Project Title: Yellow Flashing Effect on Yield in Wheat

Project Location(s): Scott, Saskatchewan; R.M. #380; Legal land description: SE 19-39-20 W3

Project start and end dates (month & year): May 2023 to February 2024

Project Collaborators:

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Objective:

The objective is to evaluate the effect of group one and two herbicide injury on wheat yield when applied at different growth stages and times of the day. Followed by assessing the visual damage to wheat and its correlation to yield.

Methodology:

The trial was arranged as a randomized complete block design (RCBD) with thirteen treatments replicated four times at Scott (WARC), SK in 2023 (Table 1). Treatments consisted of an herbicide free check, a group one herbicide (Horizon) or a group two herbicide (Simplicity), applied at three different times of day (early morning, mid-afternoon, dusk) and at two crop stages (two-four leaf, flag leaf).

Seeding occurred on May 15, 2023 using certified AAC Viewfield wheat at 112 lb/ac at a depth of 1 inch. A fertilizer blend of 73-17-7-3 was side banded at 150 lb/ac as per spring soil test recommendations. The herbicide free check was hand weeded throughout the growing season to ensure any treatment effects were due to differences in herbicide applications as opposed to differences in weed pressure. Horizon was applied at the recommended rate of 376 mL/ac and Simplicity at 200 mL/ac. All applications also had a tank mix of Stellar X (group 2, 4) and MCPA (group 4) herbicide. Simplicity had a water conditioner added as per label recommendations. Pesticides throughout the growing season were conducted with best management practices in mind and at the discretion of the site manager (Appendix A1). The trial was straight combined on August 30th using a Wintersteiger plot combine.

Table 1. Treatment list for Yellow Flashing Effect on Yield in Wheat at Scott, SK in 2023.

TRT #	Product	Time of Day	Wheat Stage
1	N/A (herbicide free check)	-	-
2	Horizon (376 mL/ac)	Early morning	2-4 leaf
3		Mid-afternoon	
4		Dusk	
5		Early morning	Flag leaf
6		Mid-afternoon	
7		Dusk	
8	Simplicity (0.20 L/ac)	Early morning	2-4 leaf
9		Mid-afternoon	
10		Dusk	
11		Early morning	Flag leaf
12		Mid-afternoon	
13		Dusk	

Data Collection:

Soil samples were collected in the spring of 2023 at two depth increments (0-6 inches and 6-24 inches). The Canadian Weed Science Society (CWSS) evaluation of crop tolerance scale was used at 7, 14 and 21 days after application (DAA) (Figure 1). During each application spray conditions were recorded, which included date, time, temperature, wind speed, wind direction and relative humidity. NDVI was collected using a hand-held Trimble Greenseeker at the boot stage. Yields were determined from cleaned harvested grain samples and corrected to 14.5% moisture content. Grain protein was collected as an indicator of seed quality. Weather data was collected from a Government of Canada on-site weather station and growing degree days were based on 5°C.

B) Evaluation of Crop Tolerance

The same basic principals apply. The evaluation should again be done with a comparison to the untreated check.. In most cases, however, the untreated check suffer from the competition of the weeds and therefore one should include hand weeded check plots.

Phytotoxicity Range	Assessment of Injury	Suggested Interval size
0-9%	Slight Discoloration and/or Stunting	2%
10%	Just acceptable	
11-30%	Not Acceptable	5%
< 30%	Severe	10%

- 10% or less is considered acceptable injury

Initial Damage of up to 10% will generally be outgrown and will disappear with time. The impact of these low levels of injury will not be reflected in yield losses.

Figure 1. Canadian Weed Science Society (CWSS) rating scale to determine crop tolerance (%).

Statistical Analysis:

The data was statistically analyzed using R Studio (ver.2021.9.2.382) (RStudio Team, 2022) to determine the effects of herbicide product, plant stage at application, time of application, and their interactions on visual phytotoxicity ratings, NDVI, yield, and protein of wheat planted at Scott. A random intercept mixed effects model was used with product, plant stage, and time of application as fixed effects and replication as a random effect. When analysis of variance (ANOVA) indicated significant differences ($p < 0.05$), means were separated using the estimated marginal means comparison. Analysis excluding the control treatment was initially done to analyze the interaction between fixed effects using a two-way ANOVA. Further analysis of fixed effects was analyzed between all treatments using a one-way ANOVA.

Weather:

The beginning of the 2023 growing season had little residual soil moisture as drought conditions persisted throughout 2021 and 2022. As a result, conditions were very dry and cool for the first month of the growing season. The average temperature in April was lower than the long-term average, but increased in May. Total precipitation was considerably lower than the long-term average in April and May. June experienced above average temperatures and precipitation. Majority of the precipitation in June occurred in two days, June 6th and 18th, with 33.6 and 20.7 mm of rainfall, respectively. June temperatures were unusually high, with the highest temperature of 32.0 °C on June 5th. July had average temperatures but below average precipitation, resulting in poor growing conditions. August had below average temperatures and precipitation. From April to August there was 87 mm less precipitation than the long-term average. Due to the warm conditions in May, June and July there was 210 more growing degree days (based on 5°C) compared to the long-term average. The higher temperature, decreased precipitation, and low residual soil moisture in the spring, resulted in poor growing conditions throughout the growing season (Table 2).

Table 2. Mean monthly temperature, precipitation, and growing degree day accumulated from April to August 2023 at Scott, SK.

Year	April	May	June	July	August	Average
----------*Temperature (°C)* ----------						
2023	0.4	14.9	17.2	17.1	17.4	13.4
Long-term[z]	3.8	10.8	14.8	17.3	16.3	12.6
----------*Precipitation (mm)*----------						
2023	5.4	16.6	81.1	29.7	31.7	164.5
Long-term[z]	24.4	38.9	69.7	69.4	48.7	251.1
----------*Growing Degree Days 5°C*----------						
2023	20	297	367	376	389	1450
Long-term[z]	44	171	295	381	350	1240

[z]Long-term average (1985-2014)

Results

Soil Samples

Spring soil samples were collected and analysed at Agvise Laboratories. Spring soil test results indicated that nitrogen (N) levels were 6 lb/ac at the 0-6” depth, 4 lb/ac at the 6-12” depth and 30 lb/ac at the 12-24” depth. Resulting in a total of 40 lb/ac from 0-24”. Phosphorous was measured using an Olsen extraction and showed high levels of available phosphorous (19 ppm). The soil pH was 6.4 at the 0-6” depth and organic matter was 4.0%.

Spray Application Conditions

Table 3. Information regarding date, time, temperature (°C), wind speed (km/hr), wind direction and relative humidity (%) for in-crop herbicide applications to wheat at Scott, SK in 2023.

Date	Crop Stage	Time	Temperature	Wind Speed (km/hr)	Wind Direction	Relative Humidity
June 7th	2-4 leaf	9:00 am	18.5 °C	8.0	North	51%
		2:45 pm	24.5 °C	5.5	North	37%
		9:30 pm	21.0 °C	3.5	North	12%
June 28th	Flag leaf	9:00 am	18.8 °C	12.0	North	54%
		3:00 pm	23.3 °C	13.0	North East	32%
		9:00 pm	19.5 °C	3.0	North	49%

Evaluation of Visual Phytotoxicity Ratings

Analysis of all treatments showed similar trends at 7 DAA (p=0.719), 14 DAA (p=0.554), and 21 DAA (p=0.807) (Figure 2). Similarly, there were no significant differences for the response of phytotoxicity ratings to product, plant stage, or time of application at 7, 14, and 21 DAA (Appendix A2). This statistical indifference could be attributed to precipitation events and low temperatures, even at the mid-afternoon application timing (Table 3). The highest phytotoxicity rating of 2% was seen with Horizon, 2-4 leaf, at mid-afternoon and dusk (Figure 3 & 4). Throughout all ratings, crop injury did not exceed 2%. This could be due to the rainfall that occurred on June 6th, one day prior to the first application (2-4 leaf) and on June 30th, two days after the second application (flag leaf), reducing risk of crop injury. According to the Canadian Weed Science Society (CWSS) scale to determine crop tolerance (Figure 1), the phytotoxicity range from 0-9% has an assessment of injury of slight discoloration and/or stunting. The suggested interval size is 2%; therefore, ratings were either a 0 or 2% due to the very minor injury. These ratings accurately describe the injury seen at all three timings (7, 14 and 21 DAA).

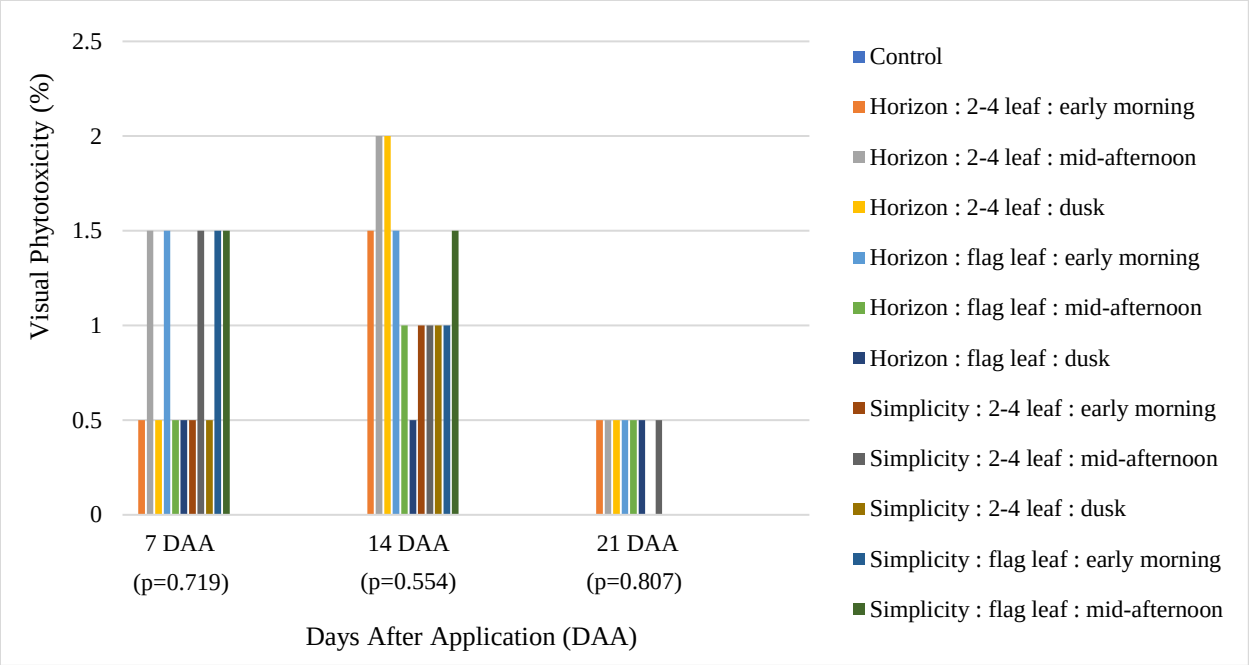

Figure 2. The effect of product (Horizon vs Simplicity), stage (2-4 leaf vs flag leaf) and timing (early morning, mid-afternoon, dusk) on visual phytotoxicity ratings (%) at 7, 14 and 21 days after application (DAA) at Scott, SK 2023. Significance at $p<0.05$ using estimated marginal means comparison.

Figure 3. TRT 3 (Horizon, mid morning) (left), and TRT 9 (Simplicity, mid morning) (right) 14 days after application at the 2-4 leaf stage.

Figure 4. TRT 4 (Horizon, dusk) (left), and TRT 10 (Simplicity, dusk) (right) 14 days after application at the 2-4 leaf stage.

NDVI

NDVI is an indicator of vegetative health based on how plants reflect certain ranges of the electromagnetic spectrum. Healthy plants, with an abundance of chlorophyll and cell structures, actively absorb red light and reflect NIR and thereby have a higher NDVI value. A value between 0.66 to 1.0 indicates very healthy plants, 0.33 to 0.66 indicates moderately healthy plants, 0 to 0.33 indicates unhealthy plants and 0 to -1 indicates a dead plant (UP42, 2020). NDVI readings were collected on June 30th, two days after the second herbicide application (flag leaf). NDVI values for the thirteen treatments were not significantly different ($p=0.433$) (Figure 3). Analysis of product, stage, and time also determined significant differences (Appendix A2). The NDVI values for all treatments ranged between 0.57 and 0.77; indicating that all plants were moderately healthy to very healthy (Appendix A3). The moderately healthy to healthy NDVI ratings corresponds with low phytotoxicity ratings, such that low ratings result in healthy plants. If more phytotoxicity damage had occurred, lower NDVI numbers would be expected.

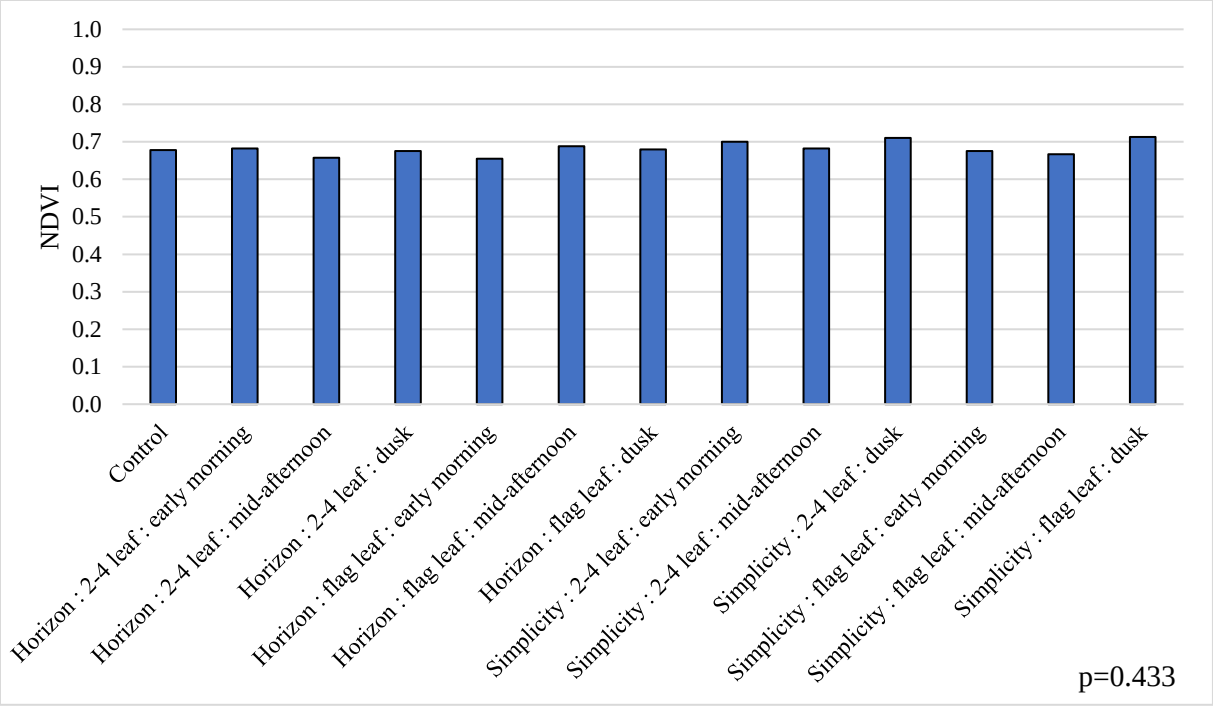

Figure 3. The effect of product (Horizon vs Simplicity), stage (2-4 leaf vs flag leaf) and timing (early morning, mid-afternoon, dusk) on NDVI (0-1) of wheat at Scott, SK 2023. Significance at $p<0.05$ using estimated marginal means comparison.

Yield

There was a significant interaction between product and plant stage ($p=0.031$) and a significant effect of product on yield ($p=0.016$). This indicates that the products applied in this study resulted in different yields and these results varied depending on the growth stage they were applied. The highest yields occurred with the application of Simplicity at the flag leaf stage (56.7 bu/ac), and this was not significantly different than Simplicity applied at the 2-4 leaf stage, as indicated by the post-hoc results. This suggests that Simplicity can improve yields when applied at a later plant stage, although the results in this study were not significantly different. However, Simplicity applied at flag leaf did result in significantly higher yields than both applications of Horizon. These results show that Simplicity applied at a later stage is less harmful to yields than applications of Horizon. There was no statistical difference between applications of Simplicity at the 2-4 leaf stage and applications of Horizon applied at either plant stage.

There was no statistical significance found for the effects of stage ($p=0.160$), time ($p=0.388$), product x time ($p=0.334$), stage x time ($p=0.680$) and product x stage x time interaction ($p=0.697$) (Appendix A2). Yields for all treatments ranged from 45.5 to 58.2 bu/ac (Appendix A3). The highest yield was recorded for the application of Simplicity at the flag leaf stage in the mid-afternoon (58.2 bu/ac) and the lowest was recorded for the application of Horizon at the flag leaf stage at dusk (45.5 bu/ac).

Overall, these results show that the time of day these products are applied does not significantly

affect yields; however, the products applied and the stage these products were applied at had marginal effects on yield. The combination with the least detrimental effects to yield was the application of Simplicity at the flag leaf stage. These results indicate that overall crop tolerance to Simplicity is higher and that the crop is tolerable to later season applications up to flag leaf.

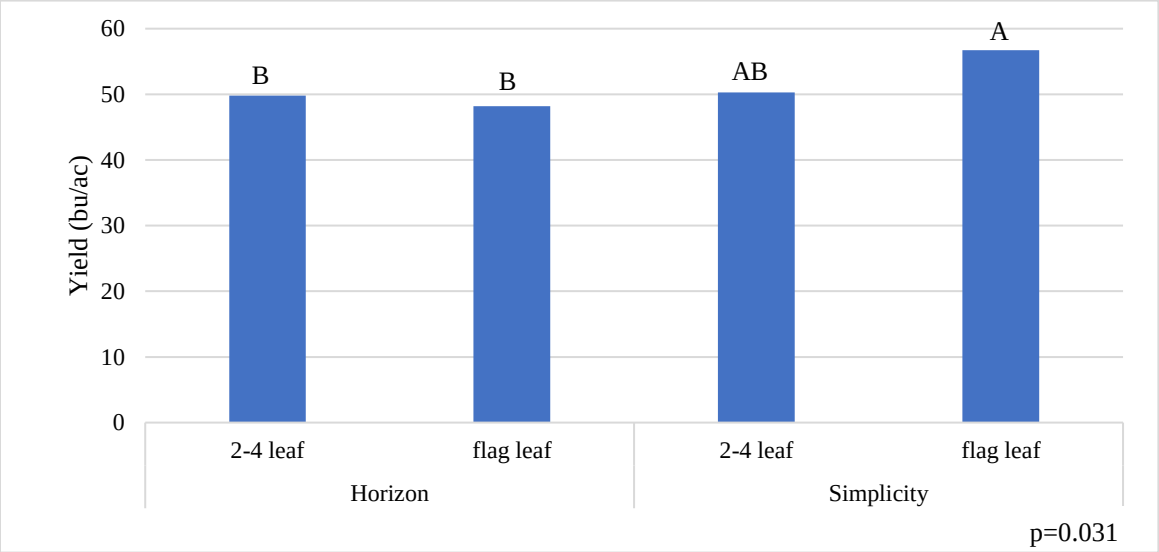

Figure 4. The effect of product (Horizon vs Simplicity) and plant stage (2-4 leaf vs flag leaf) on yield (bu/ac) of wheat at Scott, SK 2023. Different letters indicate significance at $p<0.05$ using estimated marginal means comparison.

Protein

The effect of product ($p=0.021$), and the interaction between product and plant stage ($p=0.042$) had significant impacts on protein of wheat (Appendix A2). Throughout all treatments, proteins ranged between 12.3 – 14.0% (Appendix A3). Horizon, flag leaf, early morning resulted in the highest protein (14.0%) and Simplicity, flag leaf, dusk had the lowest (12.3%). Mean protein was highest for Horizon applied at flag leaf stage (13.6%), followed by Horizon applied at 2-4 leaf stage (13.5%), Simplicity applied at 2-4 leaf stage (13.4%), and lastly, Simplicity applied at flag leaf stage (12.6%) (Figure 5). Considering the inverse trend observed in the data between yields and protein, these results may be more indicative of the relationship between yield and protein than the actual treatment effects.

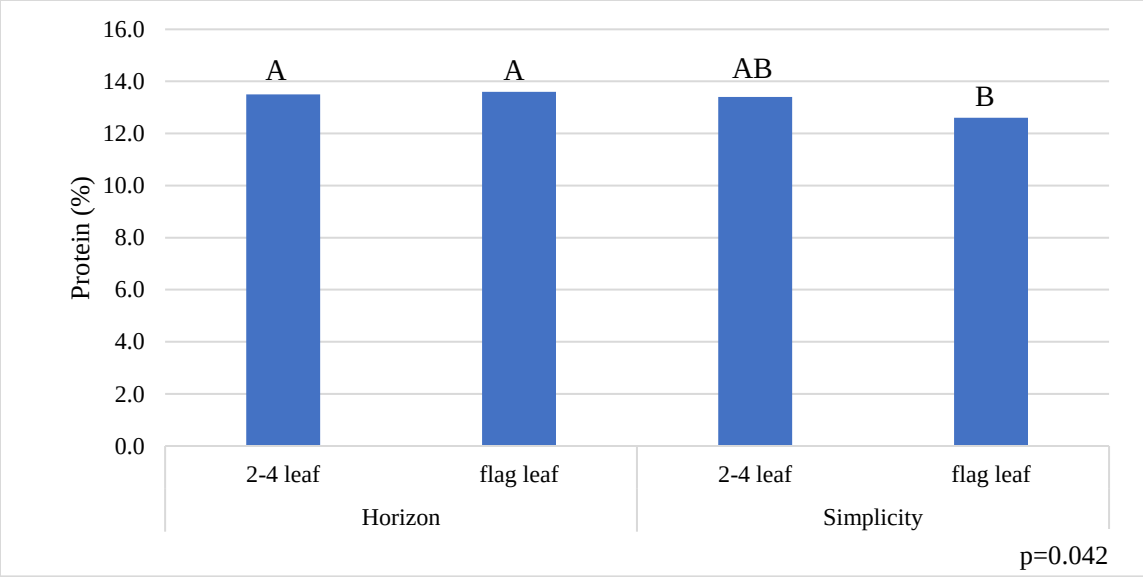

Figure 5. The effect of product (Horizon vs Simplicity) and plant stage (2-4 leaf vs flag leaf) on protein (%) of wheat at Scott, SK 2023. Different letters indicate significance at $p<0.05$ using estimated marginal means comparison.

Conclusion

In 2023, a thirteen-treatment RCBD trial was conducted at Scott, SK. This trial aimed to evaluate the effect of group one and two herbicide injury on wheat yield when applied at different growth stages and times of the day. According to the 2023 Saskatchewan Guide to Crop Protection, Horizon, a group 1 herbicide (clodinafop), is registered for application prior to the emergence of the 4th tiller in wheat. While Simplicity, a group 2 herbicide (pyroxsulam), is registered for application between the 3-leaf stage and prior to the emergence of the flag leaf. The results of this study found that NDVI values and phytotoxicity ratings indicated healthy plants for all treatments. This suggests that the products and their application timings did not cause any negative impacts on plant growth. There were slight differences in yield responses between products and growth stage applications. Mean yields were highest for Simplicity at flag leaf (56.7 bu/ac), followed by Simplicity at 2-4 leaf (50.3 bu/ac), Horizon at 2-4 leaf (49.8 bu/ac), and Horizon at flag leaf (48.2 bu/ac). However, only applications of Horizon were significantly lower in yield than applications of Simplicity at the flag leaf stage. These results indicate that overall crop tolerance to Simplicity is greater than Horizon. Additionally, there were no yield penalties when herbicides were applied at the flag leaf stage. Generally, herbicides are more effective when applied at earlier crop stages, but this may vary depending on environmental conditions and weed pressure. Inverse trends were observed between yield and protein, which may be more indicative of the relationship between yield and protein than the actual treatment effects. Ultimately, this study suggests that crop tolerance to Simplicity is greater than Horizon and applications of both herbicides at the flag leaf stage did not negatively impact yields. It is important to

consider that environmental conditions before and after spray applications were optimal for herbicide absorption and metabolism. Results of this study may vary depending on environmental conditions during spraying. Therefore, it would be beneficial to further investigate these effects in different environmental conditions.

Acknowledgements:

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Appendices

Appendix A1. Agronomic information and dates of operations for "Yellow Flashing Effect on Yield in Wheat" completed at Scott, SK in 2023.

Agronomic Information	Product	Rate	Date
Fertilizer	73-17-7-3	150 lbs/ac	May 15, 2023
Seed	AAC Viewfield	112 lbs/ac	May 15, 2023
Seed Treatment	-	-	-
Herbicides: Pre-plant In-Crop (2-4 leaf) In-Crop (Flag leaf)	 Glyphosate 540 & AIM As per protocol As per protocol	 1 L/ac & 35 mL/ac As per protocol As per protocol	 May 14, 2023 June 7, 2023 June 28, 2023
Fungicide	Caramba	400 mL/ac	July 13, 2023
Insecticide	Decis	60 mL/ac	July 7, 2023
Desiccation	Glyphosate 540, Heat LQ & Merge	1 L/ac, 59 mL/ac & 200mL/ac	August 15, 2023
Harvest	-	-	August 30, 2023

Appendix A2. p values for product (Horizon vs. Simplicity), plant stage (2-4 leaf vs flag leaf), time of application (early morning, mid-afternoon, dusk), and their interactions on visual phytotoxicity ratings (%) performed 7, 14, and 21 days after application (DAA), NDVI (0-1), yield (bu/ac) and protein (%) of wheat at Scott, 2023.

Effect	Phytotoxicity Ratings (%)			NDVI	Yield	Protein
	7 DAA	14 DAA	21 DAA	(0-1)	(bu/ac)	(%)
Product	0.625*	0.136	0.068	0.097	**0.016**	**0.021**
Stage	0.625	0.136	0.758	0.815	0.160	0.111
Time	0.151	0.392	0.864	0.229	0.388	0.370
Product x Stage	0.567	0.152	0.645	0.363	**0.031**	**0.042**
Product x Time	0.719	0.918	0.807	0.305	0.334	0.403
Stage x Time	0.112	0.339	0.807	0.194	0.680	0.144
Product x Stage x Time	0.719	0.554	0.807	0.433	0.697	0.525

*Bold numbers indicate significance at $p<0.05$ using estimated marginal means comparison.

Appendix A3. Mean values for visual phytotoxicity ratings (%) performed 7, 14, and 21 days after application (DAA), NDVI (0-1), yield (bu/ac) and protein (%) of wheat sprayed with different herbicide (Horizon vs. Simplicity), plant stage (2-4 leaf vs. flag leaf), and timing (early morning, mid-afternoon, dusk) at Scott, SK., 2023.

Treatments	7 DAA	14 DAA	21 DAA	NDVI	Yield	Protein
	--------Phytotoxicity %------			0-1	bu/ac	%
Control	0.5	0.5	0.5	0.677	46.3	13.9
Horizon: 2-4 leaf: early morning	0.5 a	1.5 a	0.5 a	0.682 a	51.2 a	13.2 a
Horizon: 2-4 leaf: mid-afternoon	1.5 a	2.0 a	0.5 a	0.657 a	50.3 a	13.8 a
Horizon: 2-4 leaf: dusk	0.5 a	2.0 a	0.5 a	0.675 a	47.8 a	13.4 a
Horizon: flag leaf: early morning	1.5 a	1.5 a	0.5 a	0.655 a	48.8 a	14.0 a
Horizon: flag leaf: mid-afternoon	0.5 a	1.0 a	0.5 a	0.688 a	50.2 a	13.1 a
Horizon: flag leaf: dusk	0.5 a	0.5 a	0.5 a	0.680 a	45.5 a	13.7 a
Simplicity: 2-4 leaf: early morning	0.5 a	1.0 a	0.0 a	0.700 a	46.3 a	13.5 a
Simplicity: 2-4 leaf: mid-afternoon	1.5 a	1.0 a	0.5 a	0.682 a	51.6 a	13.7 a
Simplicity: 2-4 leaf: dusk	0.5 a	1.0 a	0.0 a	0.710 a	53.1 a	13.0 a
Simplicity: flag leaf: early morning	1.5 a	1.0 a	0.0 a	0.675 a	56.2 a	12.9 a
Simplicity: flag leaf: mid-afternoon	1.5 a	1.5 a	0.0 a	0.667 a	58.2 a	12.7 a
Simplicity: flag leaf: dusk	0.5 a	0.5 a	0.0 a	0.713 a	55.8 a	12.3 a
p-value	0.719	0.554	0.807	0.433	0.697	0.525
SEM	0.5	0.5	0.4	0.024	3.6	0.5

*Different letters within each column indicate significance at $p<0.05$ using estimated marginal means comparison. SEM, standard error of the mean.

References

RStudio Team (2022). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.