–13.65	4.93 ± 4.77	0.67–12.04
	R076	14.07 ± 11.29	0.90–28.36	12.47 ± 9.99	0.81–24.84	11.45 ± 12.43	1.07–28.30	10.10 ± 10.97	0.93–24.94
	All locations	7.91 ± 8.30	0.39–28.36	6.96 ± 7.33	0.34–24.84	8.18 ± 8.36	0.62–28.30	7.16 ± 7.31	0.55–24.94

N = 5 for all means and ranges except those for "all locations," for which *N* = 20.
The concentrations were adjusted for extraction efficiency.

4.2 Concentrations of PAT in Tissues at Several Developmental Stages

Tables 7 and 8 show the means and ranges of PAT concentrations measured in SYHT0H2 soybean leaves, roots, forage, and seed on a DW and FW basis. The concentrations of PAT in individual samples of the various tissues analyzed are reported in Appendix F. All concentrations reported in this section were adjusted for extraction efficiency as described above.

Analysis of nontransgenic soybean tissue extracts confirmed the absence of performance-inhibiting plant-matrix effects on the PAT ELISA method.

Variability of PAT concentrations was observed among replicate samples, as indicated by the wide ranges and large standard deviations (Tables 7 and 8). The highly variable concentrations could not be attributed to the study conduct as several levels of bias control were implemented throughout the study.

TABLE 7 Concentrations of PAT in leaves of SYHT0H2 soybean plants of four growth stages grown at four different locations on a dry-weight and a fresh-weight basis

Stage	Site code	Mesotrione + glufosinate applied				No mesotrione + glufosinate applied			
		µg/g DW		µg/g FW		µg/g DW		µg/g FW	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
V4	R073	51.38 ± 52.12	8.61–136.29	11.07 ± 11.86	1.67–30.87	39.96 ± 28.29	16.00–84.77	10.68 ± 8.06	4.34–24.38
	R074	74.44 ± 46.89	4.46–133.83	17.86 ± 10.86	1.73–31.53	77.42 ± 80.56	3.31–167.97	18.87 ± 18.85	1.04–41.43
	R075	33.74 ± 31.63	3.61–85.02	7.72 ± 7.17	0.96–19.30	57.23 ± 58.54	16.68–149.10	13.11 ± 13.58	3.58–34.59
	R076	41.59 ± 32.91	1.70–91.83	9.91 ± 8.25	0.37–22.78	34.22 ± 36.71	0.89–92.61	7.58 ± 7.57	0.22–18.98
	All locations	50.29 ± 41.46	1.70–136.29	11.64 ± 9.73	0.37–31.53	52.21 ± 53.28	0.89–167.97	12.56 ± 12.55	0.22–41.43
V8	R073	10.82 ± 11.42	0.55–28.02	2.54 ± 2.56	0.13–6.12	11.08 ± 10.59	2.04–27.65	2.55 ± 2.41	0.47–6.40
	R074	9.99 ± 4.74	4.91–16.12	2.61 ± 1.30	1.32–4.54	37.06 ± 36.32	2.30–83.43	9.37 ± 9.23	0.66–21.17
	R075	19.29 ± 7.52	9.33–30.21	5.15 ± 2.27	2.27–8.62	20.59 ± 15.56	2.46–40.46	5.95 ± 4.56	0.68–12.24
	R076	33.82 ± 22.42	17.21–70.95	8.14 ± 5.17	4.28–17.03	23.25 ± 19.31	3.71–52.78	6.33 ± 5.33	1.00–14.38
	All locations	18.48 ± 15.70	0.55–70.95	4.61 ± 3.74	0.13–17.03	23.00 ± 22.84	2.04–83.43	6.05 ± 5.97	0.47–21.17
V10	R073	24.91 ± 9.38	15.58–35.31	6.92 ± 2.63	4.44–9.91	58.04 ± 40.45	4.77–115.86	16.06 ± 10.92	1.41–31.35
	R074	37.38 ± 42.02	14.61–112.26	9.51 ± 10.56	3.62–28.32	22.88 ± 9.24	7.28–31.64	6.03 ± 2.44	1.91–8.16
	R075	74.09 ± 69.71	10.17–172.52	18.44 ± 17.02	2.70–40.85	40.97 ± 38.93	9.35–106.82	9.85 ± 9.38	2.37–25.69
	R076	75.98 ± 55.57	13.57–154.98	17.41 ± 13.04	3.13–35.94	31.03 ± 22.30	14.36–68.14	7.09 ± 5.56	3.13–16.56
	All locations	53.09 ± 50.91	10.17–172.52	13.07 ± 12.15	2.70–40.85	38.23 ± 31.10	4.77–115.86	9.76 ± 8.21	1.41–31.35
R6	R073	36.28 ± 56.50	5.29–136.75	12.43 ± 19.95	1.61–47.90	44.25 ± 40.15	5.09–101.58	13.58 ± 12.42	1.61–30.84
	R074	41.25 ± 45.85	2.59–101.70	10.46 ± 11.37	0.64–24.43	29.31 ± 27.24	2.74–67.29	7.28 ± 6.60	0.73–16.25
	R075	12.83 ± 13.03	2.17–29.66	3.02 ± 2.95	0.53–6.55	15.88 ± 14.34	6.65–41.28	4.07 ± 3.65	1.73–10.54
	R076	45.22 ± 38.52	6.84–94.50	12.95 ± 10.92	1.98–26.39	28.20 ± 22.98	0.77–62.99	7.97 ± 6.42	0.22–17.45
	All locations	33.89 ± 40.36	2.17–136.75	9.71 ± 12.43	0.53–47.90	29.41 ± 27.51	0.77–101.58	8.23 ± 8.09	0.22–30.84

$N = 5$ for all means and ranges except those for "all locations," for which $N = 20$.
The concentrations were adjusted for extraction efficiency.

TABLE 8 Concentrations of PAT in V8 roots, R6 roots, R6 forage, and R8 seed of SYHT0H2 soybean plants grown at four different locations on a dry-weight and a fresh-weight basis

Tissue Type	Site code	Mesotrione + glufosinate applied				No mesotrione + glufosinate applied			
		µg/g DW		µg/g FW		µg/g DW		µg/g FW	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Roots (V8)	R073	12.08 ± 17.03	<LOD[a]–41.21	2.63 ± 3.60	<LOD[a]–8.75	15.81 ± 19.12	1.25–41.66	3.77 ± 4.53	0.30–9.96
	R074	7.26 ± 5.84	1.54–16.94	1.66 ± 1.46	0.36–4.12	19.95 ± 18.07	0.33–45.51	4.22 ± 3.78	0.07–8.92
	R075	9.85 ± 6.62	2.65–20.16	2.34 ± 1.61	0.63–4.89	22.31 ± 18.53	0.61–45.06	4.94 ± 4.13	0.14–10.21
	R076	31.73 ± 22.39	9.62–66.60	6.67 ± 4.09	3.21–12.53	26.57 ± 21.36	1.75–46.07	6.16 ± 5.29	0.37–11.98
	All locations	15.23 ± 16.78	<LOD[a]–66.60	3.33 ± 3.36	<LOD[a]–12.53	21.16 ± 18.17	0.33–46.07	4.77 ± 4.21	0.07–11.98
Roots (R6)	R073	14.91 ± 10.39	0.88–25.26	4.16 ± 2.94	0.27–7.38	9.31 ± 7.83	0.45–19.04	2.30 ± 2.00	0.13–4.97
	R074	6.76 ± 5.07	2.28–14.61	1.93 ± 1.53	0.64–4.40	11.35 ± 11.19	2.26–29.35	3.21 ± 3.22	0.66–8.45
	R075	10.04 ± 8.03	1.00–22.97	2.62 ± 2.19	0.29–6.14	6.09 ± 7.49	0.47–17.92	1.51 ± 1.81	0.14–4.39
	R076	12.88 ± 12.57	0.31–30.00	3.73 ± 3.89	0.10–9.43	9.73 ± 9.09	0.32–23.87	2.57 ± 2.33	0.07–6.16
	All locations	11.15 ± 9.21	0.31–30.00	3.11 ± 2.71	0.10–9.43	9.12 ± 8.50	0.32–29.35	2.40 ± 2.29	0.07–8.45
Forage (R6)	R073	50.81 ± 18.78	19.07–68.10	14.51 ± 5.54	5.37–20.15	11.47 ± 5.88	2.13–17.51	3.28 ± 1.69	0.62–5.20
	R074	13.25 ± 10.83	6.15–32.47	3.44 ± 2.81	1.62–8.42	21.53 ± 18.50	4.22–43.26	5.46 ± 4.67	1.06–10.73
	R075	22.29 ± 12.06	8.41–37.70	5.79 ± 3.17	2.13–9.62	14.31 ± 19.86	1.50–49.41	3.60 ± 5.05	0.37–12.53
	R076	32.58 ± 22.81	1.27–63.37	8.39 ± 6.04	0.36–16.73	29.36 ± 25.16	1.12–60.91	7.80 ± 6.86	0.29–16.46
	All locations	29.73 ± 21.08	1.27–68.10	8.03 ± 5.99	0.36–20.15	19.17 ± 18.61	1.12–60.91	5.03 ± 4.88	0.29–16.46
Seed (R8)	R073	5.86 ± 7.09	0.07–16.13	5.17 ± 6.26	0.06–14.27	0.38 ± 0.61	0.07–1.47	0.34 ± 0.54	0.06–1.31
	R074	2.77 ± 4.40[b]	<LOQ[b]–10.58	2.39 ± 3.80[b]	<LOQ[b]–9.12	4.94 ± 4.30	0.15–9.29	4.27 ± 3.75	0.13–8.09
	R075	0.59 ± 0.42[b]	<LOQ[b]–1.02	0.52 ± 0.37[b]	<LOQ[b]–0.91	1.13 ± 1.23	0.07–2.50	1.00 ± 1.09	0.06–2.21
	R076	6.33 ± 6.60	0.21–16.30	5.59 ± 5.81[b]	0.19–14.27	4.35 ± 6.14	0.09–14.85	3.85 ± 5.43	0.08–13.13
	All locations	3.89 ± 5.45[b]	<LOQ[b]–16.30	3.42 ± 4.79[b]	<LOQ[b]–14.27	2.70 ± 4.04	0.07–14.85	2.36 ± 3.55	0.06–13.13

N = 5 for all means and ranges except those for "all locations," for which *N* = 20.
The concentrations were adjusted for extraction efficiency.
[a] LOD = 0.060 µg/g DW for roots. The LOD value was used for the sample that was less than the LOD to compute the mean and SD for both DW and FW sample sets.
[b] LOQ = 0.060 µg/g DW for seed. The LOQ value was used for the sample that was less than the LOQ to compute the mean and SD for both DW and FW sample sets.

4.3 Data Quality and Integrity

No circumstances occurred during the conduct of this study that would have adversely affected the quality or integrity of the data generated.

5.0 CONCLUSION

The concentrations of AvHPPD-03 and PAT measured in this study represent the levels of these proteins in SYHT0H2 soybean in various tissue types and developmental stages across four different field environments. Concentrations of AvHPPD-03 and PAT were quantifiable in all SYHT0H2 soybean tissues types analyzed.

6.0 REFERENCES

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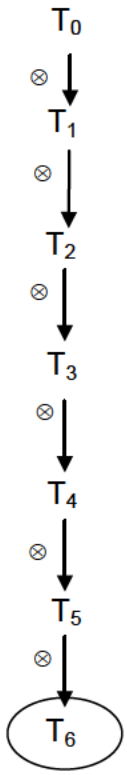
APPENDICES SECTION

APPENDIX A Pedigree Chart of the Test Substance



Transformation recipient line is nontransgenic soybean variety 'Jack'

T_0 = original transformant
⊗ = self-pollination
The generation used in this study is denoted with a circle

APPENDIX B Field Trial Summary

Prepared by: [redacted], Traits Field Program*

Study Number: TK0050018

Permit Number(s): Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA)
Expediente No.: 126492/10-1 and 226426/10

Test and Control Seed

Field Entry ID	Seed Identification	Material Identification
E01	Control – nontransgenic	10SG025036
E02	Test - SYHT0H2 untreated	10SG025049
E03	Test - SYHT0H2 treated with trait-specific herbicides	10SG025049

Trial Location Details

Site Code	Field Trial No.	Location Name	GPS Coordinates[a]	
			Latitude	Longitude
R076	10DE R076-50018L01	Salto	-34.232028	-60.255972
R075	10DE R075-50018L02	Ines Indart	-34.269917	-60.493056
R073	10DE R073-50018L03	Gahan	-34.366167	-60.135417
R074	10DE R074-50018L05	Los Angeles	-34.391472	-60.186111

[a] Geographical positioning system (GPS) coordinates (one corner of the test site) converted from degrees: minutes: seconds to decimal via Federal Communications Commission (FCC) converter (http://transition fcc.gov/mb/audio/bickel/DDDMMSS-decimal.html).

*Operator and Consumer Safety, Product Safety, Syngenta Crop Protection, LLC

Field Trial Personnel

Trial Site Code (Farm Name, City)	Contact Person and Address	Other Contributing Personnel
R076 (Costantini Hnos, Salto)	Alejandra Costantini IBCA Hipolito Yrigoyen 439 Salto (2741) Provincia de Buenos Aires, Argentina	Alberto Lopez Inda Perez Miguel Richard Carringer Nadia Paola Abregu
R075 (El Paraiso, Inés Indart)	Alejandra Costantini IBCA Hipolito Yrigoyen 439 Salto (2741) Provincia de Buenos Aires, Argentina	Nerina Giovagnoli Alberto Lopez Inda Perez Miguel Richard Carringer Nadia Paola Abregu
R073 (El Eucalipto, Gahan)	Nerina Giovagnoli IBCA Hipolito Yrigoyen 439 Salto (2741) Provincia de Buenos Aires, Argentina	Alejandra Constantini Alberto Lopez Inda Perez Miguel Richard Carringer
R074 (Don Pepe, Los Angeles)	Nerina Giovagnoli IBCA Hipolito Yrigoyen 439 Salto (2741) Provincia de Buenos Aires, Argentina	Alejandra Constantini Alberto Lopez Inda Perez Miguel Richard Carringer Nadia Paola Abregu

TRIAL LAYOUT/TEST SYSTEM

Test system:	Soybean
Experimental design:	One plot per entry
Number of plots:	3 plots

Constituients of each plot:

Length:	approximately 5 m
Row spacing:	70 cm
Number of rows:	4
Planting rate (seeds/row):	approximately 105

Soil characterization, field preparation, planting and harvest dates

Site Code	Soil Type (USDA)	Previous Crop (1 yr)	Field Preparation	Fertilization[a]		Planting Date	Harvest Date
R076	Silt Loam	Corn	no tillage	13-Dec-10	3-30-0-9-14 (N-P-K-S-Ca)	13-Dec-10	06-Apr-11
R075	Silt Loam	Corn	no tillage	21-Dec-10	3-30-0-9-14 (N-P-K-S-Ca)	21-Dec-10	10-Apr-11
R073	Silty Clay Loam	Wheat Soybean	no tillage	14-Dec-10	3-30-0-9-14 (N-P-K-S-Ca)	14-Dec-10	07-Apr-11
R074	Silt Loam	Peas Soybeans	no tillage	14-Dec-10	3-30-0-9-14 (N-P-K-S-Ca)	14-Dec-10	07-Apr-11

[a] Percent by weight of nitrogen, phosphorous, potassium, sulphur and calcium (N-P-K-S-Ca).

PESTICIDE APPLICATIONS

Maintenance Pesticides Used

Site Code	Maintenance Pesticides			
	Purpose[a]	Active Ingredient (ai)	Date Applied	kg ai/Ha
R076	H	glyphosate	13-Dec-10	1.216
	H	sulfentrazone	13-Dec-10	0.4
	H	s-metolachlor	13-Dec-10	0.96
	I	zetamethrin	6-Jan-11	0.018
	I	chlorpyriphos	6-Jan-11	0.336
	I	chlorpyriphos	24-Jan-11	0.384
	I	zetamethrin	24-Jan-11	0.018
	F	picoxystrobin/cyproconazole	2-Feb-11	0.06 + 0.024
	I	thiamethoxam/zetamethrin	21-Feb-11	0.028 + 0.0396
	I	thiamethoxam/zetamethrin	9-Mar-11	0.028 + 0.0396
R075	H	glyphosate	21-Dec-10	1.216
	H	sulfentrazone	21-Dec-10	0.4
	H	s-metolachlor	21-Dec-10	0.96
	I	thiamethoxam/zetamethrin	2-Jan-11	0.028 + 0.0396
	I	chlorpyriphos	2-Jan-11	0.288
	I	thiamethoxam/zetamethrin	24-Jan-11	0.028 + 0.0396
	I	chlorpyriphos	24-Jan-11	0.288
	D	picoxystrobin/cyproconazole	31-Jan-11	0.06 + 0.024
	I	thiamethoxam/zetamethrin	24-Feb-11	0.028 + 0.0396
	I	thiamethoxam/zetamethrin	10-Mar-11	0.028 + 0.0396

[a]H (herbicide); I (insecticide); F (fungicide)

Maintenance Pesticides Used (Continued)

Site Code	Maintenance Pesticides			
	Purpose[a]	Active Ingredient (ai)	Date Applied	kg ai/Ha
R073	H	glyphosate	14-Dec-10	1.216
	H	sulfentrazone	14-Dec-10	0.4
	H	s-metolachlor	14-Dec-10	0.96
	I	thiamethoxam/zetamethrin	5-Jan-11	0.028 + 0.0396
	I	chlorpyriphos	5-Jan-11	0.288
	I	chlorpyriphos	25-Jan-11	0.384
	I	zetamethrin	25-Jan-11	0.018
	F	picoxystrobin/cyproconazole	2-Feb-11	0.06 + 0.024
	I	thiamethoxam/zetamethrin	21-Feb-11	0.028 + 0.0396
	I	thiamethoxam/zetamethrin	19-Mar-11	0.028 + 0.0396
R074	H	glyphosate	14-Dec-10	1.216
	H	sulfentrazone	14-Dec-10	0.4
	H	s-metolachlor	14-Dec-10	0.96
	I	chlorantraniliprole	10-Jan-11	0.006
	I	chlorpyriphos	10-Jan-11	0.384
	I	chlorpyriphos	5-Feb-11	0.384
	I	zetamethrin	5-Feb-11	0.018
	F	picoxystrobin/cyproconazole	5-Feb-11	0.06 + 0.024
	I	thiamethoxam/zetamethrin	24-Feb-11	0.028 + 0.0396
	I	thiamethoxam/zetamethrin	10-Mar-11	0.028 + 0.0396

[a]H (herbicide); I (insecticide); F (fungicide)

Trait-specific herbicides used

Site Code	Field Entry ID	Crop Growth Stage[a]	Mesotrione		Glufosinate[b]	
			Date Applied	kg ai/Ha	Date Applied	kg ai/Ha
R076	E03	V3-V4	13-Jan-11	0.105	13-Jan-11	0.322
R075	E03	V3-V4	17-Jan-11	0.105	17-Jan-11	0.322
R073	E03	V3-V4	11-Jan-11	0.105	11-Jan-11	0.322
R074	E03	V3	15-Jan-11	0.105	15-Jan-11	0.322

[a] Pederson (2009)
[b]Ammonium sulphate (spray adjuvant) added to spray solution at 0.01 g/mL (1% wt/v).

Ecological stress evaluation

Site Code	Ecological Stress Summary
R076	Minimal stress from insects; mild stress from disease; mild abiotic stressors.
R075	Mild stress from insects; mild stress from disease; mild abiotic stressors.
R073	Mild stress from insects; mild stress from disease; minimal abiotic stressors.
R074	Minimal stress from insects; mild stress from disease; minimal abiotic stressors.

SAMPLE COLLECTION AND SHIPPING CONDITIONS

V4 Leaf Samples

Site Code	Crop Growth Stage	Sample Type	Date of Sampling	Elapsed Time from Collection to Dry Ice	Shipping Date	Condition of Samples Throughout Shipment
R076	V4	leaf	18-Jan-11	<10 min	4-Mar-11	frozen on dry ice
R075	V4	leaf	21-Jan-11	<10 min	4-Mar-11	frozen on dry ice
R073	V4	leaf	15-Jan-11	<10 min	4-Mar-11	frozen on dry ice
R074	V4	leaf	18-Jan-11	<10 min	4-Mar-11	frozen on dry ice

[a] Pederson (2009)

V8 Samples

Site Code	Crop Growth Stage	Sample Type	Date of Sampling	Elapsed Time from Collection to Dry Ice	Shipping Date	Condition of Samples Throughout Shipment
R076	V8	leaf	29-Jan-11	<10 min	4-Mar-11	frozen on dry ice
		root	29-Jan-11	<10 min	4-Mar-11	frozen on dry ice
R075	V8	leaf	3-Feb-11	<10 min	4-Mar-11	frozen on dry ice
		root	3-Feb-11	<10 min	4-Mar-11	frozen on dry ice
R073	V8	leaf	29-Jan-11	<10 min	4-Mar-11	frozen on dry ice
		root	29-Jan-11	<10 min	4-Mar-11	frozen on dry ice
R074	V8	leaf	1-Feb-11	<10 min	4-Mar-11	frozen on dry ice
		root	1-Feb-11	<10 min	4-Mar-11	frozen on dry ice

[a] Pederson (2009)

V10 Samples

Site Code	Crop Growth Stage	Sample Type	Date of Sampling	Elapsed Time from Collection to Dry Ice	Shipping Date	Condition of Samples Throughout Shipment
R076	V10	leaf	11-Feb-11	<10 min	11-Apr-11	frozen on dry ice
R075	V10	leaf	14-Feb-11	<10 min	11-Apr-11	frozen on dry ice
R073	V10	leaf	9-Feb-11	<10 min	11-Apr-11	frozen on dry ice
R074	V10	leaf	11-Feb-11	<10 min	11-Apr-11	frozen on dry ice

[a] Pederson (2009)

R6 Samples

Site Code	Crop Growth Stage	Sample Type	Date of Sampling	Elapsed Time from Collection to Dry Ice	Shipping Date	Condition of Samples Throughout Shipment
R076	R6	leaf	2-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		root	2-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		forage	2-Mar-11	<10 min	30-May-11	frozen on dry ice
R075	R6	leaf	7-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		root	7-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		forage	7-Mar-11	<10 min	30-May-11	frozen on dry ice
R073	R6	leaf	4-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		root	4-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		forage	4-Mar-11	<10 min	30-May-11	frozen on dry ice
R074	R6	leaf	7-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		root	7-Mar-11	<10 min	11-Apr-11	frozen on dry ice
		forage	7-Mar-11	<10 min	30-May-11	frozen on dry ice

[a] Pederson (2009)

R8 Samples

Site Code	Crop Growth Stage	Sample Type	Date of Sampling	Elapsed Time from Collection to Dry Ice	Shipping Date	Condition of Samples Throughout Shipment
R076	R8	seed	6-Apr-11	<10 min	9-May-11	frozen on dry ice
R075	R8	seed	10-Apr-11	<10 min	9-May-11	frozen on dry ice
R073	R8	seed	7-Apr-11	<10 min	9-May-11	frozen on dry ice
R074	R8	seed	7-Apr-11	<10 min	9-May-11	frozen on dry ice

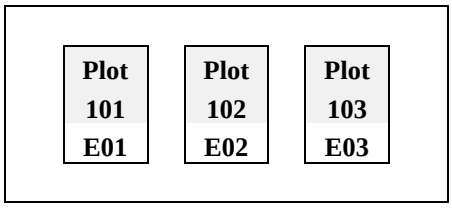
[a] Pederson (2009)

Weather summary/irrigation

Site Code	Weather Period (2010-2011)	Mean Minimum Air Temperature (°C)	Mean Maximum Air Temperature (°C)	Total Rainfall[a] (cm)
R076	December	15.9	35.0	5.9
	January	15.4	33.0	13.4
	February	17.7	30.8	16.0
	March	11.7	31.3	4.0
	April	10.9	29.0	11.4
R075	December	13.9	32.4	4.3 +
	January	19.9	27.3	14.8 +
	February	14.4	28.2	13.1
	March	10.2	28.5	7.2
	April	8.0	24.7	2.6
R073	December	13.6	32.6	5.3
	January	14.6	31.5	14.1
	February	14.0	28.1	12.3
	March	9.8	27.8	6.1
	April	8.9	27.4	5.2
R074	December	15.4	36.2	4.0
	January	15.1	33.6	18.3
	February	14.1	28.3	12.3
	March	9.6	28.1	4.0
	April	8.1	25.2	8.0

[a]irrigation applied indicated by '+'

Field diagram



References

Pederson, P. 2009. *Soybean Growth and Development.* Ames, IA: Iowa State University, University Extension. 28 pp.

APPENDIX C AvHPPD-03 Quantification Procedure

Reagents and buffers used for extraction and enzyme-linked immunosorbent assay (ELISA) analysis of AvHPPD-03

Buffer / Item	Constituents
Phosphate-buffered saline with 0.05% Tween® 20 (PBST)	138 mM sodium chloride, 2.7 mM potassium chloride, 10.1 mM disodium phosphate, 1.8 mM potassium dihydrogen phosphate, pH 7.4, 0.05% Tween® 20
Qualiplate™ Kit for HPPD in Soy	96-well plate precoated with anti-AvHPPD-03 antibody, AvHPPD-03 enzyme conjugate solution, substrate solution

AvHPPD-03 Extraction

PBST buffer was added to lyophilized plant tissue at a ratio of 3 ml of buffer to 30 mg of sample. The samples were mixed, placed on wet ice, homogenized in an Omni-Prep Multi-Sample Homogenizer, and centrifuged at 2°C to 8°C to form a pellet. The supernatants were removed and stored at -20°C ± 5°C until analysis.

AvHPPD-03 Quantification

The appropriate number of 96-well plates pre-coated with the capture antibody, the appropriate amounts of antibody/enzyme conjugate solution, and substrate solution were removed from storage at 2°C to 8°C and allowed to equilibrate to room temperature (all aforementioned items are provided in the Qualiplate™ ELISA Kit for HPPD in Soy). The tube containing the substrate solution was covered to prevent exposure to light. Dilutions of each sample extract and the ELISA standard (prepared using protein reference substance AvHPPD-03-0209 [Winslow 2009]), prepared in PBST, were applied to the plates at a volume of 50 µl/well. The plates were incubated at room temperature for 30 minutes while shaking. The plates were then washed five times prior to addition of the AvHPPD-03 enzyme conjugate (50 µl/well) and incubated at room temperature for 30 minutes while shaking. The plates were then washed five times prior to addition of the substrate solution (100 µl/well). The plates were covered to prevent exposure to light during incubation at room temperature for five minutes while shaking. The colorimetric reaction was stopped by the addition of 1 N hydrochloric acid (100 µl/well) and absorbance was measured using a dual-wavelength spectrophotometer at 450 nm and 650 nm. The results were analyzed with Molecular Devices SoftMax Pro® GxP Microplate Data Compliance Software, v. 5.4.1. The 650-nm reference measurement was subtracted from the 450-nm measurement prior to further analysis. Concentrations were interpolated from a standard curve generated using a four parameter algorithm.

Validation of AvHPPD-03 ELISA Extraction Efficiency and Sensitivity

Protein extraction efficiency and method sensitivity (minimum dilution factor, limit of detection [LOD], and limit of quantitation [LOQ]) were validated for each type of sample outside the scope of this study (Read 2011).

Minimum dilution factor. The minimum dilution factor for each sample type was determined by analysis of a dilution series of nontransgenic extracts spiked with a known quantity of AvHPPD-03 reference protein. The most concentrated dilution of spiked sample extract that yielded a percent recovery between 70% and 120% and was followed by two subsequent dilutions with recoveries in the same range was selected as the minimum acceptable dilution factor.

Limit of detection. The LOD for each sample type was evaluated by comparison of the mean optical density (OD) plus three standard deviations of the unspiked nontransgenic sample extract with the mean OD of the nontransgenic sample extract spiked with AvHPPD−03 reference protein. The measured LOD is the lowest spike concentration with an OD greater than the mean OD plus three standard deviations of the unspiked nontransgenic sample extract.

The LOD (micrograms per gram of sample) was calculated by the following formula:

$$\left(\frac{\text{LOD (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Limit of quantitation. The LOQ for each sample type was evaluated by spiking of nontransgenic sample extracts with known concentrations of AvHPPD-03 reference protein and measurement of the percent recovery of AvHPPD-03 protein. The LOQ was the lowest spike concentration of AvHPPD-03 that resulted in recovery of between 70% and 120% of nominal value and was greater than or equal to the LOD.

The percent recovery for each spiked sample was calculated by the following formula:

$$\left(\frac{\text{mean protein concentration of spiked extract (ng/ml)}}{\text{spiked protein concentration (ng/ml)}}\right) \times 100$$

The LOQ (micrograms per gram of tissue) was calculated by the following formula:

$$\left(\frac{\text{LOQ (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Extraction efficiency. The efficiency of the AvHPPD-03 extraction method was evaluated in soybean leaf, root, and seed through exhaustive protein extractions from transgenic samples. Each extraction was analyzed by ELISA to determine the concentration of AvHPPD-03 present.

The extraction efficiencies (percent) were calculated by the following formula:

$$\left(\frac{\text{Amount of AvHPPD-03 (ng/ml) from 1st extraction}}{\text{Total AvHPPD-03 (ng/ml) from all extractions}}\right) \times 100$$

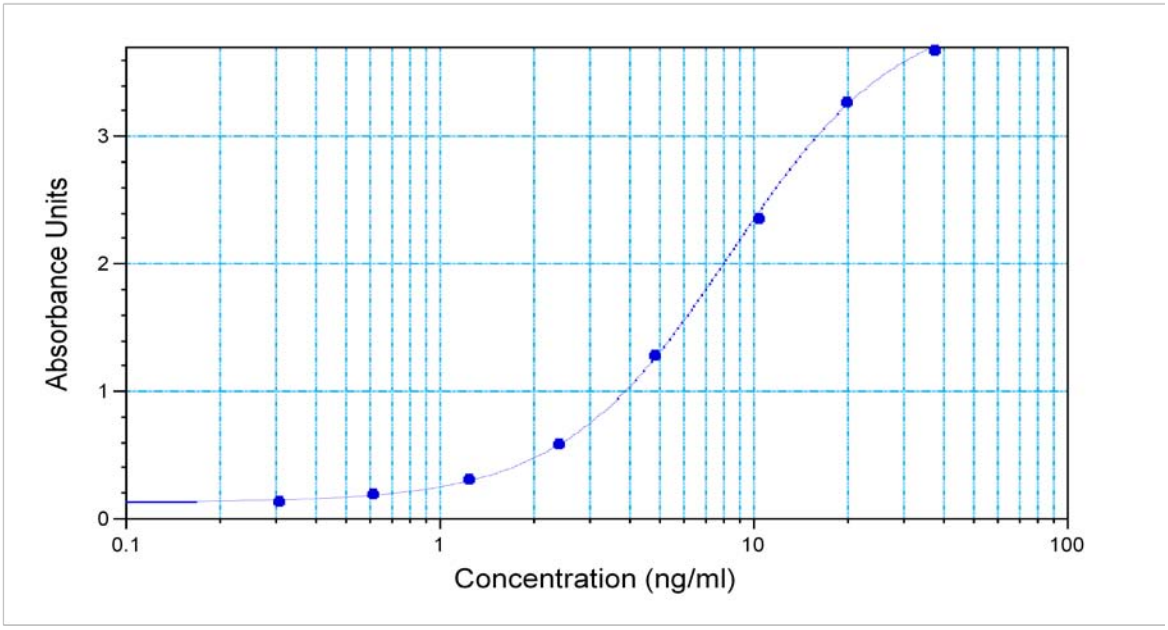
Extraction efficiency and method sensitivity data are summarized in the following table.

Minimum dilution factor, LOD, LOQ and extraction efficiency for each matrix

Sample Type	Minimum Dilution Factor	Extraction Efficiency	LOD (µg/g DW)	LOQ (µg/g DW)
Soybean leaf	1	81%	0.0313	0.0625
Soybean root	1	81%	0.0313	0.250
Soybean seed	1	94%	0.0313	0.125

DW = dry weight.
All values were determined outside the scope of this study during the validation of the AvHPPD-03 quantitation method (Read 2011).

Representative Standard Curve. The concentrations used to produce the ELISA standard curve were 40, 20, 10, 5, 2.5, 1.25, 0.625, and 0.313 ng/ml. A representative standard curve for the AvHPPD-03 ELISA is shown below.



References

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Winslow S. 2009. *Characterization of Microbially Produced Test Substance Containing* p-*Hydroxyphenylpyruvate Dioxygenase Protein (AvHPPD-03) and Certificate of Analysis.* Report No. SSB-041-09 (unpublished). Research Triangle Park, NC: Syngenta Biotechnology, Inc.

APPENDIX D PAT Quantification Procedure

Reagents and buffers used for extraction and enzyme-linked immunosorbent assay (ELISA) analysis of PAT

Buffer / Item	Constituents
Phosphate-buffered saline with 0.05% Tween® 20 (PBST)	138 mM sodium chloride, 2.7 mM potassium chloride, 10.1 mM disodium phosphate, 1.8 mM potassium dihydrogen phosphate, pH 7.4, 0.05% Tween® 20
Qualiplate™ Kit for Liberty Link PAT / *pat*	96-well plate precoated with anti-PAT antibody, PAT enzyme conjugate solution, substrate solution

PAT Extraction

PBST extraction buffer was added to lyophilized plant tissue at a ratio of 3 ml of buffer to 30 mg of tissue. The samples were mixed, homogenized in an Omni Prep Multi-Sample Homogenizer, and centrifuged at 2°C to 8°C to form a pellet. The supernatants were removed and stored at -20°C ± 5°C until analysis.

PAT Quantification

The appropriate number of 96-well plates pre-coated with the capture antibody, the appropriate amounts of antibody/enzyme conjugate solution, and substrate solution were removed from storage at 2°C to 8°C and allowed to equilibrate to room temperature (all aforementioned items are provided in the Qualiplate™ Kit for Liberty Link PAT / *pat*). The tube containing the substrate solution was covered to prevent exposure to light. The PAT enzyme conjugate solution was applied to each well at a volume of 50 µl/well. Immediately following the addition of the enzyme conjugate solution, dilutions of each tissue extract and the appropriate serial dilutions of the ELISA standard (prepared using protein reference substance, PAT-0109 [Seastrum 2009]), prepared in PBST, were applied to the pre-coated plates at a volume of 50 µl/well. The plates were mixed in a rapid circular motion on the benchtop for 10 seconds and incubated at room temperature for one hour. The plates were washed five times with PBST in a microplate washer. After washing the plates, the substrate solution was added at a volume of 100 µl/well. The plates were covered to prevent exposure to light during incubation at room temperature for 15 minutes. The colorimetric reaction was stopped by the addition of 1N hydrochloric acid (100 µl/well) and absorbance was measured using a dual-wavelength spectrophotometer at 450 nm and 650 nm. The results were analyzed with Molecular Devices SoftMax Pro® GxP Microplate Data Compliance Software, v. 5.4.1. The 650-nm reference measurement was subtracted from the 450-nm measurement prior to further analysis. Concentrations were interpolated from a standard curve generated using a quadratic curve fitting algorithm.

Validation of PAT ELISA Extraction Efficiency and Sensitivity

Protein extraction efficiency and method sensitivity (minimum dilution factor, limit of detection [LOD], and limit of quantitation [LOQ]) were validated for each type of sample outside the scope of this study (Read 2011).

Minimum dilution factor. The minimum dilution factor for each sample type was determined by analysis of a dilution series of nontransgenic extracts spiked with a known quantity of PAT reference protein. The most concentrated dilution of spiked sample extract that yielded a percent recovery between 70% and 120% and was followed by two subsequent dilutions with recoveries in the same range was selected as the minimum acceptable dilution factor.

Extraction efficiency. The efficiency of the PAT extraction method was evaluated in soybean leaf, root, and seed through exhaustive protein extractions from transgenic samples. Each extraction was analyzed by ELISA to determine the concentration of PAT present. The extraction efficiencies (percent) were calculated by the following formula:

$$\left(\frac{\text{Amount of PAT (ng/ml) from 1st extraction}}{\text{Total PAT (ng/ml) from all extractions}}\right) \times 100$$

Limit of detection (LOD). The LOD for each sample type was evaluated by comparison of the mean optical density (OD) plus three standard deviations of the unspiked nontransgenic sample extract with the mean OD of the nontransgenic sample extract spiked with AvHPPD–03. The measured LOD is the lowest spike concentration with an OD greater than the mean OD plus three standard deviations of the unspiked nontransgenic sample extract.

The LOD (micrograms per gram of tissue) was calculated by the following formula:

$$\left(\frac{\text{LOD (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Limit of quantitation (LOQ). The LOQ for each sample type was evaluated by spiking of nontransgenic sample extracts with known concentrations of PAT reference protein and measurement of the percent recovery of PAT protein. The LOQ was the lowest spike concentration of PAT that resulted in recovery of between 70% and 120% of nominal value and was greater than or equal to the LOD.

The percent recovery for each spiked sample was calculated by the following formula:

$$\left(\frac{\text{mean protein concentration of spiked extract (ng/ml)}}{\text{spiked protein concentration (ng/ml)}}\right) \times 100$$

The LOQ (micrograms per gram of tissue) was calculated by the following formula:

$$\left(\frac{\text{LOQ (ng/ml)} \times \text{dilution factor} \times \text{volume of extraction buffer (ml)}}{\text{amount of tissue extracted (g)}}\right) \div 1000$$

Extraction efficiency and method sensitivity data are summarized in the following table.

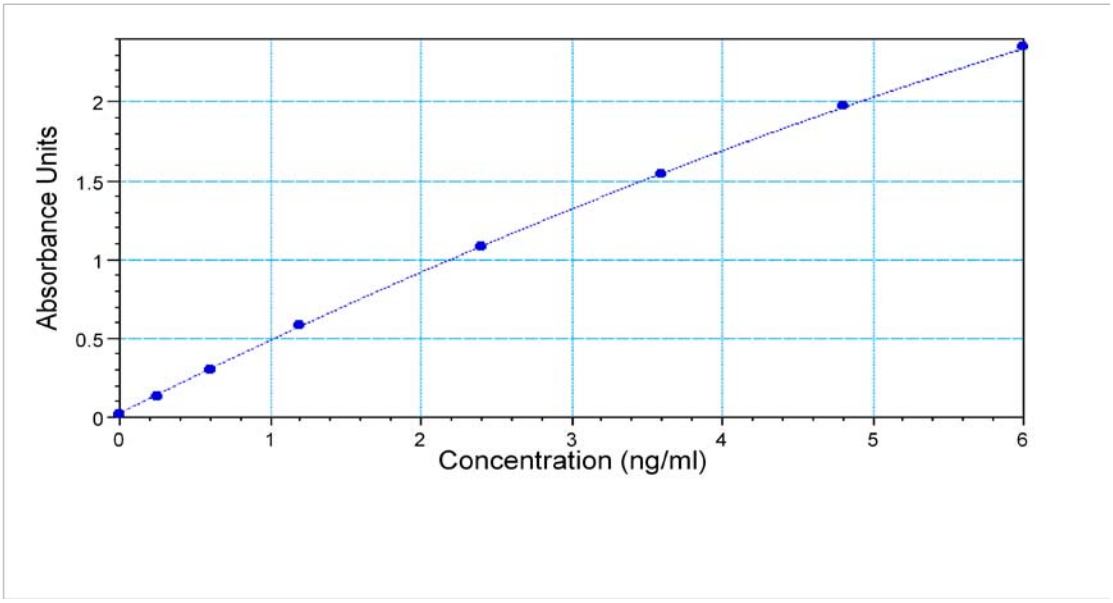
Minimum dilution factor, protein extraction efficiency, LOD, and LOQ for each sample type for the PAT ELISA

Tissue Type	Minimum Dilution Factor	Extraction Efficiency	LOD (µg/g DW)	LOQ (µg/g DW)
Soybean leaf	1	77%	0.025	0.025
Soybean root	1	75%	0.060	0.060
Soybean seed	1	94%	0.025	0.060

DW = dry weight.
All values were determined outside the scope of this study during the validation of the PAT quantitation method (Read 2011).

Representative Standard Curve. Concentrations used to produced the ELISA standard curve: 6, 4.8, 3.6, 2.4, 1.2, 0.6, 0.25, and 0 ng/ml. The representative standard curve for the

PAT ELISA is depicted below.



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Read A. 2011. *Validation of the Enzyme-linked Immunosorbent Assay Method for Quantitation of Phosphinothricin Acetylytransferase (PAT) Protein in Soybean Leaf, Root, and Seedl*. Report No. TK0059686 (unpublished). Research Triangle Park, NC: Syngenta Crop Protection, LLC.

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APPENDIX E Concentrations of AvHPPD-03 in Individual Samples

Concentrations of AvHPPD-03 measured in each of the replicate leaf samples of SYHT0H2 soybean on a dry-weight (DW) and a fresh-weight (FW) basis

Growth Stage	Mesotrione + glufosinate applied [a]								No mesotrione + glufosinate applied							
	Site R073		Site R074		Site R075		Site R076		Site R073		Site R074		Site R075		Site R076	
	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW
V4	199.64	42.43	850.49	207.21	215.35	44.80	92.95	19.05	196.85	58.22	226.32	70.34	235.63	50.55	50.88	12.45
	877.37	185.19	178.51	42.06	242.32	64.71	89.23	19.64	235.51	50.27	20.23	6.39	331.16	73.39	222.83	54.47
	437.02	86.31	191.40	46.89	545.17	132.40	239.95	61.24	265.96	73.67	383.77	92.94	585.46	135.84	174.93	42.55
	124.83	24.20	141.41	54.97	479.98	108.96	633.30	157.11	77.26	20.96	350.14	86.37	130.72	31.77	151.96	31.14
	468.33	106.07	388.14	88.95	176.79	40.54	96.86	22.43	258.99	74.47	307.57	70.34	494.38	111.48	139.41	32.58
V8	96.55	22.93	167.19	38.98	270.67	65.96	358.57	75.64	56.71	13.21	193.23	49.07	271.82	72.40	344.43	93.81
	238.70	52.16	88.79	22.37	204.40	56.09	238.00	57.12	335.55	79.94	227.80	57.72	100.34	29.72	136.56	36.97
	307.28	81.37	83.08	22.35	334.84	84.15	236.31	58.78	53.77	11.72	136.26	39.16	369.51	109.82	237.75	54.93
	114.10	26.94	99.88	27.31	342.45	88.49	165.65	44.45	147.34	36.88	184.03	43.48	183.60	50.95	116.06	31.73
	90.26	22.74	200.07	56.41	160.19	45.72	179.90	47.46	205.55	47.55	232.47	59.00	386.15	116.85	340.69	98.18
V10	206.13	60.33	145.75	38.63	257.41	68.64	321.28	70.75	281.46	74.46	132.65	34.20	232.08	53.84	263.58	56.03
	187.52	53.55	222.70	56.19	333.93	79.06	108.66	25.07	134.59	36.42	159.23	40.91	165.69	38.69	149.85	36.42
	160.30	45.68	244.82	60.99	169.45	42.21	122.56	29.25	178.21	52.66	131.57	34.60	302.90	72.84	141.12	31.81
	157.75	42.72	240.05	59.54	128.49	34.11	238.87	50.67	85.55	24.09	110.51	30.48	240.63	58.88	55.96	12.11
	216.85	55.44	169.70	45.67	495.19	117.19	246.00	57.04	141.24	41.64	101.16	27.97	148.39	37.60	146.36	31.94
R6	69.91	20.11	94.62	26.36	73.37	17.95	235.38	65.74	75.75	21.94	38.57	10.14	136.37	35.84	95.54	25.06
	74.97	22.80	219.09	58.41	37.47	9.27	209.09	61.79	255.30	75.67	173.93	43.20	48.77	12.45	75.75	22.65
	91.60	28.33	76.97	19.11	83.25	22.31	84.82	24.55	190.93	57.96	63.69	16.54	25.96	6.76	16.94	4.93
	209.78	73.48	175.11	42.07	94.02	20.75	133.51	38.19	61.02	19.35	48.30	12.93	64.03	16.07	179.22	52.07
	70.47	23.78	131.70	34.32	31.15	7.59	23.61	6.82	191.94	61.31	123.52	29.84	74.33	19.07	166.52	46.14

DW = dry weight, FW = fresh weight.
All concentrations were rounded to two decimal places for consistency.
The concentrations presented were adjusted to account for extraction efficiency.
[a] Mesotrione and glufosinate were applied to the SYHT0H2 soybean plot at the V3–V4 growth stage at the labeled rates of 0.105 kg ai/ha and 0.322 kg ai/ha, respectively.

Concentrations of AvHPPD-03 measured in each of the replicate root, forage, and seed samples of SYHT0H2 soybean on a dry-weight (DW) and a fresh-weight (FW) basis

Tissue Type	Mesotrione + glufosinate applied [a]								No mesotrione + glufosinate applied							
	Site R073		Site R074		Site R075		Site R076		Site R073		Site R074		Site R075		Site R076	
	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW
Roots (V8)	12.69	2.72	39.33	9.08	76.28	18.28	76.32	16.82	15.43	3.74	67.72	12.23	62.14	15.28	144.86	39.40
	124.71	26.47	65.98	16.04	56.03	13.33	266.68	50.18	64.12	15.20	129.13	25.31	114.14	21.71	15.44	3.24
	61.84	14.19	51.01	12.29	75.20	17.20	71.35	12.23	71.70	17.14	16.66	3.71	102.20	23.85	85.23	13.34
	44.04	10.00	27.52	5.04	93.44	22.00	115.10	26.73	38.85	10.06	45.30	10.38	44.45	10.13	97.12	20.97
	5.90	1.58	55.96	11.99	77.72	18.85	15.80	5.27	76.97	18.04	73.84	18.46	201.47	45.65	123.11	26.52
Roots (R6)	61.38	18.12	29.44	7.62	5.32	1.55	6.48	2.16	43.48	9.29	32.85	9.00	7.42	1.99	6.08	1.29
	10.72	2.90	24.64	6.82	51.84	13.85	63.72	20.03	24.61	6.40	5.75	1.62	4.75	1.39	6.19	1.93
	58.30	16.03	24.54	7.39	9.53	2.34	58.18	14.34	1.50	0.42	2.95	0.87	32.58	7.89	4.46	1.20
	75.50	20.17	6.42	1.99	50.94	11.01	4.26	1.21	69.95	18.26	62.44	17.99	6.58	1.91	28.96	6.98
	5.99	1.85	14.96	4.07	38.04	11.09	25.79	7.23	7.36	1.80	47.82	13.39	24.13	5.91	30.21	7.80
Forage (R6)	111.53	31.82	77.26	20.11	99.70	26.82	34.73	9.88	100.75	26.93	48.82	12.47	49.03	12.01	16.76	4.31
	171.79	48.79	67.25	17.38	78.38	21.01	85.49	22.76	50.49	14.28	67.31	17.28	58.40	14.30	164.01	44.32
	100.22	28.22	99.81	25.89	78.58	20.06	165.73	44.00	32.36	9.37	148.05	38.26	63.27	15.60	146.96	39.39
	119.75	32.98	43.77	11.20	101.57	25.70	130.24	34.39	106.70	31.66	163.10	40.44	55.96	14.45	84.90	19.74
	107.00	31.66	46.17	12.15	76.28	18.74	64.61	14.72	38.33	11.33	80.84	20.29	63.96	16.22	53.20	14.63
Seed (R8)	3.46	3.07	2.58	2.22	3.19	2.85	0.90	0.81	4.48	3.95	19.32	16.85	8.34	7.38	2.46	2.13
	0.55	0.49	8.39	7.25	3.37	2.93	5.00	4.35	0.62	0.55	5.55	4.73	13.65	12.04	21.15	18.70
	0.39	0.34	0.60	0.52	1.37	1.21	28.36	24.84	2.11	1.87	2.51	2.19	3.79	3.33	4.27	3.78
	20.89	18.48	7.36	6.32	2.71	2.41	14.99	13.29	0.62	0.56	20.61	17.51	1.40	1.25	1.07	0.93
	15.38	13.47	14.24	12.28	3.48	3.05	21.08	19.05	10.02	8.92	12.61	10.95	0.75	0.67	28.30	24.94

All concentrations were rounded to two decimal places for consistency.
The concentrations presented were adjusted to account for extraction efficiency.
[a] Mesotrione and glufosinate were applied to the SYHT0H2 soybean plot at the V3–V4 growth stage at the labeled rates of 0.105 kg ai/ha and 0.322 kg ai/ha, respectively.

APPENDIX F Concentrations of PAT in Individual Samples

Concentrations of PAT measured in each of the replicate leaf samples of SYHT0H2 soybean on a dry-weight (DW) and a fresh-weight (FW) basis

Growth Stage	Mesotrione + glufosinate applied [a]								No mesotrione + glufosinate applied							
	Site R073		Site R074		Site R075		Site R076		Site R073		Site R074		Site R075		Site R076	
	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW
V4	13.20	2.81	92.52	22.54	15.58	3.24	43.26	8.87	17.82	5.27	25.72	7.99	16.68	3.58	0.89	0.22
	35.94	7.59	133.83	31.53	3.61	0.96	1.70	0.37	48.61	10.38	3.31	1.04	20.82	4.61	45.24	11.06
	62.86	12.41	68.16	16.70	24.22	5.88	44.00	11.23	32.61	9.03	28.08	6.80	149.10	34.59	9.16	2.23
	8.61	1.67	4.46	1.73	85.02	19.30	91.83	22.78	16.00	4.34	167.97	41.43	16.72	4.06	92.61	18.98
	136.29	30.87	73.21	16.78	40.25	9.23	27.18	6.30	84.77	24.38	162.04	37.06	82.85	18.68	23.22	5.43
V8	0.55	0.13	11.58	2.70	9.33	2.27	38.90	8.21	2.04	0.47	18.23	4.63	31.82	8.47	52.78	14.38
	28.02	6.12	11.86	2.99	17.69	4.85	70.95	17.03	3.51	0.84	68.39	17.33	18.33	5.43	3.71	1.00
	14.89	3.94	4.91	1.32	21.20	5.33	17.21	4.28	15.25	3.32	2.30	0.66	9.90	2.94	9.09	2.10
	9.96	2.35	5.47	1.50	18.03	4.66	21.89	5.87	6.96	1.74	12.94	3.06	2.46	0.68	29.10	7.96
	0.68	0.17	16.12	4.54	30.21	8.62	20.14	5.31	27.65	6.40	83.43	21.17	40.46	12.24	21.57	6.22
V10	20.26	5.93	17.58	4.66	119.38	31.84	84.59	18.63	65.00	17.20	31.64	8.16	20.64	4.79	35.54	7.56
	34.72	9.91	112.26	28.32	172.52	40.85	13.57	3.13	115.86	31.35	23.93	6.15	23.61	5.51	68.14	16.56
	15.58	4.44	24.62	6.13	48.84	12.17	93.62	22.34	63.46	18.75	24.47	6.43	106.82	25.69	19.73	4.45
	35.31	9.56	14.61	3.62	10.17	2.70	33.15	7.03	41.11	11.58	7.28	1.91	44.43	10.87	17.38	3.76
	18.66	4.77	17.83	4.80	19.56	4.63	154.98	35.94	4.77	1.41	27.06	7.48	9.35	2.37	14.36	3.13
R6	7.18	2.06	9.48	2.64	24.12	5.90	94.50	26.39	17.92	5.19	3.06	0.80	9.24	2.43	17.06	4.48
	5.29	1.61	79.39	21.17	3.16	0.78	69.57	20.56	27.09	8.03	39.95	9.92	41.28	10.54	27.10	8.10
	10.97	3.39	2.59	0.64	5.03	1.35	6.84	1.98	101.58	30.84	33.53	8.71	6.65	1.73	0.77	0.22
	136.75	47.90	101.70	24.43	29.66	6.55	47.84	13.68	5.09	1.61	2.74	0.73	9.79	2.46	33.06	9.60
	21.24	7.17	13.07	3.41	2.17	0.53	7.34	2.12	69.56	22.22	67.29	16.25	12.46	3.20	62.99	17.45

DW = dry weight, FW = fresh weight.
All concentrations were rounded to two decimal places for consistency.
The concentrations presented were adjusted to account for extraction efficiency.
[a] Mesotrione and glufosinate were applied to the SYHT0H2 soybean plot at the V3–V4 growth stage at the labeled rates of 0.105 kg ai/ha and 0.322 kg ai/ha, respectively.

Concentrations of PAT measured in each of the replicate root, forage, and seed samples of SYHT0H2 soybean on a dry-weight (DW) and a fresh-weight (FW) basis

Tissue Type	Mesotrione + glufosinate applied [a]								No mesotrione + glufosinate applied							
	Site R073		Site R074		Site R075		Site R076		Site R073		Site R074		Site R075		Site R076	
	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW	µg/g DW	µg/g FW
Roots (V8)	0.26	0.06	1.54	0.36	2.65	0.63	21.23	4.68	1.25	0.30	17.92	3.24	28.03	6.89	44.04	11.98
	41.21	8.75	16.94	4.12	8.53	2.03	66.60	12.53	1.59	0.38	45.51	8.92	31.76	6.04	1.75	0.37
	6.86	1.57	5.85	1.41	11.61	2.66	20.90	3.58	41.66	9.96	0.33	0.07	6.09	1.42	5.51	0.86
	11.98	2.72	4.40	0.81	6.31	1.49	40.29	9.35	3.56	0.92	6.68	1.53	0.61	0.14	46.07	9.95
	<LOD[b]	—	7.56	1.62	20.16	4.89	9.62	3.21	30.99	7.26	29.33	7.33	45.06	10.21	35.50	7.65
Roots (R6)	25.00	7.38	6.49	1.68	1.00	0.29	0.31	0.10	12.51	2.67	14.41	3.95	1.71	0.46	0.32	0.07
	10.05	2.72	8.02	2.22	22.97	6.14	30.00	9.43	12.44	3.24	2.83	0.79	1.19	0.35	8.62	2.68
	13.38	3.68	14.61	4.40	7.62	1.87	13.25	3.27	0.45	0.13	2.26	0.66	9.18	2.22	3.75	1.01
	25.26	6.75	2.28	0.71	8.27	1.79	1.23	0.35	19.04	4.97	29.35	8.45	0.47	0.14	12.09	2.91
	0.88	0.27	2.37	0.64	10.34	3.01	19.61	5.50	2.09	0.51	7.90	2.21	17.92	4.39	23.87	6.16
Forage (R6)	53.29	15.20	9.04	2.35	31.80	8.55	1.27	0.36	14.90	3.98	13.97	3.57	1.50	0.37	1.12	0.29
	60.46	17.17	9.48	2.45	17.15	4.60	24.09	6.41	12.57	3.55	6.75	1.73	8.26	2.02	60.91	16.46
	19.07	5.37	32.47	8.42	37.70	9.62	41.56	11.03	2.13	0.62	39.47	10.20	8.65	2.13	50.37	13.50
	53.16	14.64	9.12	2.33	8.41	2.13	63.37	16.73	17.51	5.20	43.26	10.73	3.71	0.96	16.23	3.77
	68.10	20.15	6.15	1.62	16.39	4.03	32.59	7.42	10.26	3.03	4.22	1.06	49.41	12.53	18.15	4.99
Seed (R8)	2.78	2.47	0.50	0.43	0.93	0.83	0.21	0.19	0.15	0.14	9.27	8.09	2.50	2.21	0.26	0.23
	0.07	0.06	1.61	1.39	0.25	0.21	4.46	3.88	0.07	0.06	1.28	1.09	2.42	2.14	14.85	13.13
	0.09	0.08	<LOQ[c]	—	<LOQ[c]	—	16.30	14.27	0.10	0.09	0.15	0.13	0.57	0.50	1.99	1.76
	16.13	14.27	1.08	0.93	1.02	0.91	1.36	1.20	0.12	0.11	4.69	3.98	0.08	0.07	0.09	0.08
	10.24	8.97	10.58	9.12	0.69	0.61	9.33	8.43	1.47	1.31	9.29	8.07	0.07	0.06	4.58	4.04

All concentrations were rounded to two decimal places for consistency.
The concentrations presented were not adjusted to account for extraction efficiency.
[a] Mesotrione and glufosinate were applied to the SYHT0H2 soybean plot at the V3–V4 growth stage at the labeled rates of 0.105 kg ai/ha and 0.322 kg ai/ha, respectively.
[b] LOD = 0.060 µg/g DW for roots. LOD values were not determined on a FW basis.
[c] LOQ = 0.060 µg/g DW for seed. LOQ values were not determined on a FW basis.