

## ROUNDUP READY ALFALFA INJURY

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### ABSTRACT

Since the second release in 2011, the Roundup Ready (RR) alfalfa technology has given growers an excellent tool, allowing control of difficult weeds while increasing the flexibility of herbicide application timing. Initial screening of the technology provided excellent crop safety at all application timings. In 2014, there was an initial observation of injury to Roundup Ready alfalfa after glyphosate application was followed by frost. Symptomology observed included necrosis of individual stems, as well as stunting of the crop. Replicated field trials in 2015, 2016 and 2017 confirmed these same symptoms regularly occur when glyphosate is applied to RR alfalfa followed by frost in Northern California. The field trials also documented first cutting yield was reduced up to 0.8 ton/acre compared to the untreated control at multiple sites. Yield reduction was greatest when glyphosate was applied to alfalfa between 8 and 10 inches tall, while yield reduction was minimized when the crop was treated before it grew four inches tall after dormancy. Lower rates of glyphosate (0.77 lb a.e./acre) generally caused less injury and yield loss compared to higher rates tested (1.54 lb a.e./acre). In 2019, a multi-state researcher team evaluated this phenomenon at multiple locations in California and Utah to better determine which management practices minimize crop injury from glyphosate. Another objective was to investigate a hypothesis that the crop injury is caused by *Pseudomonas syringae* (bacterial stem blight).

\*\* This research is a continuation of work initially conducted by Steve Orloff, who has since passed on. Steve's terrific scientific insight and constant wise cracks are surely missed.

### BACKGROUND/INTRODUCTION

Roundup Ready alfalfa has been a tremendous weed control tool since its second release in 2011. It has provided a useful strategy for controlling difficult weeds in alfalfa, particularly perennial species. Initial screening of the technology throughout the 2000's found excellent crop safety at a variety of growth stages. In 2014, Steve Orloff and growers in Scott Valley observed injury to Roundup Ready alfalfa after applications of glyphosate (Roundup) were followed by frost. At the time, it was unclear what conditions, or agronomic practices, resulted in the injury occurring, and it was not known what role the glyphosate application played.

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During the field season of 2015, initial field trials were conducted, which replicated crop injury observed in 2014. The trials found significant yield differences between alfalfa treated with glyphosate followed by a frost, compared to an untreated control. During the 2016 and 2017 growing seasons, numerous replicated field trials were conducted throughout the Intermountain Region of California evaluating a low rate and high rate of glyphosate applied at various heights after the alfalfa broke dormancy. While some trial locations had minimal crop injury, crop injury at many locations resulted in a significant alfalfa yield reduction after a single application of glyphosate followed by frost. No visible injury or yield reductions occurred when applications were made to alfalfa shorter than 2 inches in height. However, applications to alfalfa 4 inches and taller resulted in noticeable injury weeks after application. Overall, the most severe injury occurred when the high rate of glyphosate (1.54 lb a.e./acre (Roundup Powermax 44oz/acre)) was applied to alfalfa plants between 6 and 8 inches. Multiple frost events occurred following glyphosate applications making it difficult to correlate injury severity with the timing of frost after glyphosate application.

A puzzling aspect in all studies was the crop injury observed is not the typical symptomology associated with a glyphosate treatment. Following frost after application, individual alfalfa stems would curl over and die, forming a shepherd's crook (Photo One and Two). Stems and plants would continue to develop and show this symptomology for weeks after treatment. Additionally, some of the alfalfa plants developed chlorosis and stunting following the application, resulting in yield loss. Injury was not always readily apparent at first glance, as stems in the understory often showed the worst symptoms.

The shepherd's crook symptomology on the affected alfalfa stems looked eerily similar to symptoms caused by bacterial stem blight. *Pseudomonas syringae* is a common bacterium found many places. It has a protein that mimics the crystalline structure of ice and helps start the formation of ice. When water freezes, it needs a starting point for ice crystals to form, which the bacteria provide. After ice formation occurs, damage to the plant tissue allows a pathway for the bacteria to enter the tissue of the plant, causing infection. *Pseudomonas syringae* and frost damage have been studied extensively in a variety of annual crops. However, it has not been the focus of much research, until recently, within alfalfa. Initial trials in 2017 began to investigate the possibility of *Pseudomonas syringae* playing an increased role in crop injury after applications of glyphosate, but trial results were inconclusive. Interestingly, a second bacterial species, *Pseudomonas viridiflava* was identified from these trials that also causes symptoms of bacterial stem blight.

While the 2015-2017 field trials found treating alfalfa early caused the least amount of crop injury, it was still unknown exactly what management practices minimized injury as treatments were not standardized across studies. In 2019, a standardized study was conducted at several locations in California and Utah to replicate previous trials, to test new agronomic practices, and to continue to investigate the role of *Pseudomonas syringae* in injury observed.

Three trial locations were selected in the Intermountain Region of California: Tulalake, Scott Valley and the Honey Lake Valley, with one location in Utah. Two experiments were implemented at each location. The first experiment evaluated glyphosate applied at a low or high rate to alfalfa

at six growth stages. This experiment included additional treatments at a taller crop growth stage compared to earlier studies to determine if a late application could avoid crop interaction with frost. The second experiment focused on weekly applications of a bactericide to try to suppress and possibly eliminate *Pseudomonas syringae* populations on the leaf surface of the crop with and without glyphosate application.

## METHODS

Treatments were standardized across locations. Each experiment used a randomized complete block design with four replications. Individual plot size was 10\*20 ft. Herbicide applications were made with a CO<sub>2</sub> pressured backpack sprayer delivering a carrier volume of 20 gal/acre. Roundup Powermax was the glyphosate product used at all locations. Temperature loggers were placed in the trial area at all locations to capture hourly temperatures in the leaf canopy and corresponding frost events. Visual crop injury, number of injured stems, crop height, and yield were measured at timing of the first cutting. Only crop height and yield were measured at the Utah location.

## RESULTS

Observed results for the crop height application trial can be observed in Tables 1-4. Table 1 depicts the visual injury assessment data before the first cutting. Variable results were observed across the three sites in California. The Tulelake site and Honey Lake Valley site showed statically significant visual injury compared to the untreated check when applications of the high rate of glyphosate (Roundup Powermax 44 oz/ac) were made to alfalfa at the 6", 8", and 12" growth stages. Average crop height in inches at the time of first cutting can be observed for all four sites in Table 2. No statistical differences in crop height were observed at the Utah or Scott Valley locations. The Tulelake site had four glyphosate treatments between the 6" and 16" application height that showed a two-inch reduction in crop height compared to the untreated check. The Honey Lake Valley site had a four-inch reduction in crop height where the high rate of glyphosate (Roundup Powermax 44 oz/ac) was applied at 6", 8" and 12". The number of injured alfalfa stems were counted at all three California sites (Table 3). Very little injury occurred at the Scott Valley site, with no statistical differences between treatments. Conversely, at the Tulelake site many stems were injured with no differences between treatments. The Honey Lake Valley site had a moderate number of stems that showed injury, with increased numbers compared to the untreated check when the high rate of glyphosate (Roundup Powermax 44 oz/ac) was applied at the 8" and 12" crop height. While there were numerical difference in crop yield at all sites (Table 4) only the Honey Lake Valley site showed statistical differences. There was a 0.4-0.45 dry tons/acre yield reduction at the Honey Lake Valley site when the high rate of glyphosate (Roundup Powermax 44 oz/ac) was applied to the crop at 6" and 8".

Results from the bactericide trial can be found in Tables 5-8. Results for the Scott Valley site were not reported because of complications at that location. At both the Tulelake and Honey Lake Valley sites, statically significant visual injury occurred in both treatments containing glyphosate,

regardless of bactericide treatment (Table 5). Crop height at time of harvest was statically insignificant between treatments at the Utah site (Table 6). The Tulelake site showed a statistical reduction in crop height where glyphosate was applied alone compared to the untreated check. Bactericide treatments with and without glyphosate had similar heights to the untreated check in Tulelake. Crop height was statically lower in the glyphosate treatments, with and without bactericide at the Honey Lake Valley site. Crop yield showed a similar trend as crop height for each of the three sites. No yield differences between treatments were observed at the Utah site (Table 8). The Honey Lake Valley site had a 0.59-0.62 tons/acre yield reduction for both glyphosate treatments compared to the untreated check. The Tulelake site had a significant yield reduction of 0.42 tons/acre when glyphosate was applied alone, with no statistical difference in yield occurring between the bactericide, bactericide + glyphosate, and untreated check.

Temperatures below 32 °F registered on the data loggers can be found in Graph 1. The Scott Valley site had 25 total days where frost occurred, with a temperature dropping down to 21.8 °F on May first, with five frost events occurring after that date. The Utah site had 25 total days where frost occurred, with a low of 25.5 °F occurring on May second, with eight frost events occurring after that date. The Honey Lake Valley site experienced 24 total days of frost, with a low temperature of 20.7 °F on May first, with eight frost events occurring after that date. Tulelake was the coldest site, with 36 total frost events, a low of 10.5 °F on May first, and 14 frost events occurring after that date.

## DISCUSSION

The 2019 experiments confirmed results found in previous trials, namely crop injury following glyphosate and frost was variable across sites and in some instances first cutting yield was reduced by more than 0.5 ton/acre. The 2019 studies also confirmed the recommendation to make Roundup applications to alfalfa at growth stages under 4 inches to minimize the risk of injury. If there is concern of weeds emerging after the glyphosate application, a product with pre-emergent activity should be tank-mixed with glyphosate to control weeds that have not germinated. Further, when applications are made to alfalfa greater than 4 inches tall, if targeted species will be controlled with the 22 oz. rate, there is evidence to suggest there is less risk of crop injury than with the 44 oz. rate. Trials in 2019 also included applications at larger crop growth stages than previous research to avoid interaction with frost. In these later treatments it was noted that unacceptable weed control occurred, as significant weed growth occurred before herbicide application was made.

Results from the bactericide trial were promising at the Tulelake location, but inconclusive at the Honey Lake and Utah locations. Continued work will need to take place next growing season to confirm what role *Pseudomonas syringae* plays in the crop injury observed. Based on the visual symptoms observed in the plots, our working hypothesis still involves an interaction with *Pseudomonas syringae*, glyphosate, and frost.

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\*\*Visual injury assessment and number of injured stems was not recorded at the Utah trial location.

Height Trial: Visual Injury Assessment - First Cutting						
Treatment	Scott Valley		Honey Lake Valley		Tulelake	
	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report
Control	13	AB	3	E	10	D
Tricor 75% DF + Gramoxone SL 2.0	15	AB	3	DE	13	CD
Roundup Powermax 22 oz/ac-2"	10	B	8	BCDE	20	BCD
Roundup Powermax 44 oz/ac-2"	11	AB	13	ABCDE	20	BCD
Roundup Powermax 22 oz/ac-4"	13	AB	6	CDE	21	BCD
Roundup Powermax 44 oz/ac-4"	13	AB	13	ABCDE	25	ABC
Roundup Powermax 22 oz/ac-6"	14	AB	9	BCDE	24	ABC
Roundup Powermax 44 oz/ac-6"	15	AB	24	ABC	35	A
Roundup Powermax 22 oz/ac-8"	16	AB	24	ABC	31	AB
Roundup Powermax 44 oz/ac-8"	15	AB	31	A	35	A
Roundup Powermax 22 oz/ac-12"	18	A	28	AB	30	AB
Roundup Powermax 44 oz/ac-12"	16	AB	31	A	30	AB
Roundup Powermax 22 oz/ac-16"	18	AB	21	ABCD	19	BCD
Roundup Powermax 44 oz/ac-16"	16	A	30	A	21	BCD

**Table 1:** Visual injury assessment of crop damage in the first cutting (\*color coded by site for visualization) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Sites were analyzed separately. The conventional treatment was applied during dormancy (Tricor 75% DF 2/3 lb/acre + Gamoxone SL 2.0 1qt/acre), where all other applications were made at the corresponding crop height listed in the table.

Height Trial: Average Crop Height in Inches - First Cutting								
Treatment	Scott Valley		Utah		Honey Lake Valley		Tulelake	
	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report
Control	27	A	32	A	25	A	19	A
Tricor 75% DF +Gramoxone SL 2.0	26	A	31	A	24	AB	17	BC
Roundup Powermax 22 oz/ac-2"	28	A	32	A	24	ABC	19	AB
Roundup Powermax 44 oz/ac-2"	27	A	33	A	23	ABC	18	ABC
Roundup Powermax 22 oz/ac-4"	27	A	32	A	23	ABC	17	BC
Roundup Powermax 44 oz/ac-4"	27	A	32	A	22	BC	18	ABC
Roundup Powermax 22 oz/ac-6"	26	A	31	A	23	ABC	17	C
Roundup Powermax 44 oz/ac-6"	25	A	31	A	21	BC	18	ABC
Roundup Powermax 22 oz/ac-8"	24	A	31	A	21	C	17	BC
Roundup Powermax 44 oz/ac-8"	26	A	31	A	21	C	17	C
Roundup Powermax 22 oz/ac-12"	25	A	31	A	23	ABC	18	ABC
Roundup Powermax 44 oz/ac-12"	26	A	30	A	21	C	18	ABC
Roundup Powermax 22 oz/ac-16"	25	A	31	A	23	ABC	17	BC
Roundup Powermax 44 oz/ac-16"	25	A	31	A	22	BC	18	ABC

**Table 2:** Average crop height before first cutting (\*color coded by site for visualization of data) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Sites were analyzed separately.

The conventional treatment was applied during dormancy (Tricor 75% DF 2/3 lb/acre + Gamoxone SL 2.0 1qt/acre), where all other applications were made at the corresponding crop height listed in the table.

Height Trial: Number of Injured Stems/Plot - First Cutting						
Treatment	Scott Valley		Honey Lake Valley		Tulelake	
	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report
Control	1	A	0	D	19	A
Tricor 75% DF +Gramoxone SL 2.0	2	A	0	D	18	A
Roundup Powermax 22 oz/ac-2"	1	A	1	D	18	A
Roundup Powermax 44 oz/ac-2"	3	A	1	C D	20	A
Roundup Powermax 22 oz/ac-4"	1	A	1	D	18	A
Roundup Powermax 44 oz/ac-4"	2	A	2	C D	22	A
Roundup Powermax 22 oz/ac-6"	2	A	2	C D	22	A
Roundup Powermax 44 oz/ac-6"	3	A	6	B C D	18	A
Roundup Powermax 22 oz/ac-8"	2	A	4	C D	22	A
Roundup Powermax 44 oz/ac-8"	2	A	11	A B	23	A
Roundup Powermax 22 oz/ac-12"	2	A	5	B C D	19	A
Roundup Powermax 44 oz/ac-12"	5	A	17	A	19	A
Roundup Powermax 22 oz/ac-16"	2	A	5	B C D	20	A
Roundup Powermax 44 oz/ac-16"	4	A	8	B C	16	A

**Table 3:** Average number of injured stems per 0.5m<sup>2</sup> (\*color coded across site for visualization of data) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Sites were analyzed separately. The conventional treatment was applied during dormancy (Tricor 75% DF 2/3 lb/acre + Gamoxone SL 2.0 1qt/acre), where all other applications were made at the corresponding crop height listed in the table.

Height Trial: Yield in Tons/Acre - First Cutting								
Treatment	Scott Valley		Utah		Honey Lake Valley		Tulelake	
	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report
Control	2.37	A	2.88	A	2.51	A B	1.95	A
Tricor 75% DF +Gramoxone SL 2.0	2.31	A	2.73	A	2.58	A	2.02	A
Roundup Powermax 22 oz/ac-2"	2.60	A	2.91	A	2.38	A B C	2.02	A
Roundup Powermax 44 oz/ac-2"	2.41	A	2.98	A	2.42	A B C	1.88	A
Roundup Powermax 22 oz/ac-4"	2.63	A	2.88	A	2.31	A B C	1.88	A
Roundup Powermax 44 oz/ac-4"	2.24	A	2.87	A	2.16	A B C	1.90	A
Roundup Powermax 22 oz/ac-6"	2.29	A	2.67	A	2.33	A B C	1.76	A
Roundup Powermax 44 oz/ac-6"	2.19	A	2.61	A	2.09	C	1.72	A
Roundup Powermax 22 oz/ac-8"	2.19	A	2.72	A	2.10	C	1.73	A
Roundup Powermax 44 oz/ac-8"	2.28	A	2.71	A	2.05	C	1.67	A
Roundup Powermax 22 oz/ac-12"	1.99	A	2.73	A	2.34	A B C	1.91	A
Roundup Powermax 44 oz/ac-12"	1.99	A	2.66	A	2.13	B C	1.87	A
Roundup Powermax 22 oz/ac-16"	2.28	A	2.72	A	2.29	A B C	1.96	A
Roundup Powermax 44 oz/ac-16"	2.39	A	2.63	A	2.32	A B C	1.82	A

**Table 4:** Average first cutting yield (\*color coded by site for visualization of data) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Sites were analyzed separately. The

conventional treatment was applied during dormancy (Tricor 75% DF 2/3 lb/acre + Gamoxone SL 2.0 1qt/acre), where all other applications were made at the corresponding crop height listed in the table.

Because of complications at the Scott Valley location, data for that location is not shown.

<b>Bactericide Trial: Visual Assessment of Injury - First Cutting</b>				
Treatment	Honey Lake Valley		Tulelake	
	Mean	Letter Report	Mean	Letter Report
Control	0	B	15	B
Kocide DF+Manzate Max	3	B	14	B
Kocide DF+Manzate Max+Powermax 44oz/ac	29	A	31	A
Roundup Powermax 44oz/ac	33	A	39	A

**Table 5:** Visual injury assessment of crop damage in the first cutting (\*color coded by site for visualization) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Sites were analyzed separately. Bactericide treatments were applied weekly at the flowing rates, Kocide DF 2lb/ac +Manzate Max 1.6 lb/ac. Roundup Powermax was only applied once at the 8” growth stage.

<b>Bactericide Trial: Average Alfalfa Height Inches - First Cutting</b>						
Treatment	Honey Lake Valley		Tulelake		Utah	
	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report
Control	24	A	19	AB	32	A
Kocide DF+Manzate Max	24	A	20	A	32	A
Kocide DF+Manzate Max+Powermax 44oz/ac	20	B	18	B	32	A
Roundup Powermax 44oz/ac	20	B	17	C	32	A

**Table 6:** Average number of injured stems (\*color coded by site for visualization of data) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Sites were analyzed separately. Bactericide treatments were applied weekly at the flowing rates, Kocide DF 2lb/ac +Manzate Max 1.6 lb/ac. Roundup Powermax was only applied once at the 8” growth stage.

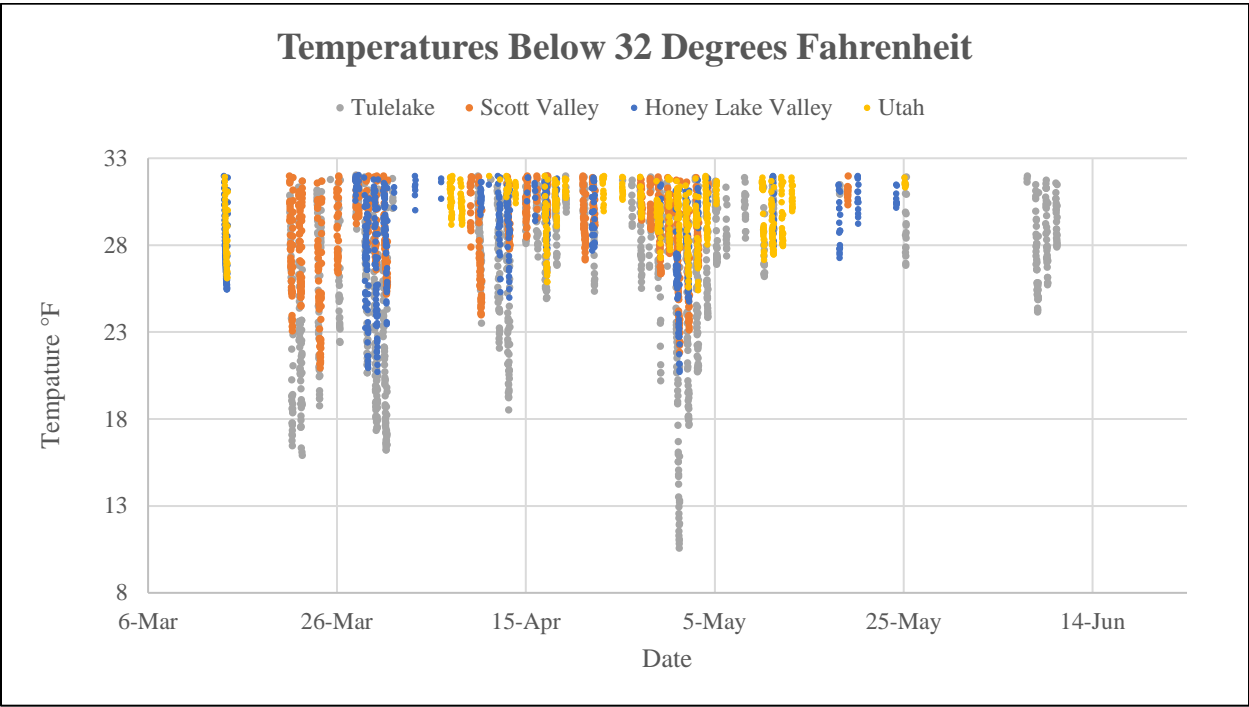
<b>Bactericide Trial: Number of Injured Stems - First Cutting</b>				
Treatment	Honey Lake Valley		Tulelake	
	Mean	Letter Report	Mean	Letter Report
Control	0	B	13	AB
Kocide DF+Manzate Max	1	B	13	AB
Kocide DF+Manzate Max+Powermax 44oz/ac	12	A	12	B
Roundup Powermax 44oz/ac	16	A	21	A

**Table 7:** Average number of injured stems (\*color coded across site for visualization of data) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Sites were analyzed separately. Bactericide treatments were applied weekly at the flowing rates, Kocide DF 2lb/ac +Manzate Max 1.6 lb/ac. Roundup Powermax was only applied once at the 8” growth stage.



Bactericide Trial: Yield in Tons/Acre - First Cutting						
Treatment	Honey Lake Valley		Tulelake		Utah	
	Mean	Letter Report	Mean	Letter Report	Mean	Letter Report
Control	2.59	A	2.13	A	2.59	A
Kocide DF+Manzate Max	2.53	A	2.39	A	2.65	A
Kocide DF+Manzate Max+Powermax 44oz/ac	2.00	B	2.11	A	2.61	A
Roundup Powermax 44oz/ac	1.97	B	1.71	B	2.48	A

**Table 8:** Yield in tons/acre (\*color coded by site for visualization of data) Letter reports indicate means with the same letter were not statically different using the Tukey HSD test. Bactericide treatments were applied weekly at the flowing rates, Kocide DF 2lb/ac +Manzate Max 1.6 lb/ac. Roundup Powermax was only applied once at the 8” growth stage.



**Graph 1:** Hobo temperature datalogger information collected for all four sites. Only values registered below 32 °F are depicted on the graph.

\*\*\*\*\* Color was added to the charts in order to better visualize trends in the numerical data and are a non-exact measure of those numerical differences. Color does not indicate statistical differences, which are indicated by the letter reports for each site.

Green colors generally represent a positive value for the associated variable, where red colors indicate a “negative” value for the associated variable. Yellows and oranges fall somewhere in the middle. The table below is an example of values for each variable visualized. High numerical values for yield and alfalfa stem height were considered positive/good, and are highlighted in green. Conversely, large numbers of injured stems and high values of visual ratings were considered negative/bad and are colored in red.

Example of Generalized Color Code: (Green=“good” value Red=“bad” value)			
Yield tons/acre	Height inches	# Injured Stems	% Visual Injury
1.7	14	0	0
1.8	15	2	2
1.9	17	4	4
2	19	6	5
2.1	21	8	8
2.2	22	10	12
2.3	23	12	15
2.4	24	14	20
2.5	25	16	22
2.6	26	18	27
2.7	28	20	32



Photo One: Shepherd’s Crook symptomology as the stems die in a glyphosate treated plot

Photo Two: General chlorosis and injured stems in a glyphosate treated plot

Photo Three: Untreated plot with no apparent symptoms

