Herbicide + PeptiGro® Compatibility Study in Soybean

(Purdue University Study) - Technical Report No. 2021.2

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Executive Summary
NF Protein LLC funded faculty at Purdue University to evaluate the physical and functional compatibility of PeptiGro® with 10 commonly used herbicides in soybean. Physical compatibility study was conducted in the laboratory and functional herbicide compatibility study was conducted in small plot field environments to determine the effectiveness of herbicide-specific weed control between herbicide-only" vs. "herbicide + PeptiGro®" on both selected grass and broadleaf weeds. These studies determined that adding PeptiGro® to commonly used herbicides in soybeans was physically compatible with all herbicide solutions evaluated and PeptiGro® had no impact on the effectiveness of respective-herbicide weed control.

Objectives
The objectives of this research study were to:

1) Evaluate physical mixing compatibility in the laboratory of 10 commonly used soybean post emergence foliar herbicides when tank mixed with PeptiGro® vs. respective herbicide *per se* mixed in water.

2) Evaluate herbicide effectiveness *per se* vs. herbicides tank mixed with PeptiGro® for agronomic effectiveness of weed control and rate of weed kill over time. The dose rate used for each herbicide was the normally recommended rate for weed control in soybean. PeptiGro dose rate was 1 quart/ac.

3) Evaluate the impacts of herbicide only vs. herbicide + PeptiGro on grain moisture, grain test weight, and grain yield (bu./ac. @13%).

PeptiGro® Product
Amino acids are the building blocks of plant proteins. PeptiGro® is a liquid mixture of beneficial amino acids and short-chain polypeptides that helps plants maximize yield in a variety of favorable and unfavorable environments, like times of stress from drought, heat, and herbicide injury. PeptiGro® is an exciting, new, proactive tool to use in your foliar and in-furrow fertilizer program. Based on PeptiGro® yield performance measured with >10,000 data points in 71 corn and 71 soybean trials across 10 States in 2020 and 2021, PeptiGro® provides a 2+ bu./ac. benefit in corn and a 1+ bu./ac. benefit in soybeans and can be applied as a standalone product in-furrow or foliar applied or can be tank mixed with herbicides, fungicides, and other foliar fertilizers.

Implementation
Study was conducted near West Lafayette, IN in 2021 on soybeans (*Glycine max*, varieties AG29XF1 & STINE 32EA12, with attributes of XtendFlex and Enlist) on silt loam soil (name was Toronto-Millbrook Complex with 97% sand, 2% silt, 1% clay, 0.9% OM, pH 6.8, and CEC of 10.9 meq/100g) using a planting density of 140,000 seeds/ac. for soybeans in 15-inch row spacing with good soil fertility levels. Foliar applications were applied at 15 gal./ac. volume using a compressed CO_2 backpack sprayer at V5 stage (~30-33 days after emergence; avg. soybean height of 7 inches; avg. weed height was 3-7; avg. weed density was 10/ft^2). In the trial, Giant ragweed (*Ambrosia trifida*) was assessed at 7, 14, and 21 days after application (DPA) to determine the effectiveness of soybean-specific herbicide weed control (0-100% complete weed control) for "herbicide only (Control)" vs. "herbicide + PeptiGro®" treatments.

Experimental Design & Treatments

SPLIT-PLOT DESIGN: Main Factor A (Soybean Herbicides) and Sub-Factor B (Herbicide-only (Control) vs. Herbicide + PeptiGro®). Trial had 8 replications.

Factor	Level	Herbicide	Rate/A
A	1	Roundup (glyphosate)	2 QT/A
A	2	Enlist One (2,4-D)	1 QT/A
A	3	Liberty (glufosinate)	1 QT/A
A	4	DiFlex (dicamba)	10 FL OZ/A
A	5	SelectMax (clethodim)	16 FL OZ/A
A	6	Classic (clorimuron)	0.66 FL OZ/A
A	7	Pursuit (imazethapyr)	4 FL OZ/A
A	8	Cobra (lactofen)	12 FL OZ/A
A	9	Flexstar (fomesafen)	21 FL OZ/A
A	10	FirstRate (cloransulam-methyl)	6 FL OZ/A
B	1	Control (herbicide only)	As above
B	2	+ PeptiGro® (tank mixed with herbicide)	1 QT/A

List of Treatments in Soybean Trial (Purdue)

TRT No.	Effect	Level	Herbicide
1	factor B[factor A]	[1]1	Roundup (glyphosate) - Control
2	factor B[factor A]	[1]2	Roundup (glyphosate) + PeptiGro
3	factor B[factor A]	[2]1	Enlist One (2,4-D) - Control
4	factor B[factor A]	[2]2	Enlist One (2,4-D) + PeptiGro
5	factor B[factor A]	[3]1	Liberty (glufosinate) - Control
6	factor B[factor A]	[3]2	Liberty (glufosinate) + PeptiGro
7	factor B[factor A]	[4]1	DiFlex (dicamba) - Control
8	factor B[factor A]	[4]2	DiFlex (dicamba) + PeptiGro
9	factor B[factor A]	[5]1	SelectMax (clethodim) - Control
10	factor B[factor A]	[5]2	SelectMax (clethodim) + PeptiGro
11	factor B[factor A]	[6]1	Classic (clorimuron) - Control
12	factor B[factor A]	[6]2	Classic (clorimuron) + PeptiGro
13	factor B[factor A]	[7]1	Pursuit (imazethapyr) - Control
14	factor B[factor A]	[7]2	Pursuit (imazethapyr) + PeptiGro
15	factor B[factor A]	[8]1	Cobra (lactofen) - Control
16	factor B[factor A]	[8]2	Cobra (lactofen) + PeptiGro
17	factor B[factor A]	[9]1	Flexstar (fomesafen) - Control
18	factor B[factor A]	[9]2	Flexstar (fomesafen) + PeptiGro
19	factor B[factor A]	[10]1	FirstRate (cloransulam-methyl) - Control
20	factor B[factor A]	[10]2	FirstRate (cloransulam-methyl) + PeptiGro

Soybean Field Plot Layout. TRT No. is lower number in cells; 8 REPs and empty white cells represent border plots

surrounding trial.
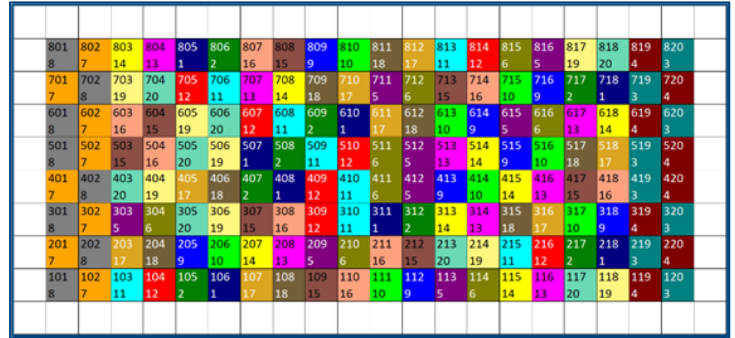
Physical Compatibility Measurements

After 10 second shake/mix time of PeptiGro® with each herbicide, two observation timings were taken:

a. 10-15 min of sit time to allow participates to settle out (take notes/photos as needed).
b. 24 hours of sit time to allow participates to settle out (take notes/photos as needed).

Field Data Measurements

The following characteristics were evaluated to determine the effectiveness of herbicide-specific weed control between herbicide-only" vs. "herbicide + PeptiGro®" on both selected grass and broadleaf weeds:

1. STANDCNT_V2: total plant number in center 2 rows of 4-row plot at V2 stage.

2. VIGOR: rating 0-9 (index/scale) taken at V2 stage.

3. MAX.WHORL HEIGHT_V5: average maximum leaf whorl height of 5 plants in row 1 at V5 stage.

4. Giant ragweed (*Ambrosia trifida*) was assessed at 7, 14, and 21 days after application (DPA): measured as a rating 0-100% relative to 100% complete weed control.

5. Grain Moisture (MOICON): 0-100% moisture content of seed.

6. Grain Test Weight (WEITES): weight in lb. of 1 bushel.

7. Grain Yield: BU/A @13%

Statistical Analysis

Outliers were determined and values converted to missing data points by the Box-Whisker definition where an outlier is defined as a data point that is located above 1.5 times the upper 75th percentile or located 1.5 times below the lower 25th percentile. Data were analyzed with SAS JMP v.16 Statistical Software using Restricted Maximum Likelihood (REML) method to estimate Fit Model variance components. Tukey HSD test (alpha 0.1) was performed to compare individual mean differences for main Factor A (herbicides) and for Subfactor B (herbicide-only as Control vs. herbicide + PeptiGro®). LSMean$_{(0.1)}$ values and Treatment Fixed Effects Prob(F) values are presented. Treatment means not sharing a Letter in common are significantly different. For measuring significance of Factor B effects (± PeptiGro®), compare Letters within common Factor A (herbicide) level.

Results

Herbicide + PeptiGro® Physical Compatibility Measurements – PeptiGro®. was physically compatible with all 10 herbicides (two examples are shown below; other herbicide compatibility data not shown).

Example 1: Roundup + PeptiGro (left bottle) vs. Roundup only (right bottle)

Example 2: Enlist One + PeptiGro (left bottle) vs. Enlist One only (right bottle)

@15 min	@24 hrs.	@15 min	@24 hrs.

Field Data Measurements

1. STANDCNT_V2. **Non-significant herbicide effect** (Factor A) and **non-significant PeptiGro® effect** (Factor B, nested in Factor A). Data not shown.

2. VIGOR. All plots were rated as 5 out of 1-9 index/scale (statistics not applicable).

3. MAX.WHORL HEIGHT_V5. **Non-significant herbicide effect** (Factor A) and **non-significant PeptiGro® effect** (Factor B, nested in Factor A). Data not shown.

4. Herbicide control of Giant ragweed (*Ambrosia trifida*) was assessed at 7, 14, and 21 days after application (DPA) using 0-100% rating with 100% being complete weed control.

   a. Giant ragweed @ 7 DPA – **Significant Herbicide Effect** (Factor A).
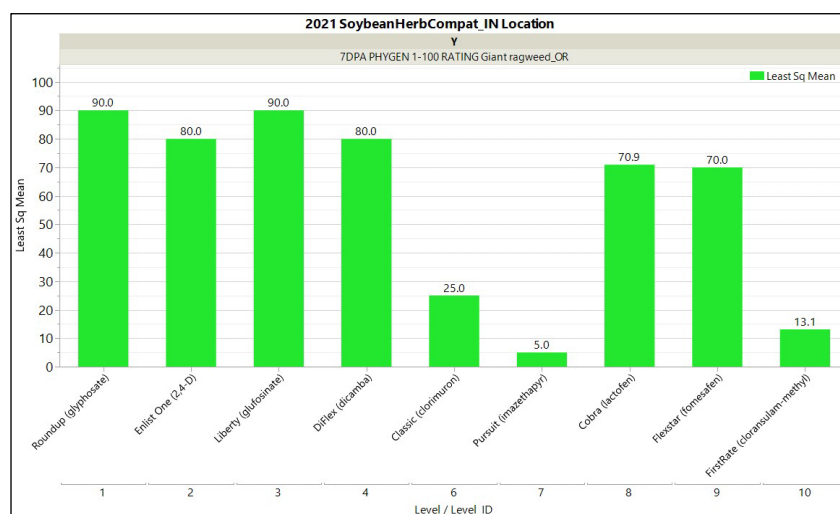
Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
factor A	8	8	119	4274.421	<.0001*
factor B[factor A]	9	9	119	0.6828	0.7232

factor A

LSMeans Differences Tukey HSD

α= 0.100 Q= 2.89652

Level	Least Sq Mean	Letters
1	90.0	A
2	80.0	B
3	90.0	A
4	80.0	B
6	25.0	D
7	5.0	F
8	70.9	C
9	70.0	C
10	13.1	E

Levels not connected by same letter are significantly different.

b. Giant ragweed @ 7 DPA – **Non-Significant PeptiGro® Effect** (Factor B nested in Factor A). For measuring significance of Factor B effects (± PeptiGro®), compare Letters within common Factor A (herbicide) level.
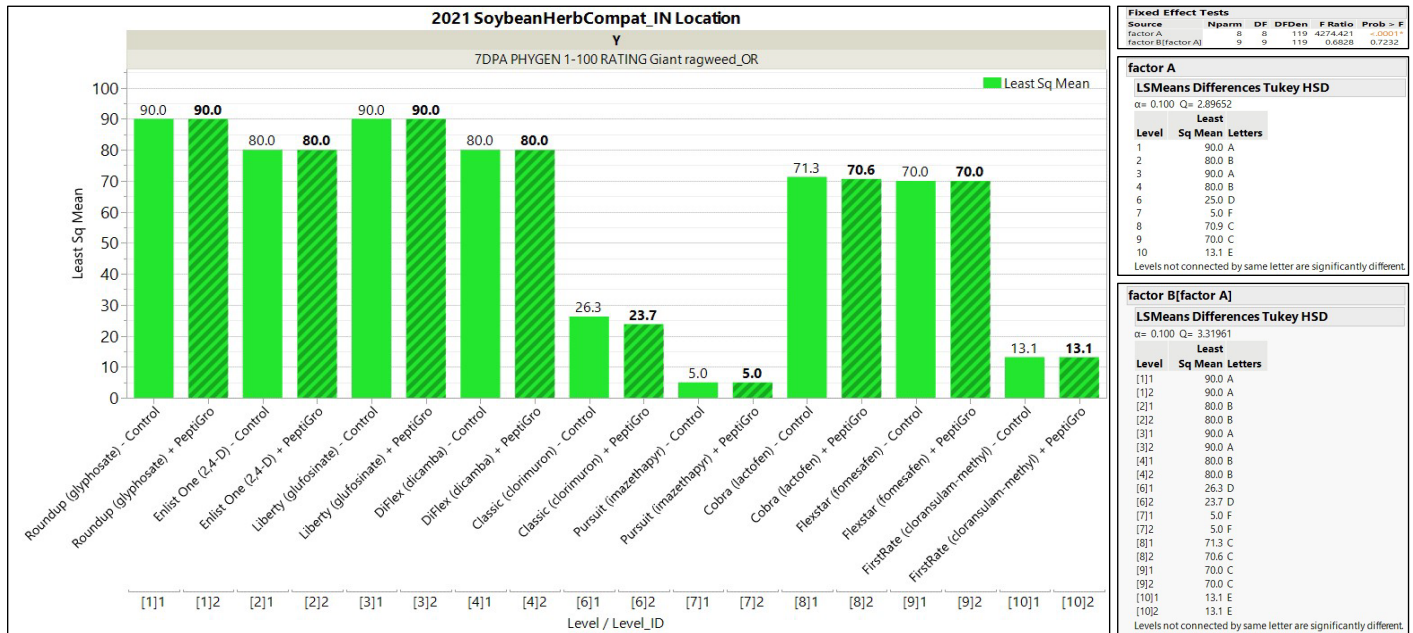
c. Giant ragweed @ 14 DPA – **Significant Herbicide Effect** (Factor A).
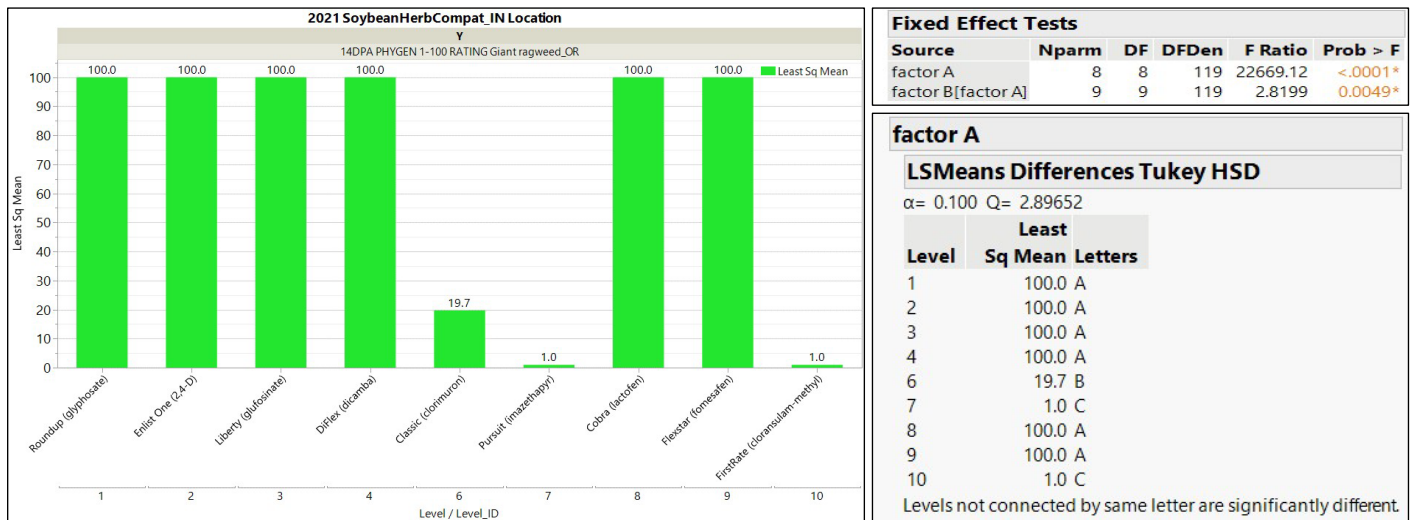
d. Giant ragweed @ 14 DPA – **Mainly Non-Significant PeptiGro® Effect** (Factor B nested in Factor A; only significant PeptiGro® effect was for Classic herbicide [6]). For measuring significance of Factor B effects (± PeptiGro®), compare Letters within common Factor A (herbicide) level.

2021 SoybeanHerbCompat_IN Location
Y
14DPA PHYGEN 1-100 RATING Giant ragweed_OR

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
factor A	8	8	119	22669.12	<.0001*
factor B[factor A]	9	9	119	2.8199	0.0049*

factor A
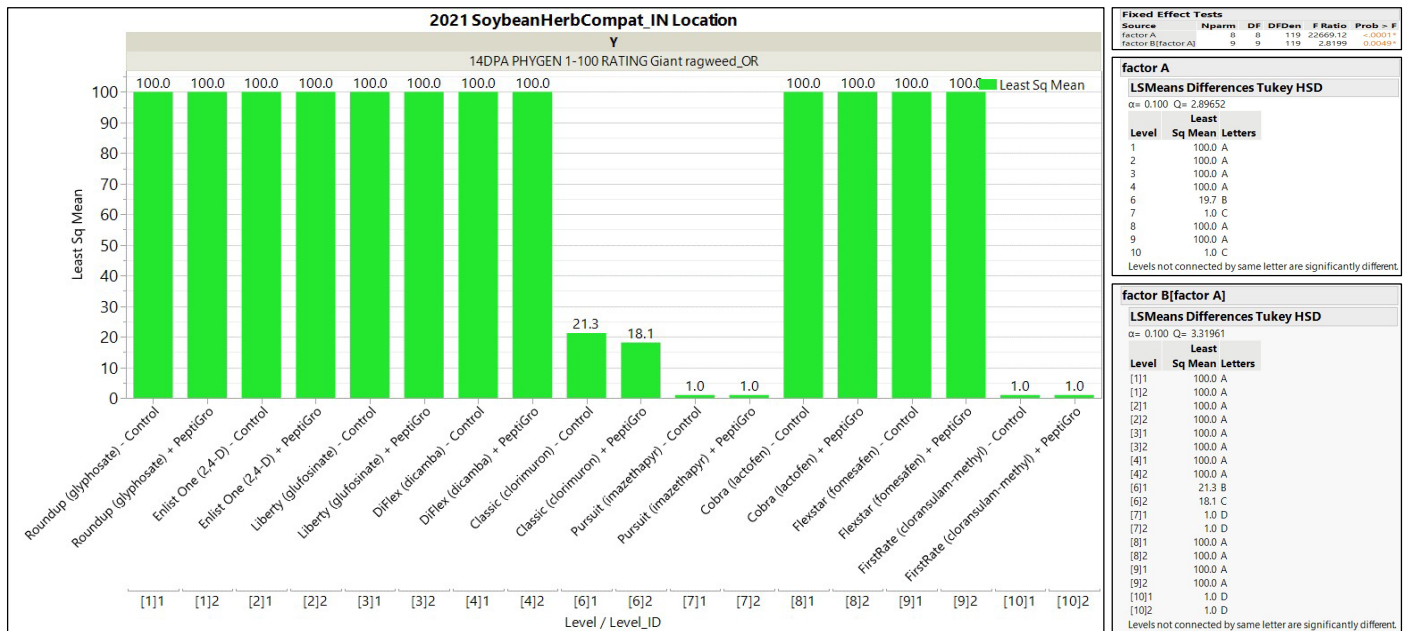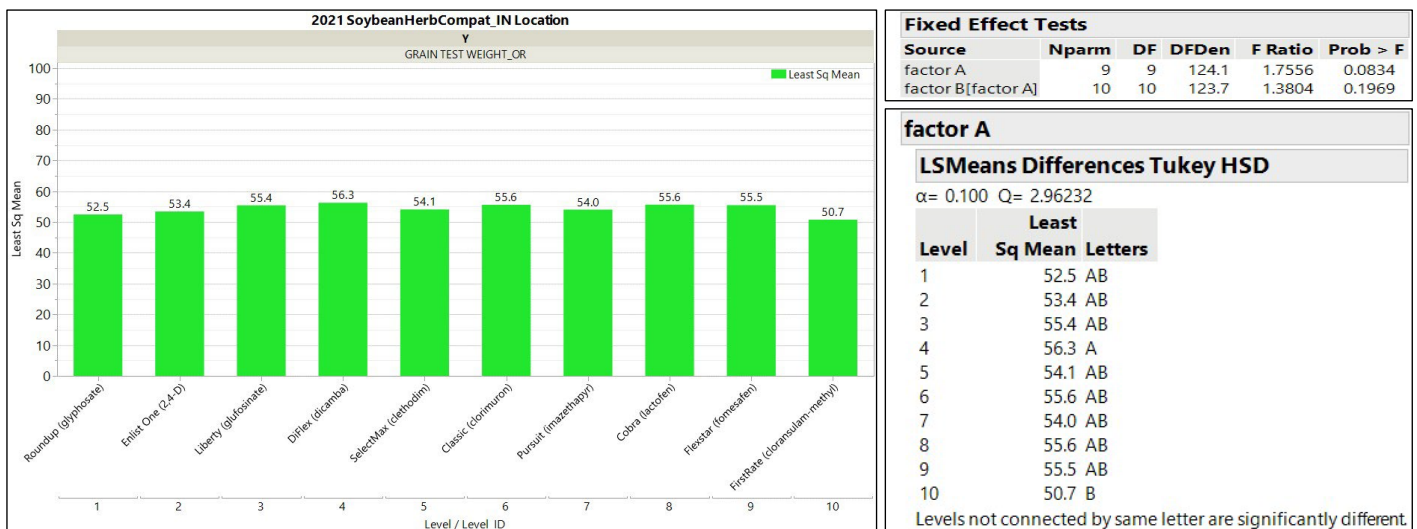LSMeans Differences Tukey HSD
α= 0.100 Q= 2.89652

Level	Least Sq Mean	Letters
1	100.0	A
2	100.0	A
3	100.0	A
4	100.0	A
6	19.7	B
7	1.0	C
8	100.0	A
9	100.0	A
10	1.0	C

Levels not connected by same letter are significantly different.

factor B[factor A]
LSMeans Differences Tukey HSD
α= 0.100 Q= 3.31961

Level	Least Sq Mean	Letters
[1]1	100.0	A
[1]2	100.0	A
[2]1	100.0	A
[2]2	100.0	A
[3]1	100.0	A
[3]2	100.0	A
[4]1	100.0	A
[4]2	100.0	A
[6]1	21.3	B
[6]2	18.1	C
[7]1	1.0	D
[7]2	1.0	D
[8]1	100.0	A
[8]2	100.0	A
[9]1	100.0	A
[9]2	100.0	A
[10]1	1.0	D
[10]2	1.0	D

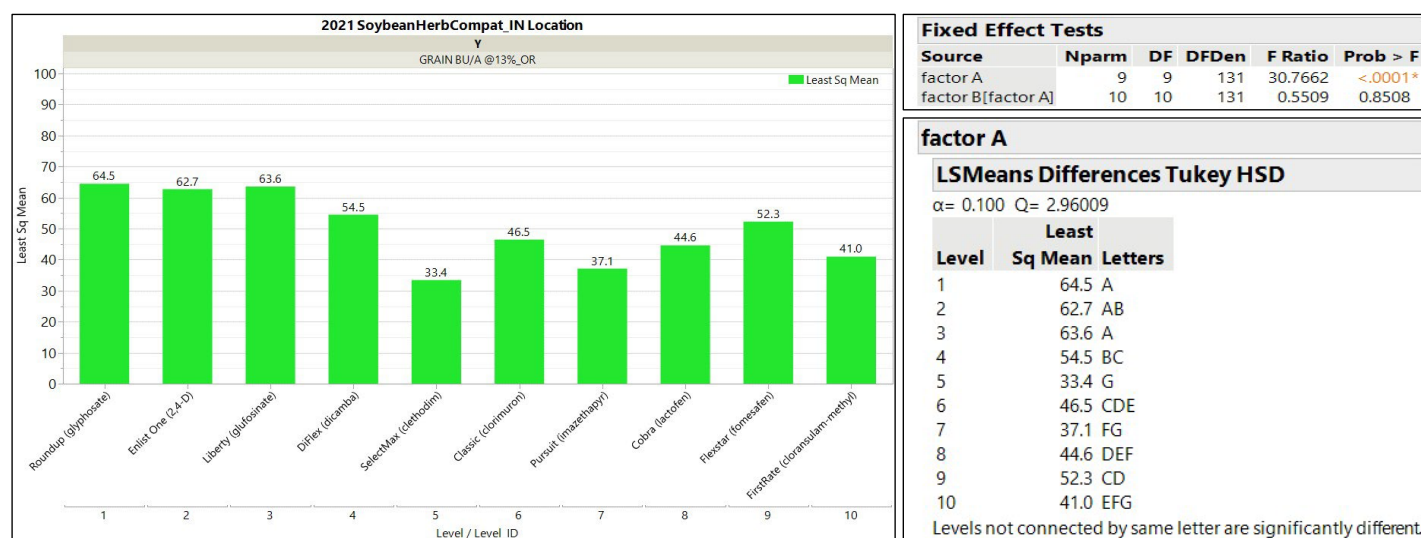Levels not connected by same letter are significantly different.

e. Giant ragweed @ 21 DPA – **Data not shown.** No statistical analysis due to lost degrees of freedom (DFDen) and loss of ability to determine Error Variance in REML model to measure F Ratio and Prob > F. Avg. value for herbicide [6] was 10.0, herbicide [7] was 1.0, and herbicide [10] was 1.0; all others were 100%.

5. Grain Moisture (MOICON). **Non-significant herbicide effect** (Factor A) and **non-significant PeptiGro® effect** (Factor B, nested in Factor A). Data not shown.

6. Grain Test Weight (WEITES) – **Significant Herbicide Effect** (Factor A) and **non-significant PeptiGro® effect** (Factor B, nested in Factor A; data not shown).

2021 SoybeanHerbCompat_IN Location
Y
GRAIN TEST WEIGHT_OR

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
factor A	9	9	124.1	1.7556	0.0834
factor B[factor A]	10	10	123.7	1.3804	0.1969

factor A
LSMeans Differences Tukey HSD
α= 0.100 Q= 2.96232

Level	Least Sq Mean	Letters
1	52.5	AB
2	53.4	AB
3	55.4	AB
4	56.3	A
5	54.1	AB
6	55.6	AB
7	54.0	AB
8	55.6	AB
9	55.5	AB
10	50.7	B

Levels not connected by same letter are significantly different.

7. Grain Yield. **Significant herbicide effect** (Factor A) and non-significant PeptiGro® effect - only 8 REPs; (Factor B, nested in Factor A; data not shown; **for PeptiGro® yield performance in 2020 and 2021 across 71 soybean trials in 10 states, refer to PeptiGro® brochure on Cibari Biosciences website**).

2021 SoybeanHerbCompat_IN Location — GRAIN BU/A @13%_OR (Least Sq Mean)

Bar values: Roundup (glyphosate) 64.5; Enlist One (2,4-D) 62.7; Liberty (glufosinate) 63.6; DiFlex (dicamba) 54.5; SelectMax (clethodim) 33.4; Classic (cloransulon) 46.5; Pursuit (imazethapyr) 37.1; Cobra (lactofen) 44.6; Flexstar (fomesafen) 52.3; FirstRate (cloransulam-methyl) 41.0

Fixed Effect Tests

Source	Nparm	DF	DFDen	F Ratio	Prob > F
factor A	9	9	131	30.7662	<.0001*
factor B[factor A]	10	10	131	0.5509	0.8508

factor A

LSMeans Differences Tukey HSD

α= 0.100 Q= 2.96009

Level	Least Sq Mean	Letters
1	64.5	A
2	62.7	AB
3	63.6	A
4	54.5	BC
5	33.4	G
6	46.5	CDE
7	37.1	FG
8	44.6	DEF
9	52.3	CD
10	41.0	EFG

Levels not connected by same letter are significantly different.

Conclusions from this Trial

Ten commonly used soybean herbicides (Factor A in trial design) differed in their ability to control Giant ragweed (measured at 7, 14, and 21 DPA). Tank mixing of PeptiGro® with each of the 10 different soybean herbicides was physically compatible and results from a field study showed PeptiGro® (Factor B in trial design) had no impact on the functional ability of 10 herbicides to control weeds. Degree of weed control for herbicides *per se* was significantly different and subsequent weed pressure levels impacted grain yields. See other herbicide + PeptiGro compatibility studies in corn and soybeans on cibaribiosciences.com website.

Key Conclusions across all 4 different Herbicide + PeptiGro® Compatibility Studies (corn and soybean studies at Purdue University and corn and soybean studies at University of Missouri)

- In each study, PeptiGro® was physically compatible when tank-mixed with each herbicide tested.

- In each study, main effect of herbicide (Factor A) was significant; indicating that corn and soybean herbicides differed in their ability to control weeds and degree of weed control significantly impacted subsequent grain yields.

- In each study, PeptiGro® effect (Factor B, nested in Factor A) was non-significant for all agronomic variables measured including degree of herbicide weed control taken 3 times between 7-35 DPA; indicating that PeptiGro® had no impact on the functional ability of each herbicide to control target weeds.

- Individual study reports can be downloaded on cibaribiosciences.com.