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Responses of seed yield, quality, and composition to the harvest-aid paraquat in soybean grown in Mississippi

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Abstract

Paraquat is used as a harvest-aid to desiccate green tissues for increasing harvest efficiency and maintaining seed quality. However, its application can cause significant crop damage and yield loss if applied too early. Limited information is available on determining the optimum time for applying paraquat. Therefore, the objectives of this study were to investigate the effects of the timing (critical stages of seed-fill) of paraquat application on soybean seed yield, seed quality (germination, viability, hard-seed, and seed damage), and seed composition. Field experiments were conducted in 2019 and 2020 at Stoneville, MS. Paraquat was applied at a rate of 0.56 kg a.i. ha^{-1} at growth stages R6 (full seed-fill), R6.5 (pod cavities completely filled with seeds), or R7 (yellow color/beginning maturity). Cultivars P46A57BX and P48A60X were used. The results showed that the application of paraquat at R6 or R6.5 resulted in significant yield loss for both cultivars in both years, whereas application at R7 resulted in significant yield loss for P46A57BX in both years, but in only 1 yr for P48A60X. Seed germination and viability were significantly increased over the control in 2020 for both cultivars at all three application stages, but with mixed effects in 2019. No seed damage that would result in dockage was observed in any treatment, as seed damage for all treatments was below 2%. Application of paraquat at R6 resulted in significantly higher seed protein, oleic acid, raffinose, and stachyose but lower oil and sucrose. This research demonstrated that the harvest-aid paraquat significantly reduced seed yield, increased seed protein, oleic acid, raffinose, and stachyose when applied before growth stage R7. Therefore, producers should use caution when applying paraquat for harvest efficiency before R7, as they will also likely reduce seed yield the earlier paraquat is applied.

Abbreviations: DAP, days after planting; ESPS, Early Soybean Production System; FGIS, Federal Grain Inspection Service.

1 | INTRODUCTION

Soybean seed is a source of protein, oil, carbohydrates, and other nutrients for human health and livestock nutrition. High protein and oil content are desirable as is high sucrose, which contributes to flavor and taste (Hou et al., 2009). High oleic acid and low linoleic and linolenic acids are desirable as they contribute to oil stability (Hou et al., 2009). Low raffinose and stachyose are also desirable because high levels of raffinose and stachyose lower the nutritive value of the soy meal due to their indigestibility by humans and animals, causing flatulence or diarrhea in nonruminants (Liu, 1997). Also, high raffinose and stachyose inhibit mineral uptake and increase flatulence (Obendorf et al., 1998). Therefore, it is essential to understand factors affecting the level of these nutrients in seeds.

Although seed composition is genetically controlled, environmental and growing conditions such as temperature (Dardanelli et al., 2006; Piper & Boote, 1999), water stress (Rotundo & Westgate, 2009), and diseases (Bellaloui et al., 2008, 2012) can also influence seed composition. Chemical stresses, including herbicides (Zobiole et al., 2010, 2012), applied during seed development can also significantly affect seed protein, oil, fatty acids, and sugars. Hence, understanding how the accumulation of seed components responds to environmental stresses and growing conditions during seed filling period is important when considering the application of the harvest-aid desiccant. The use of harvest-aids such as paraquat to desiccate green tissue for harvest efficiency and to maintain high seed quality (reduced seed moisture, foreign material, green seed and green pods, and seed damage) has become common, especially for the Early Soybean Production System (ESPS) in the mid-South, including Mississippi (Bellaloui et al., 2020; Boudreaux & Griffin, 2011; Ellis et al., 1998; Griffin et al., 2010; Ratnayake & Shaw, 1992a, 1992b). Although transitioning to the ESPS resulted in higher seed yield under both irrigated and nonirrigated conditions (Heatherly, 1999), the shift toward the use of early-maturing soybean cultivars in the ESPS that mature under hot temperatures resulted in increases in green stems, green pods, and late-season weed infestation (Griffin et al., 2010). Increases in green pods and weeds complicate harvest, resulting in reduced seed quality and increased seed moisture, foreign matter, and damaged seed. These will all penalize producers when the seed is sold at grain elevators (Grichar et al., 2020; Griffin et al., 2010; Pereira et al., 2020). Thus, the use of harvest-aids to desiccate green tissues for achieving uniformly dry plants at harvest improves harvest efficiency, reduces elevator discounts, and thereby increases net returns.

High moisture in late-season weeds was shown to damage harvested soybean seeds, reduce harvest efficiency (Griffin et al., 2010), and increase postharvest losses due to bacterial and fungal growth, and aflatoxin contamination (Stichler & Livingston, 2021). Studies have also shown that the application of paraquat after soybean seed has reached physiological maturity resulted in a reduced number of green stems, pods, and retained green leaves, making it possible to harvest 1–2 wk earlier than nontreated soybean (Griffin et al., 2010). In addition, the application of paraquat reduced seed moisture, foreign material, and seed damage (Griffin et al., 2010), contributing to harvest efficiency and improved seed quality (reduced seed moisture, foreign material, green seed, green pods, and seed damage) (Ratnayake & Shaw, 1992a, 1992b; Boudreaux & Griffin, 2008; Boudreaux & Griffin, 2011; Grichar et al., 2020; Griffin et al., 2010; Pereira et al., 2020).

The intent of crop harvest-aid application is to rapidly dry vegetative and reproductive plant tissues, including seeds, without affecting seed yield and seed quality (Ratnayake & Shaw, 1992a, 1992b; Soltani et al., 2013). Yet, the application of paraquat can cause significant crop yield losses and seed damage if applied too early, such as at the R5 or early R6 (Fehr & Caviness, 1977) growth stages. Previous research showed that application of paraquat at a rate of 0.84 kg a.i. ha^{-1} and glyphosate at rate of 0.560 kg a.i. ha^{-1} at R5, R6, and R7 can be safely applied at R7 growth stage without reducing seed yield or quality (Ratnayake & Shaw, 1992a, 1992b). Others have reported that application of harvest-aids, such as glyphosate, paraquat, ametryn, or sodium chloride, after seeds reach physiological maturity (R6.5 or 50% average seed moisture) did not affect soybean yield (Boudreaux & Griffin, 2011; Griffin et al., 2010). In addition, other studies found that soybean yields were reduced with paraquat application at the R5 or R6 growth stages, but not at R7 or R8 (Ratnayake & Shaw, 1992a, 1992b; Ross & Barber, 2018). However, application of glyphosate at 23 and 29 d before harvest when 5–30% of leaves have senesced reduced

Core Ideas

- Application of paraquat at R7 or earlier resulted in yield loss.
- Application of paraquat at R6 resulted in significantly higher protein, oleic acid, raffinose, and stachyose.
- Application of paraquat at R6 resulted in significantly lower oil and sucrose.
- New knowledge to producers about harvest-aids use is introduced.
- Application of paraquat on seed germination was significant but inconsistent between years.

seed yield by 18% (Azlin & McWhorter, 1981). Thus, as the literature does not agree as to the effects of applying desiccants or to the best application time, further research is needed to determine the optimum time for application of harvest-aids in terms of seed yield and quality. Especially in question are the effects of applications of desiccants at R6.5 and R7.

Information on the effects of paraquat on seed composition (protein, oil, fatty acids, and sugars) is scarce (Bellaloui et al., 2020), and what is available is either inconsistent or contradictory. Therefore, this information could be important in the future if a premium were paid at the delivery point for seed composition profile or if soybeans were used in seed production (Heatherly, 2018). Application of paraquat, glyphosate, and ametryn resulted in a decrease of seed oil content when applied 3 and 4 wk before harvest date (Whigham & Stoller, 1979), but there was no decrease in oil content when application was 2 wk before the harvest date of nontreated soybean. The application of ametryn 4 wk before harvest led to a decrease in oil content compared with the other treatments. Alternately, protein content increased with the application of ametryn at low and high rates 3 to 4 wk before actual harvest (Whigham & Stoller, 1979). These researchers concluded that the application of a harvest-aid before physiological maturity significantly altered seed composition (Whigham & Stoller, 1979). However, others have shown that the application of paraquat (0.28 kg a.i. ha^{-1}), carfentrazone-ethyl (1.015 kg a.i. ha^{-1}), sodium chlorate ($NaClO_3$; 6.72 kg a.i. ha^{-1}), and glyphosate (2.0 kg ae ha^{-1}) at R6 or R7 resulted in higher seed protein and oleic acids but did not decrease seed oil. They also reported that harvest-aid effects on seed composition constituents, especially protein, oil, and oleic acid differed, depending on year and growth stage at application (Bellaloui et al., 2020).

Based on the above studies, the timing of harvest-aid application is crucial (Toledo et al., 2014). Application needs to be after seed physiological maturity to avoid yield loss (Boudreaux & Griffin, 2011) due to the incomplete remobilization of photo-assimilates from their production sources (leaves and stems) to sinks (seeds) (Pereira et al., 2020). Otherwise, yield losses and seed damage may be expected.

In summary, although several studies have been conducted to evaluate the effects of paraquat application on yield and seed quality, their results have been inconsistent. In addition, the effects of paraquat on seed composition have not been well investigated, and what information is available is not consistent. Therefore, the aim of this research was to investigate the effect of the timing (during the critical stages near the end of seed-fill: R6, R6.5, and R7) of paraquat application (Figure 1) on soybean seed yield, seed composition constituents (seed protein, oil, fatty acids, and sugars), and seed quality (seed germination and seed damage).

FIGURE 1 Soybean cultivar P46A57BX response 6 d after the application of paraquat at R6 (7 Aug. 2019) to the two center rows of the plots

2 | MATERIALS AND METHODS

2.1 | Planting and field conditions

A field experiment was conducted in 2019 and 2020 in Stoneville, MS. The experiment was designed as a randomized complete block design with ten replications. Two recent maturity group IV commercial soybean cultivars (P46A57BX and P48A60X) (Pioneer brand products, 2020, St. Louis, MO) were used. The cultivars used in this study were selected based on their current use by growers in the Early Soybean Production System. The experiment was rain-fed. Plots were eight rows wide and 18.3 m long, with a row-spacing of 101.6 cm. Soybeans were planted on 1 May 2019 and 13 May 2020. Paraquat (Gramoxone SL 2.0; Syngenta) was applied at the higher rate recommended on the label for the use of paraquat as a harvest-aid (0.56 kg a.i. ha^{-1}) at growth stages R6, R6.5, or R7. These stages represent critical stages of seed-fill. Soybean without a paraquat application was used as the control. The application of paraquat should not exceed 0.56 kg a.i. ha^{-1} (Syngenta, 2016). A surfactant was tank mixed with the paraquat (1% of Fire Zone methylated seed oil; Syngenta). As the two cultivars had similar maturities, paraquat application for both cultivars was on 7 Aug. 2019 for R6; 22 Aug. 2019 for R6.5; and 5 Sept. 2019 for R7. For 2020, application dates were on 20 Aug. 2020 for R6; 24 Aug. 2020 for R6.5; and 11 Sept. 2020 for R7. To avoid weathering effects, a 1.52-m subsample of each of the two center rows of each plot was timely hand-harvested at full maturity (R8) for seed composition, germination, and seed damage analyses. Sampling at R8 in 2019 occurred on 6 Sept. for R6; 10 Sept. for R6.5; 17 Sept. for R7; and on 30 Sept. for R8 (control). Sampling at R8 in 2020 occurred on 14 Sept. for R6; 17 Sept. for R6.5;

21 Sept. for R7; and on 6 Oct. for R8 (control). The remainder of the center rows of each plot were harvested using a plot combine (Kincaid 8 XP) equipped with a load cell (Harvest Master H2 Grain Gauge) and moisture meter, allowing the harvest of the two center rows of each plot with their weights separately. Growth stages R6, R7, and R8 were classified as per Fehr and Caviness (1977) using samples of 10 random plants per plot at each staging. Plots were staged twice weekly as needed. When five or more of the sampled plants per plot were at a given stage, the plot was considered to be at that stage. Growth stage R6.5 was likewise estimated for all plots, except that Whiting et al. (1988) was used to estimate R6.5, which was when all pod cavities on the four uppermost nodes of the main stem were completely filled with seeds. Maximum and minimum air temperatures in 2019 and 2020, along with precipitation data, were obtained from Mississippi State University Extension, Delta Agricultural Weather Center at http://deltaweather.extension.msstate.edu/weather-station-result/.

2.2 | Seed germination, viability, hard-seed, and seed damage

Seed germination, viability, and hard seed were performed on 200 seeds by the State Seed Testing Laboratory, Mississippi State, MS. The test was conducted using the protocol of the Association of Official Seed Analysts (2001) and detailed by Smith et al. (2008) and Bellaloui et al. (2017). Seed damage grading was conducted using Federal Grain Inspection Service (2013) standards, as detailed by Bellaloui et al. (2017).

2.3 | Seed protein, oil, fatty acids, and sugars

Mature seeds were collected at R8 and were analyzed for protein, oil, fatty acid, and sugar (glucose, raffinose, and stachyose) contents. Seed composition constituents were analyzed with a Diode Array Feed Analyzer AD 7200 (Perten, Springfield, IL, USA). Briefly, seeds were ground by a Laboratory Mill 3600 (Perten) and approximately 25 g of ground seed were analyzed for protein, oil, and fatty acid contents according to (Bellaloui et al., 2009a, 2009b; Bellaloui et al., 2014; Wilcox & Shibles, 2001). Calibration equations were initially developed by the University of Minnesota and upgraded by the Perten company using Perten's Thermo Galactic Grams PLS IQ software. Equations were established based on AOAC methods (AOAC, 1990a, 1990b). Protein, oil, and sugars (glucose, raffinose, and stachyose) were expressed on a dry-matter basis (Bellaloui et al., 2009b; Bellaloui et al., 2010; Bellaloui et al., 2014; Boydak et al., 2002; Wilcox & Shibles, 2001); fatty acids palmitic, stearic, oleic, linoleic, and linolenic were expressed on a total-oil basis.

2.4 | Experimental design and statistical analyses

The experiment was set up as a randomized complete block design with 10 replications. Each block contained all treatments (two cultivars and all paraquat timing applications: R6, R6.5, R7, or control). An analysis of variance (ANOVA) was conducted using Proc Glimmix (SAS software, Version 9.4; SAS Institute). Year, cultivar, paraquat treatment at different stages, and their interactions were considered as fixed effects. Replicates within a year were considered as random effects. Mean comparisons were conducted by Fisher's protected least significant difference (LSD) test and the level of significance of $P \leq .05$ was used. Pearson correlation coefficients (R and P values) between soybean seed yield and seed quality components with days after planting (DAP) to R8 in 2019, 2020, and across years and across the two cultivars (cultivars P46A57BX and P48A60X) were conducted using Prism ver. 9.1.2 (GraphPad Software). Level of significance was $P \leq .05$.

3 | RESULTS

3.1 | Environmental conditions during each experiment year

Temperatures during the main critical stages of flowering, pod-set, and seed-fill coincide with June, July, August, and September (Table 1). It is clear that during these months, temperatures were different from month to month in each year and between years. For example, maximum and minimum temperatures in July were 32.7 and 22.0 ˚C, respectively, in 2019, compared with 33.7 and 22.8C, respectively, in 2020. However, for August, maximum and minimum temperatures were 34.2 and 22.2 ˚C, respectively, in 2019, compared with maximum and minimum temperatures of 32.5 and 20.8 ˚C, respectively, in 2020. Precipitation (mm) was higher in May, June, and July in 2019 than in 2020. However, in August and September, the precipitation was higher in 2020 than in 2019. Total precipitation in 2019 was higher than in 2020 and they were different in amount and pattern (Table 1).

3.2 | ANOVA for seed yield, germination, hard seed, viability, and damage

The ANOVA showed that year, cultivar, and treatment were the main factors affecting seed yield (Table 2). Also, year × treatment interactions were significant for yield. Cultivar, treatment, and year × treatment were significant for germination, hard seed, and viability (Table 2). Although year,

TABLE 1 Mean monthly maximum (Max.) and minimum (Min.) air temperatures (˚C), average temperatures, and mean monthly precipitation (Prec.) (mm) and total during the growing period of soybean in 2019 and 2020

	2019				2020			
Month	**Max.**	**Min.**	**Average**	**Prec.**	**Max.**	**Min.**	**Average**	**Prec.**
April	23.1	12.1	17.6	217.9	22.2	11.5	16.9	125.7
May	29.3	18.7	24.0	286.5	28.0	15.9	22.0	46.5
June	31.4	19.9	25.7	172.0	31.1	20.2	25.7	160.5
July	32.7	22.0	27.3	121.2	33.7	22.8	28.3	66.8
August	34.2	22.2	28.2	91.9	32.5	20.8	26.6	173.2
September	35.7	21.0	28.3	8.1	29.7	18.2	24.0	141.2
Total				897.6				714.0

[a]Data were obtained from Mississippi State University Extension. Delta Agricultural Weather Center at http://deltaweather.extension.msstate.edu/weather-station-result/.

TABLE 2 Analysis of variance (*F* and *P* values) for the effect of year, cultivar, and paraquat treatment at the R6, R6.5, or R7 growth stages, or R8 control, and their interactions on soybean seed yield, germination, hard seed, viability, and seed damage

		Yield		Germination		Hard seed		Viability		Seed damage (FGIS)	
Effect	**No. df**	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
		kg ha^{-1}		%							
Year (Y)	1	184	***	4.32	ns	4	ns	2.01	ns	95.23	***
Cultivar (C)	1	4.21	*	29.03	***	18.11	***	17.4	***	0.41	ns
Y × C	1	3.73	*	0.04	ns	0.52	ns	0.01	ns	1	ns
Treatment (T)	3	177	***	19.5	***	9.62	***	55.95	***	4.88	***
Y × T	3	21.8	***	19.29	***	16.15	***	39.91	***	6.06	***
C × T	3	0.43	ns	0.96	ns	0.71	ns	0.51	ns	1.38	ns
Y × C × T	3	1.45	ns	0.92	ns	1.86	ns	0.25	ns	1.05	ns
Residual		86179		145		35.34		87.44		0.14	

Note. The experiment was conducted at Stoneville, MS, in 2019 and 2020. FGIS, Federal Grain Inspection Service; ns, not significant.
*Significant at $P \leq .05$., **Significant at $P \leq .01$. ,***Significant at $P \leq .001$.

treatment, and year × treatment were significant for seed damage, all values for seed damage were lower than 2%, which is generally below the level at which damage results in discounting of payments to producers (i.e., damage >2% would result in discounting). The magnitudes of the *F* values for year and treatment effects on yield were substantially larger than that of cultivar, whereas the *F* values for cultivar and treatment were much larger than that of year on germination, hard seed, and viability. For seed damage, the *F* value of year was very large compared with those for cultivar or treatment.

3.3 | ANOVA of seed protein, oil, fatty acids, and sugars

The ANOVA showed that year, cultivar, and treatment (timing of paraquat application) were the main factors affecting seed protein, oil, and linoleic acid (Tables 3 and 4). Except for palmitic acid, all other seed composition constituents were significantly affected by treatment. Year × treatment interactions were significant for protein, oil, palmitic, stearic, and linoleic acids, and sugars (Tables 3 and 4). Cultivar × treatment was not significant for these seed composition constituents, except for oil. Year × cultivar × treatment interactions were significant for oil and stachyose. Because year interacted with cultivar and treatment for some seed components, means are presented by year and cultivar for each treatment (paraquat application stage) along with the LSD for treatment comparisons (Table 5). In terms of relative magnitudes of *F* tests (Table 3), application treatment had the largest effect on protein (82.7) and oil (256), but cultivar also had a very large effect (80.66) on oil. Cultivar also had the largest effect on palmitic (40.5) and oleic (9.82) acids, but year had the largest effect on stearic acid (68.4) (Table 3). Treatment had the largest effect on linolenic acid, sucrose, raffinose, and stachyose (Table 4), whereas year had the largest effect (86) on

TABLE 3 Analysis of variance (*F* and *P* values) for the effect of year, cultivar, and paraquat treatment at the R6, R6.5, or R7 growth stages, or R8 control, and their interactions on soybean seed protein, oil, and fatty acids palmitic, stearic, and oleic

Effect	df	Protein *F*	Protein *P*	Oil *F*	Oil *P*	Palmitic *F*	Palmitic *P*	Stearic *F*	Stearic *P*	Oleic *F*	Oleic *P*
		%									
Year (Y)	1	14.1	***	15.08	***	23.8	***	68.4	***	0	ns
Cultivar (C)	1	18.3	***	80.66	***	40.5	***	27.4	***	9.82	**
Y × C	1	1.49	ns	0	ns	*	ns	0.45	ns	1.09	ns
Treatment (T)	3	82.7	***	256	***	0.42	ns	7.93	***	122	***
Y × T	3	7.59	***	13.36	***	3.5	*	8.02	***	0.98	ns
C × T	3	0.15	ns	10.66	***	0.71	ns	0.19	ns	0.61	ns
Y × C × T	3	1.01	ns	3.18	*	0.46	ns	0.59	ns	0.16	ns
Residuals		0.662		0.2528		0.2914		0.0332		1.997	

Note. The experiment was conducted at Stoneville, MS, in 2019 and 2020. ns, not significant.
* Significant at $P \leq .05$. ** Significant at $P \leq .01$. *** Significant at $P \leq .001$.

TABLE 4 Analysis of variance (*F* and *P* values) for the effect of year (Y), cultivar (C), and paraquat treatment (T) at the R6, R6.5, or R7 growth stages, or R8 control, and their interactions on soybean seed linoleic acid, linolenic acid, sucrose, raffinose, and stachyose

Effects	df	Linoleic *F*	Linoleic *P*	Linolenic *F*	Linolenic *P*	Sucrose *F*	Sucrose *P*	Raffinose *F*	Raffinose *P*	Stachyose *F*	Stachyose *P*
		%				mg g^{-1}					
Year (Y)	1	86.0	***	20.4	***	0.39	ns	363	***	3.07	ns
Cultivar (C)	1	55.4	***	0.91	ns	19.9	***	0.61	ns	0.1	ns
Y × C	1	1.2	ns	9.5	**	0.03	ns	2.01	ns	1.27	ns
Treatment (T)	3	77.9	***	139	***	177	***	2494	***	149	***
Y × T	3	26.5	***	1.56	ns	8.15	***	1137	***	6.02	***
C × T	3	0.63	ns	0.45	ns	0.46	ns	0.68	ns	0.91	ns
Y × C × T	3	0.85	ns	1.14	ns	1.96	ns	0.52	ns	3.07	*
Residuals		1.308		0.3989		12.3247		0.1136		5.5714	

Note. The experiment was conducted at Stoneville, MS, in 2019 and 2020.
*Significant at $P \leq .05$.
**Significant at $P \leq .01$.
***Significant at $P \leq .001$.

linoleic acid, followed by treatment (77.9) and cultivar (55.4) (Table 4).

3.4 | Effects of application-timing on seed yield, germination, hard seed, viability, and damage

Results for seed yield and seed quality traits among paraquat applications within cultivars and years are shown in Table 5 and differences between applications and no application in Table 6. Soybean seed yield increased significantly on a continuum for both cultivars in 2019 as reproductive period was increased from R6 through R8 (Table 5). That is, reproductive period and seed yield were both increased by delaying paraquat application, as required for each application treatment. The same significant continuous yield increases were observed in 2020 for both cultivars, except that there was no significant change in seed yield between R7 and the control for P48A60X. In addition, significant increases in 100-seed weight (used as a measure for seed size) for both cultivars were observed in 2019 and 2020, as the length of the seed-filling period was increased by delaying the application of paraquat from R6 through the R8 control (Table 5). Hence, lengthening the time of seed fill through delaying the application of paraquat always resulted in significant increases in 100-seed weight, which were almost always associated with significant increases in seed yield. Conversely, shortening the seed-filling period through the application of paraquat always resulted in smaller seed weight, which was almost always

TABLE 5 Effect of paraquat[a] at the R6, R6.5, or R7 growth stages on soybean seed yield, seed quality, and seed composition constituents for cultivars P46A57BX and P48A60X in 2019 and 2020

	P46A57BX					P48A60X				
Variable	R6	R6.5	R7	Control	LSD	R6	R6.5	R7	Control	LSD
2019										
Yield, kg ha^{-1}	1815	2986	3565	3740	69.50	1858	3226	3848	4047	84.6
Germination, %	28.30	47.90	55.60	47.60	3.78	30.89	62.60	67.40	68.20	3.27
Hard seed, %	1.30	21.90	14.40	16.60	2.00	1.44	15.60	9.70	5.60	1.83
Viability, %	29.60	69.80	70.00	64.10	2.10	32.33	78.20	77.10	73.80	2.03
Seed damage (FGIS), %	1.19	0.98	0.42	0.62	0.11	1.02	0.48	0.51	0.75	0.16
100-seed weight, g	9.35	10.83	15.21	16.14	0.28	9.94	10.04	14.40	15.76	0.26
Protein, %	40.83	38.84	38.79	39.11	0.20	42.05	39.51	39.37	39.85	0.24
Oil, %	21.88	24.27	24.77	25.31	0.13	21.61	23.37	23.85	24.16	0.12
Palmitic, %	9.70	10.06	10.00	10.10	0.21	10.23	10.48	10.56	11.01	0.21
Stearic, %	4.24	4.53	4.53	4.59	0.06	4.35	4.65	4.71	4.81	0.07
Oleic, %	27.21	20.89	22.06	22.09	0.55	27.88	21.55	22.16	22.80	0.57
Linoleic, %	53.50	55.81	54.68	54.83	0.48	51.90	53.90	53.78	52.17	0.41
Linolenic, %	7.73	8.27	7.71	7.80	0.23	5.86	8.53	8.40	8.56	0.21
Sucrose, mg g^{-1}	21.29	34.59	39.20	35.24	1.02	21.06	38.52	41.62	40.10	1.04
Raffinose, mg g^{-1}	9.87	7.70	8.05	7.91	0.10	9.93	7.87	8.13	8.21	0.10
Stachyose, mg g^{-1}	44.30	32.16	33.94	34.11	0.86	46.05	32.40	33.91	33.56	0.65
2020										
Yield, kg ha^{-1}	1760	2101	2746	2963	111	1879	2201	2814	2701	187.3
Germination, %	50.29	68.63	57.50	34.33	5.35	66.50	81.50	63.50	51.80	7.50
Hard seed, %	11.29	5.88	12.33	11.17	2.69	4.66	3.33	9.67	7.20	1.99
Viability, %	61.57	74.50	69.83	45.50	4.90	71.17	84.83	73.17	59.00	6.62
Seed damage (FGIS), %	0.00	0.00	0.00	0.30	0.05	0.02	0.00	0.00	0.38	0.05
100-seed weight, g	8.33	9.66	13.37	14.79	0.20	8.09	9.45	12.94	14.28	0.14
Protein, %	41.09	37.21	38.50	39.30	0.40	41.25	37.68	39.41	39.54	0.30
Oil, %	20.94	24.60	25.04	24.27	0.27	21.10	24.08	23.17	23.25	0.21
Palmitic, %	10.76	10.84	10.58	10.62	0.15	11.61	11.20	11.28	11.20	0.16
Stearic, %	4.09	4.16	4.20	4.12	0.05	4.38	4.33	4.35	4.28	0.06
Oleic, %	26.76	20.93	21.32	22.00	0.34	27.63	22.68	21.85	23.38	0.37
Linoleic, %	53.21	58.35	59.08	58.55	0.26	51.86	57.12	57.67	57.18	0.31
Linolenic, %	4.96	8.35	7.42	7.63	0.18	4.98	7.98	7.35	7.10	0.21
Sucrose, mg g^{-1}	18.96	34.83	34.15	39.90	1.46	24.03	38.27	35.33	42.32	1.63
Raffinose, mg g^{-1}	17.31	7.37	7.80	7.47	0.14	17.46	7.25	7.60	7.52	0.17
Stachyose, mg g^{-1}	42.39	32.33	34.02	33.37	0.85	40.24	31.33	32.55	36.20	1.03

Note. The experiment was conducted at Stoneville, MS. FGIS, Federal Grain Inspection Service.

[a] Paraquat was applied at the concentration recommended by the label for the use of paraquat as a harvest-aid (0.56 kg a.i. ha^{-1}) at growth stages R6, R6.5, or R7. Soybean with no applied paraquat was used as the control. LSD = least significant difference test, significant at $P \leq .05$. Within each row, the difference between two values is statistically significant if it equals or exceeds the corresponding LSD value. FGIS includes grain damage due to multiple factors, including mold, heat, green seed, stink bug, and purple stain. A common level of grain damage that could result in discounting at grain elevators is the 2% level, meaning that damage > 2% would result in discounting of payments to producers.

associated with reduced seed yield. As would be expected, seed yield and 100-seed weight were highly correlated in 2019 ($R = 0.85$) and 2020 ($R = 0.97$) (data not shown).

Table 6 shows the difference between the means of each paraquat application timing (R6, R6.5, and R7) and the control (no paraquat application, R8) for both cultivars and both years for seed yield and seed quality traits. In 2019, for both cultivars, all applications of paraquat had a significant effect compared with no paraquat application on seed yield, 100-seed weight, hard seed, viability and seed damage (Table 6).

TABLE 6 Comparison between yield and seed quality parameters at each stage of paraquat application and the control (no paraquat application) using the respective LSD values shown in Table 5

	P46A57BX						P48A60X					
	Difference between control and respective application timing						Difference between control and respective application timing					
Variable	R6		R6.5		R7		R6		R6.5		R7	
2019												
Yield, kg ha^{-1}	1925	SIG	754	SIG	175	SIG	2189	SIG	821	SIG	199	SIG
100-seed weight, g	6.79	SIG	5.31	SIG	0.93	SIG	5.82	SIG	5.72	SIG	1.36	SIG
Germination, %	19.30	SIG	–0.30	ns	–8.00	SIG	37.31	SIG	5.60	SIG	0.80	ns
Hard seed, %	15.30	SIG	–5.30	SIG	2.20	SIG	4.16	SIG	–10.00	SIG	–4.10	SIG
Viability, %	34.50	SIG	–5.70	SIG	–5.90	SIG	41.47	SIG	–4.40	SIG	–3.30	SIG
Seed damage, %	–0.57	SIG	–0.36	SIG	0.20	SIG	–0.27	SIG	0.27	SIG	0.24	SIG
2020												
Yield, kg ha^{-1}	1203	SIG	862	SIG	217	SIG	822	SIG	500	SIG	–113	ns
100-seed weight, g	6.46	SIG	5.13	SIG	1.42	SIG	6.19	SIG	4.83	SIG	1.34	SIG
Germination, %	–15.96	SIG	–34.30	SIG	–23.17	SIG	–14.70	SIG	–29.70	SIG	–11.70	SIG
Hard seed, %	–0.12	ns	5.29	SIG	–1.16	ns	2.54	SIG	3.87	SIG	–2.47	SIG
Viability, %	–16.07	SIG	–29.00	SIG	–24.33	SIG	–12.17	SIG	–25.83	SIG	–14.17	SIG
Seed damage, %	0.30	SIG	0.30	SIG	0.30	SIG	0.36	SIG	0.38	SIG	0.38	SIG

[a]This is a control minus the value at each stage; negative values in the table mean that at that stage the trait value was greater than that at R8 (the control). ns, not significant; SIG, significant.

Only the difference in germination at R6.5 for P46A57BX and at R7 for P48A60X were not significantly different. Results were similar for these parameters in 2020 with only seed yield at R7 for P48A60X and hard seed at R6 and R7 for P46A57BX not showing significant differences at each paraquat application and the no-application control (Table 6).

For both cultivars and both years, the difference in seed yield between the time of paraquat application and the control sharply increased the earlier paraquat was applied. For 100-seed weight, for both cultivars and both years, the application of paraquat consistently showed that the control had greater seed weight than did that of each paraquat application. The trend for the other parameters was inconsistent either between application timing and/or between cultivars and years. Although, it is interesting that for 2020 for both cultivars, application timing always resulted in a significant increase in germination and a significant decrease in seed damage relative to the control (Table 6). The differences in effect between application timing and no paraquat application over years was likely influenced by differences in environmental conditions.

The cultivar × year × treatment combination that produced the highest seed germination was not consistent. However, it is noteworthy that in only one case (P48A60X in 2019) did untreated plots have the highest germination percentage (68.2%), and that was not significantly different from the R7 percentage (67.4%) of the same year and cultivar (Table 5 and Table 6). In 2019, the highest percentages of germination were achieved at R7 or later, but in 2020, the highest percentages of germination were achieved at R6.5 (Table 5 and Table 6).

Hard seed values were also variable across years, treatments, and cultivars, with values ranging from 1.3 to 21.9% in 2019 and from 3.3 to 12.3% in 2020 (Table 5 and Table 6). For hard seed in 2019, both cultivars had the lowest values for the R6 treatment, but the highest values for the R6.5 treatment. This was not true for 2020 and differed by cultivar. P46A57BX produced the most hard-seed in the R6, R7, and R8 treatments, but P48A60X produced the highest hard seed from the R7 treatment.

Seed viability represents the sum of seed germination and hard seed percentages and assumes that hard seed are alive. For all combinations of cultivar, treatment, and year, total seed viability was less than 80% in every instance, except one (P48A60X in 2020 had 84.3% viability) (Table 5 and Table 6), which illustrates the challenge of producing high-quality seed beans in the ESPS. Seed damage among treatments for both cultivars was significantly different, but not meaningful, as the Federal Grain Inspection Service (FGIS) standards seed damage rating was lower than 2% in all cases (Table 5 and Table 6). The FGIS includes grain damage due to multiple factors, including mold, heat, green seed, and stink bug. Grain elevators assess discounts on the value of grain delivered by soybean growers based on FGIS standards. This can result in a loss of revenue to growers when they sell their grain. A common level of grain damage that could result in discounting

TABLE 7 Comparison between protein, oil, fatty acids, and sugar parameters at each stage of paraquat application and the control (no paraquat application) using the respective LSD values shown in Table 5

	P46A57BX						P48A60X					
	Difference between control and respective application timing						Difference between control and respective application timing					
Variable	R6		R6.5		R7		R6		R6.5		R7	
2019												
Protein, %	−1.72	SIG	0.27	SIG	0.32	SIG	−2.20	SIG	0.34	SIG	0.48	SIG
Oil, %	3.43	SIG	1.04	SIG	0.54	SIG	2.55	SIG	0.79	SIG	0.31	SIG
Palmitic, %	0.40	SIG	0.04	ns	0.10	ns	0.78	SIG	0.53	SIG	0.45	SIG
Stearic, %	0.35	SIG	0.06	ns	0.06	ns	0.46	SIG	0.16	SIG	0.10	SIG
Oleic, %	−5.12	SIG	1.20	SIG	0.03	ns	−5.08	SIG	1.25	SIG	0.64	SIG
Linoleic, %	1.33	SIG	−0.98	SIG	0.15	ns	0.27	ns	−1.73	SIG	−1.61	SIG
Linolenic, %	0.07	ns	−0.47	SIG	0.09	ns	2.70	SIG	0.03	ns	0.16	ns
Sucrose, mg g^{-1}	13.95	SIG	0.65	ns	−3.96	SIG	19.04	SIG	1.58	SIG	−1.52	SIG
Raffinose, mg g^{-1}	−1.96	SIG	0.21	SIG	−0.14	SIG	−1.72	SIG	0.34	SIG	0.08	ns
Stachyose, mg g^{-1}	−10.19	SIG	1.95	SIG	0.17	ns	−12.49	SIG	1.16	SIG	−0.35	ns
2020												
Protein, %	−1.79	SIG	2.09	SIG	0.80	SIG	−1.71	SIG	1.86	SIG	0.13	ns
Oil, %	3.33	SIG	−0.33	SIG	−0.77	SIG	2.15	SIG	−0.83	SIG	0.08	ns
Palmitic, %	−0.14	ns	−0.22	SIG	0.04	ns	−0.41	SIG	0.00	ns	−0.08	ns
Stearic, %	0.03	ns	−0.04	ns	−0.08	SIG	−0.10	SIG	−0.05	ns	−0.07	SIG
Oleic, %	−4.76	SIG	1.07	SIG	0.68	SIG	−4.25	SIG	0.70	SIG	1.53	SIG
Linoleic, %	5.34	SIG	0.20	ns	−0.53	SIG	5.32	SIG	0.06	ns	−0.49	SIG
Linolenic, %	2.67	SIG	−0.72	SIG	0.21	SIG	2.12	SIG	−0.88	SIG	−0.25	SIG
Sucrose, mg g^{-1}	20.94	SIG	5.07	SIG	5.75	SIG	18.29	SIG	4.05	SIG	6.99	SIG
Raffinose, mg g^{-1}	−9.84	SIG	0.10	ns	−0.33	SIG	−9.94	SIG	0.27	SIG	−0.08	ns
Stachyose, mg g^{-1}	−9.02	SIG	1.04	SIG	−0.65	ns	−4.04	SIG	4.87	SIG	3.65	SIG

[a]This is a control minus the value at each stage; negative values in the table mean that at that stage the trait value was greater than that at R8 (the control). SIG = significant; ns = not significant.

at grain elevators is the 2% level, meaning that damage >2% could result in discounting of payments to growers. The timely harvest of all seed samples for all treatments just after R8 likely ensured that total seed damage was negligible and therefore not meaningful in this experiment.

3.5 | Effects of application-timing on seed protein, oil, fatty acids, and sugars

Results for seed composition traits among paraquat applications within cultivars and years is shown in Table 5, and differences between applications and no application is shown in Table 7, also within cultivars and years. Over both cultivars and both years, protein and oleic acid showed significant increases at R6 compared with the control, but significant decreases at R6.5 and R7 applications (Table 7). In 2019, oil showed significant increases at all application times over the control for both cultivars, but in 2020, there were significant decreases at the R6.5 and R7 applications for P46A57BX, as well as for the R6.5 application for P48A60X.

For palmitic and stearic acids, the differences between the control and the applications stages of paraquat were small even when significant (Table 7). In addition, differences with regard to increases or decreases compared with the controls were inconsistent between cultivars and between years (Table 7). Results for linoleic and linolenic acids were also inconsistent, although for both acids the strongest and most consistent difference was between the R6 application and the control (a significant decrease except for a nonsignificant decrease in linoleic acid in 2019 for P48A60X and linolenic acid in 2020 for P46A57BX). These inconsistencies may be the result of a combination of genetic differences and environmental factors.

Sucrose was significantly less than the control at the R6 application for both cultivars and both years, whereas raffinose and stachyose showed significant increases at R6 for both cultivars and both years. For sucrose in 2020, both cultivars also showed significant reductions at the R6.5 and R7 applications of paraquat. However, in 2019, both cultivars showed a significant increase at R7 (Table 7). Although raffinose and stachyose showed significant increases at R6 in both years, at R6.5 they either had significant increases or were not significantly different. Raffinose at R7 in both years showed a small but significant increase for 46A57BX, but for P48A60X, differences were not significant in either year. For stachyose at R7, only P48A60X in 2020 showed a significant difference with the control. For all three sugars (sucrose, raffinose, and stachyose), by far the greatest effects, compared with no paraquat application, was at the R6 paraquat application.

3.6 | Correlations between traits and with maturity

As expected, the application of paraquat hastened maturity and the DAP to maturity (R8) decreased the earlier the application of paraquat (Table 8). Within each year, data for the two cultivars were combined for the correlation analysis. DAP to R8 were 128, 132, 139, and 152 (corresponding to R6, R6.5, R7, and R8, respectively) in 2019; and 124, 127, 131, and 146 DAP to R8 in 2020. Among the traits, in 2019, yield ($R = 0.82$), 100-seed weight ($R = 0.92$), and oil ($R = 0.76$) were significantly correlated with DAP to R8 (Table 8). In 2020 yield ($R = 0.79$), 100-seed weight ($R = 0.88$), viability ($R = -0.72$), and seed damage ($R = 0.94$) were significantly correlated with DAP to R8. Sucrose ($R = 0.74$) was significantly correlated with DAP to R8 (Table 7). Oil was significantly correlated with DAP to R8 in 2019, but not in 2020, and sucrose was in 2020 but not in 2019. No other traits were significantly correlated with DAP to R8 in either year. Across years, yield ($R = 0.80$), oil ($R = 0.57$), sucrose ($R = 0.64$), and 100-seed weight ($R = 0.91$) were all significantly associated with DAP to R8 (Table 8).

4 | DISCUSSION

4.1 | General discussion

In this study we observed an increasing yield loss the earlier the paraquat was applied as a harvest aid for both cultivars and in both years; the largest decrease in yield was at R6. It was previously found that the application of glyphosate at five rates, from 0.56 to 3.36 kg a.i. ha^{-1}, 3 wk before soybean harvest decreased soybean yield (Azlin & McWhorter, 1981). Also, an application before physiological maturity was found to decrease seed weight (Clay & Griffin, 2000; Jeffery et al., 1981). In addition, it was reported that an application of a defoliant too early, such as at R5 or early R6, can result in significant yield losses (Ellis et al., 1998). Whigham and Stoller (1979) and Griffin et al. (2010) found that an application of paraquat 3 and 4 wk before harvest reduced soybean yield. Soybean yield was also reduced with paraquat applied at a rate of 0.840 kg a.i. ha^{-1} at the R5 and R6 growth stages, but not at R7 or R8 (Ratnayake & Shaw, 1992a; Ross & Barber, 2018). The reduced seed yield at the R6 application observed in our study is in agreement with previous research (Boudreaux & Griffin, 2011; Ellis et al., 1998; Griffin et al., 2010; Ratnayake & Shaw, 1992a, 1992b; Ross & Barber, 2018) but differs with those of Griffin et al. (2010) and Ratnayake and Shaw (1992a) in that seed yield in the current study was decreased from R6.5 or R7 applications. The disagreement in yield loss between our study and previous studies could be due to soybean cultivar, environmental growing conditions, and paraquat rate used. The decrease of yield at R7 application, for both years for cultivar P46A57BX and 1 yr (2019) for cultivar P48A60X, may be due to green pods still undergoing seed-fill, especially at the bottom of the canopy. Seed weight data, as estimated on 100-seed counts from timely hand-harvested plots at full maturity (R8) for each paraquat application, show that seed weight increased on a continuum from R6 to R8 for both cultivars in both years (Table 5). Therefore, when seed-fill is abruptly stopped, due to desiccants, seed yield declines as a function of a decrease in seed weight. As noted previously, 100-seed weight and seed yield were highly correlated in both 2019 ($R = 0.85$) and 2020 ($R = 0.97$). Hence, the application of paraquat as a defoliant based on growth stage determination according to Fehr and Caviness (1977) or Whiting et al. (1988) resulted in lower seed yield because some pods on plants were not yet fully mature (i.e., seed-fill was not complete) when sprayed. Using a price of US$523.86 per 1,000 kg (metric ton) of soybean (IndexMundi, 2022), our estimation, using Table 6, showed that a 1,000-ha farm would have lost over $390,000 in 2019 and this loss would have reached over $430,000 in 2020 with either cultivar if the farmer had sprayed paraquat at R6.5. If the crop had been sprayed at R6, the potential loss could have reached over a million dollars. Therefore, growers should exercise caution in applying defoliants when some pods on the plant are still green even if the stage rating is R7. They should weigh their options of applying a defoliant for increased harvest efficiency, but with possible yield loss, against greater yield with less harvest efficiency. However, waiting to spray until R7.5 could still provide complete harvest efficiency, but with much greater probability of full yield.

The seed germination, hard seed, and viability data indicate that these two cultivars may not be suitable for producing seed beans in the midsouthern United States, as seed laws in Mississippi require seed beans to have at least 80% germination to be certified (Keith & Delouche, 1999). The difference in seed germination and viability between years may be due to the differences in the environmental conditions of each year, especially heat, as 2019 was hotter than 2020 in

TABLE 8 Pearson correlation coefficients (*R* values and level of significance) between DAP to R8, seed yield, and other seed components in 2019 and 2020 across two cultivars (cultivars P46A57BX and P48A60X)

2019	DAP to R8	Yield	Protein	Oil	P	S	O	L	Lino	Suc	Raff	Stac	Germ	HS	V	SD
Yield	0.82*															
Protein	–0.47	–0.77*														
Oil	0.76*	0.87*	–0.90*													
P	0.51	0.62	–0.07	0.20												
S	0.71	0.92*	–0.58	0.66	0.85*											
O	–0.47	–0.82*	0.94*	–0.86*	–0.32	–0.75*										
L	0.02	0.25	–0.79*	0.62	–0.47	0.02	–0.67									
Lino	0.40	0.68	–0.75*	0.56	0.37	0.67	–0.76*	0.41								
Suc	0.61	0.95*	–0.82*	0.80*	0.59	0.91*	–0.90*	0.34	0.76*							
Raff	–0.53	–0.85*	0.93*	–0.89*	–0.35	–0.77*	1.00*	–0.64	–0.73*	–0.90*						
Stac	–0.54	–0.87*	0.92*	–0.86*	–0.42	–0.81*	0.99*	–0.57	–0.81*	–0.93*	0.99*					
Germ	0.56	0.90*	–0.64	0.62	0.77*	0.96*	–0.77*	0.08	0.74*	0.96*	–0.78*	–0.84*				
HS	0.20	0.50	–0.85*	0.75*	–0.08	0.38	–0.87*	0.87*	0.49	0.59	–0.87*	–0.81*	0.39			
V	0.52	0.90*	–0.83*	0.78*	0.58	0.90*	–0.94*	0.40	0.77*	0.98*	–0.94*	–0.97*	0.94*	0.69		
SD	–0.47	–0.79*	0.64	–0.66	–0.40	–0.69	0.69	–0.24	–0.43	–0.84*	0.71*	0.71*	–0.78*	–0.47	–0.80*	
SW	0.92*	0.85*	–0.55	0.80*	0.40	0.66	–0.50	0.11	0.33	0.68	–0.54	–0.54	0.59	0.23	0.55	–0.62
2020																
Yield	0.79*															
Protein	–0.08	–0.29														
Oil	0.38	0.64	–0.89*													
P	–0.24	–0.29	0.32	–0.50												
S	–0.17	0.01	0.07	–0.16	0.89*											
O	–0.38	–0.69	0.86*	–0.95*	0.47	0.15										
L	0.51	0.77*	–0.80*	0.95*	–0.54	–0.21	–0.98*									
Lino	0.37	0.58	–0.94*	0.93*	–0.32	–0.05	–0.95*	0.92*								
Suc	0.74*	0.77*	–0.66	0.78*	–0.10	0.15	–0.77*	0.81*	0.84*							
Raff	–0.54	–0.75*	0.83*	–0.91*	0.29	–0.02	0.95*	–0.95*	–0.96*	–0.93*						
Stac	–0.28	–0.60	0.88*	–0.88*	0.16	–0.15	0.91*	–0.86*	–0.96*	–0.81*	0.93*					
Germ	–0.67	–0.44	–0.40	0.05	0.53	0.62	–0.03	–0.11	0.17	–0.05	–0.05	–0.28				
HS	0.29	0.45	0.23	0.10	–0.71*	–0.61	–0.17	0.27	–0.10	–0.12	–0.02	0.15	–0.75*			
V	–0.72*	–0.40	–0.41	0.09	0.44	0.57	–0.08	–0.05	0.18	–0.09	–0.06	–0.29	0.98*	–0.61		
SD	0.94*	0.54	0.10	0.14	–0.09	–0.14	–0.11	0.24	0.15	0.58	–0.31	–0.04	–0.65	0.12	–0.74*	
SW	0.88*	0.97*	–0.21	0.57	–0.35	–0.11	–0.62	0.72*	0.50	0.75*	–0.69	–0.46	–0.58	0.51	–0.55	0.68

Note. The experiment was conducted in Stoneville, MS. DAP, days after planting; Germ, germination; HS, hard-seed; L, linoleic; Lino, linolenic; O, oleic; P, palmitic; S, stearic; Raf, raffinose; SD, seed damage; Stac, stachyose; SW, 100-seed weight; Suc, sucrose; V, viability.

*Significant correlations at the .05 probability level.

August and September (coinciding with seed-fill stage), and both years differed in amounts and patterns of rainfall and heat (Table 1). The most critical temperature differences for seed germination were likely during seed fill in August and September, but especially in September during dry down and maturation. This needs further study and should be of interest to those who produce seed beans for producers, but it appears that optimum seed germination may be achieved with a desiccant applied at R6.5 or R7, although the seed was smaller. This may be true because pods generally do not mature uniformly and those that mature first may experience weathering before the entire plot is sufficiently dry for harvest. However, application of paraquat at R6 never produced the highest seed germination percentage in any year, treatment, or cultivar in this study (Table 5 and Table 6).

The higher seed protein and oleic acid, as well as the lower oil and linolenic acid, observed in the current research, could also be due to the paraquat application. This shift to an increase in protein and oleic acid, may have led to a decrease of oil and linolenic acid, as the relationships between protein and oil, as well as between oleic acid and linolenic acid, are inversely related (Bellaloui & Mengistu, 2008; Bellaloui et al., 2008; Bellaloui et al., 2009a, 2009b; Burton, 1985).

Information on the effects of paraquat, or herbicides in general, as a defoliant on seed composition is almost nonexistent, and what is available is not well established or is not consistent. For example, an experiment was conducted to investigate the effects of harvest-aid [paraquat (1,1 '-dimethyl-4,4′ -bipyridinium ion) at 0.6 and 1.1 kg ha^{-1} at 2, 3, and 4 wk before harvest date on seed composition (Whigham & Stoller, 1979). They found that the application of harvest-aid paraquat resulted in reduced soybean seed oil content when applied 3 and 4 wk before harvesting. However, there were no seed oil differences between soybean applied 2 wk before harvest and the nonapplied soybean. This pattern was observed at both rates, although ametryn applied 4 wk before harvest date resulted in lower oil content compared with the other treatments. On the other hand, the application of these three harvest-aids resulted in higher seed protein content at both rates when the treatments were applied 3 and 4 wk before harvest date. Application of harvest-aids 2 wk before harvest date resulted in significantly higher seed protein in only ametryn. They indicated that the increase of protein content was greater than the decrease in percent oil content for similar treatments. A recent experiment was conducted to investigate the effects of paraquat (at a rate of 0.28 kg a.i. ha^{-1}), carfentrazone-ethyl, glyphosate, and sodium chlorate ($NaClO_3$) on seed protein, oil, fatty acids, sugars, and amino acids in soybean when defoliants were applied at the R6 (seed-fill) or R7 (yellow pods) growth stages (Bellaloui et al., 2020). They found that the application of paraquat, paraquat + carfentrazone-ethyl, $NaClO_3$, and carfentrazone-ethyl at R6 resulted in an increase of protein, but the application of carfentrazone-ethyl or glyphosate resulted in higher oleic acid. They also found that the effect of harvest-aids was dependent on the type of harvest-aid and the growth stage of the plant. Our current findings show that paraquat application resulted in higher protein and oleic acid, agreeing with those of Whigham and Stoller (1979) and Bellaloui et al. (2020) but disagreeing with those of Bellaloui et al. (2020), who found oil either did not change or increased.

The data indicated a decrease in sucrose and an increase in raffinose and stachyose at R6 compared with when paraquat was applied at R6.5 or R7, or the nontreated control, may be due to the differential stress response of the plants to paraquat (chemical stress) at the different stages, affecting sugar hydrolysis enzymes and carbon flux and metabolism. It was reported that resources available to a developing seed are limited by the supply and form of exudates from the maternal plant such as sugars (sucrose, glucose, fructose) (Allen et al., 2009; Kambhampati et al., 2021). One can expect alteration of the carbon pathway as a result of paraquat application and the desiccation process. No previous information on the effects of paraquat on seed sugars is available, except for one recent study that showed a decrease in all sugars when paraquat was applied at R6 (Bellaloui et al., 2020). Our results showed that sucrose decreased, agreeing with the results of Bellaloui et al. (2020), but both raffinose and stachyose increased, disagreeing with the results of Bellaloui et al. (2020). The disagreement of the findings could be due to differences in paraquat rates used, cultivars, planting dates, or locations (Valentine et al., 2017), as well as the amount and pattern of rainfall and heat in each year (Bellaloui et al., 2020) as biotic and abiotic factors can increase the severity and the stress response to paraquat. Reports of increases in raffinose and stachyose in response to stress were previously reported, although stress protection from these sugars is not their exclusive role in plants (Sengupta et al., 2017). For example, it was reported that raffinose and stachyose play a role in protecting plants from different stresses, including drought (Wang et al., 2009), seed desiccation (Koster & Leopold, 1988), cold (Zuther et al., 2004), reactive oxygen species (Nishizawa et al., 2008), and carbohydrates partitioning during stress (ElSayed et al., 2014). It has been reported that the drying of immature seeds increases the accumulation of raffinose and stachyose, and that the relationship between seed stachyose content and desiccation tolerance is positive (Blackman et al., 1992). The increase of raffinose and stachyose could be due to conversion of sucrose to raffinose and stachyose as a stress response to provide protection against chemical stress that prematurely desiccates the seed.

The effects of the harvest-aid paraquat on seed weight and seed composition constituents could be also explained as a response to source-sink alterations. Kambhampati et al. (2021) studied the changes of seed protein, oil, and sugars with seed development. They found that there was no significant decrease in total protein accumulation between R7 and R8, while oil content significantly decreased. On the other hand, sucrose accumulation was highest at R7 and remained high at that level until maturity. Raffinose and stachyose accumulated between R6 and R7 to a maximum level. Kambhampati et al. (2021) found that sucrose can be a significant carbon source through R6, after which it decreases, possibly due to raffinose and stachyose production in the seed coat at stages R7 and R7.5. Thus, the decrease of total oil and increase of oleic acid could be due to the negative effects of harvest-aid paraquat application at R6–R7 and the alteration effects of paraquat on carbon pathway metabolism and carbon flux, desaturase fatty acid enzymes, and translocation or redistribution of fatty acids. Another possibility is that paraquat, as a chemical stress factor like other herbicides, such as carfentrazone-ethyl, glyphosate, and NaClO3, could have inhibited the enzyme 5-enolpyruvyl shikimate-3-phosphate synthase (EC 2.5.1.19), resulting in the increases of shikimic acid (Amrhein et al., 1980), deregulation of carbon flow into the shikimic acid pathway (Jensen, 1985), decreased photosynthesis and nutrient availability, and the alteration of seed protein, oil and fatty acids, and carbon metabolism, as suggested by others using other herbicides such as glyphosate (Bellaloui et al., 2008).

The responses of yield loss, reduced seed weight (seed dry matter), and changes in protein, oil, fatty acids, and sugars under stress, including heat, drought, and chemicals, might be also explained in terms of alterations in carbon metabolic pathways and their effects on source-sink relationships. The resources available to a developing seed are limited by the supply and form of exudates from the maternal plant, such as sugars (sucrose, glucose, fructose), and amino acids (glutamine, asparagine, alanine) (Allen et al., 2009; Kambhampati et al., 2021). Seed composition constituents such as protein and oil are produced from the available assimilates and flux through enzymatic reactions and metabolic pathways (Allen & Young, 2013). The levels of these primary assimilates (such as sucrose, glucose, fructose, amino acids, and organic acids) decline with plant development, while their storage components (such as protein, oil, raffinose, and stachyose), and cell wall polysaccharides increase (Collakova et al., 2013; Kambhampati et al., 2021; Li et al., 2015). The levels of these constituents are mainly reported on a per gram basis. However, because the content of storage components, such as protein, oil, raffinose, and stachyose (considered “inactive/inert pools”), in developing seeds increases, the primary metabolite pools are diluted (Kambhampati et al., 2021). Therefore, it was suggested that metabolite levels must account for dilution due to reserve accumulation of storage components (Kambhampati et al., 2021). The desiccation/drying processes during plant maturation involve enzyme activities and gene expression levels (Angelovici et al., 2010), which determine the final reserve composition. Once the supply of exogeneous assimilates/substrates from the maternal plant decrease, sources of carbon necessary for biosynthetic pathways of the seed are needed (Angelovici et al., 2010; Baud & Graham, 2006).

It appears that paraquat application at R6, R6.5, and R7 resulted in source-sink alterations, limiting the carbon pathway and the carbon flow into seeds, and decreasing seed oil concentration and content. Imposing a stress treatment (paraquat) at late reproductive stages resulted in oil deceases and protein increase. This agrees with the findings of Rotundo and Westgate (2009) related to water stress, who reported that water stress resulted in an increase of seed protein content when water stress was imposed at R1–R5, due to the increase in source/sink ratio resulting from seed number per plant when stress was imposed at R1–R8 (Borras et al., 2004). It was reported that quantifying seed protein and oil in terms of concentration and not content provides little or no insight into the physiological mechanisms underlying seed quality metabolism (Rotundo & Westgate, 2009). Therefore, including seed weight in the studies would significantly account for the variations in seed quality traits during the seed-fill period (Carrera et al., 2021). Further, Rotundo and Westgate (2009) showed an increase in seed size associated with an increase in protein and decrease in oil. Our research showed that seed weight increased on a continuum from R6 to R6.5 to R7 to R8 for both cultivars in both years, and seed yield declined as a function of a decrease in seed weight. Our results showed that seed weight (100 seed-weight) and seed yield (kg ha^{-1}) increased as seed protein decreased and oil seed increased, agreeing with the well-known inverse relationship between protein and yield (Helms & Orf, 1998; Mourtzinis et al., 2017). The relationship between seed composition constituents and seed weight has not been comprehensively analyzed (Carrera et al., 2021) and could be highly complex, being associated with genotypic and environmental interactions (Carrera et al., 2021).

4.2 | Correlations of seed yield, composition, and seed damage with DAP to R8

As expected, when applying paraquat before the crop fully matured, the maturity (R8) of the crop was hastened relative to no defoliant application. In all comparisons of associations of traits, except for the control versus R7 application in 2020 for P48A60X, seed yield was significantly reduced the earlier the paraquat was applied (R6 < R6.5 < R7 < Control; Table 5 and Table 7. A strong positive correlation between increasing yield and DAP to R8 in both years individually, as well as over years, was observed (Table 8). This relationship was also reflected in the strong positive correlation between 100-seed weight and DAP to R8 in both years. This indicated that pre-R8 applications of paraquat reduced seed yield by reducing seed weight. By the time paraquat was applied (R6 was at the earliest in this study), the seed number was fixed, as the plants were well past pod set, and the seed filling period was shortened, which was the principal means of yield loss. It is interesting to note that there was also a strong positive correlation with oil content in 2019, although no significant correlation was shown in 2020. However, in neither years nor over years was there a significant correlation with protein.

In 2020, and over the 2 yr, there was a significant positive relationship between sucrose levels and DAP to R8. Also in 2020, but not in 2019, there was a significant relationship between seed viability and seed damage and DAP to R8. It must also be noted here that protein, oleic acid, raffinose, and stachyose were consistently and negatively (not significantly at the $P < .05$ threshold, but some at the $P < .10$ threshold) correlated with DAP to R8 in each year. This negative correlation may support the consistent decrease in the accumulation of these constituents with the increasing delay of paraquat application from R6 to R8 (Table 5). Hymowitz et al. (1972) showed, using 60 selected lines from maturity groups 00 through IV, that the total sugar content positively correlated with oil and that each (sugar or oil) was negatively correlated with protein content. They also found that sucrose and raffinose content were positively correlated with oil content; however, stachyose content was positively correlated with protein.

The significant relationships observed in 1 yr and not in the other year could be the result of the differences in temperature and rainfall (Table 1) between the years during the period from R6 to maturity (August-September) and their effects on the underlying biochemical processes during the final stages of seed filling. Our results are in agreement with those of Wolf et al. (1982), Maestri et al. (1998), Piper and Boote (1999), Zhang et al. (2005), Dardanelli et al. (2006), and Bellaloui et al. (2009a, 2009b, 2010, 2015), who reported that the pattern of seed composition constituents differ in each year, depending on the environmental factors, including temperature and rainfall.

It is clear that seed chemical composition is a result of complex interactions between seed inherited characteristics and the environment (Aguirrezábal et al., 2015). Therefore, further research is needed to establish an understanding about the physiological mechanisms occurring during the seed filling period under the effect of environmental variables such as temperature, water, nutrient availability, radiation, genotype, and/or management practices on soybean seed chemical composition (Carrera et al., 2021).

5 | CONCLUSIONS

Our research showed that application of paraquat at R6 or R6.5 resulted in yield loss across both years and cultivars with increasingly severe yield losses the earlier paraquat was applied. Our estimation indicated that a 1,000-ha farm would have lost over \$390,000 in 2019 and this loss would have reached over \$430,000 in 2020 with either cultivar if a farmer had sprayed paraquat at R6.5. Application of paraquat at R7 resulted in lower yield over 2 yr for cultivar P46A57BX and in 1 yr for cultivar P48A60X. As there was not uniform yield loss when application occurred at R7, producers may weigh their R7 options between increased harvest efficiency with a potential for yield loss versus less harvest efficiency with the potential for more yield. Growers may want to consider the more conservative option of spraying later at R7.5, which would still provide all the harvest efficiency benefits but with a greater probability for achieving full yield potential. The potential for mature seed damage and price discounts at the elevator should also be considered, although seed damage was not a relevant issue in the current study. If paraquat is applied before full-seed maturity and complete translocation of metabolites to seeds, yield losses may be expected. Application of paraquat at R6 or R6.5 resulted in higher protein and oleic acid and lower oil compared with the control. Applying paraquat at R6 resulted in lower sucrose and higher raffinose and stachyose in both cultivars in both years. Higher oleic acid in seeds contribute to oil stability and shelf-life. Lower seed sucrose is not desirable as it contributes to improved taste and flavor. Higher raffinose and stachyose (oligosaccharides: raffinose and stachyose) are desirable for protecting plants from different stresses, including drought, seed desiccation, cold, and reactive oxygen species. However, high levels of raffinose and stachyose are not desirable for end users as they decrease the quality of soy meal by lowering its digestibility by humans and animals, causing flatulence or diarrhea in nonruminants. Whether or not the actual levels of these sugars increase the digestibility and uptake of other nutrients than what already exists remains to be investigated. Yield loss should be a concern to growers. However, as there is currently no price premium given on commodity soybeans for seed composition, individual growers may be less affected by alterations in composition caused by premature application of desiccants.

In spite of the clear findings from the current research, the sample size of cultivars was very small, and it cannot be assumed that all soybean cultivars will respond in the same way to these treatments. It is clear that seed composition changes in response to the harvest-aid paraquat are the result of complex interactions between inherited seed characteristics and the environment (Aguirrezábal et al., 2015). Further research is still needed to understand the physiological mechanisms involved during seed filling when seed fill is prematurely shortened by desiccating the plants (Carrera et al., 2021). Therefore, before conclusive recommendations are made that are broadly applicable, additional research, using a broader range of cultivars across years and across locations, is needed.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

Nacer Bellaloui: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Software; Supervision; Validation; Writing – original draft; Writing – review & editing. James R. Smith: Conceptualization; Data curation; Investigation; Methodology; Resources; Validation; Writing – original draft; Writing – review & editing. Jeffery D. Ray: Conceptualization; Formal analysis; Investigation; Methodology; Resources; Software; Validation; Writing – original draft; Writing – review & editing. Alemu Mengistu: Investigation; Methodology; Validation; Writing – review & editing. Anne M. Gillen: Methodology; Resources; Validation; Writing – review & editing. Daniel K. Fisher: Investigation; Methodology; Resources; Validation; Writing – review & editing. Gurbir Singh: Investigation; Methodology; Resources; Software; Validation; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Allen, D. K., Ohlrogge, J. B., & Shachar-Hill, Y. (2009). The role of light in soybean seed filling metabolism. *Plant Journal*, *58*, 220–234. https://doi.org/10.1111/j.1365-313X.2008.03771.x

Allen, D. K., & Young, J. D. (2013). Carbon and nitrogen provisions alter the metabolic flux in developing soybean embryos. *Plant Physiology*, *161*, 1458–1475. https://doi.org/10.1104/pp.112.203299

Amrhein, N., Deus, B., Gehrke, P., & Steinrucken, H. C. (1980). The site of the inhibition of the shikimate pathway by glyphosate. *Plant Physiology*, *66*, 830,834. https://doi.org/10.1104/pp.66.5.830

Angelovici, R., Galili, G., Fernie, A. R., & Fait, A. (2010). Seed desiccation: A bridge between maturation and germination. *Trends in Plant Science*, *15*, 211–218. https://doi.org/10.1016/j.tplants.2010.01.003

Aguirrezábal, L., Martre, P., Pereyra-Irujo, G., Echarte, M. M., & Izquierdo, N. (2015). Improving grain quality: Ecophysiological and modeling tools to develop management and breeding strategies. *Crop Physiology*, 423–65. https://doi.org/10.1016/B978-0-12-417104-6.00017-0

AOAC. (1990a). Method 988.05. In K. Helrich (Ed.), *Official methods of analysis* (15th ed.), AOAC.

AOAC. (1990b). Method 920.39. In K. Helrich (Ed.), *Official methods of analysis* (15th ed). AOAC.

Association of Official Seed Analysts. (2001). *Rules for testing seeds*. Association of Official Seed Analysts.

Azlin, W. R., & McWhorter, C. G. (1981). Preharvest effects of applying glyphosate to soybeans (*Glycine max*). *Weed Science*, *29*, pp. 123–127. https://doi.org/10.1017/S0043174500025972

Baud, S., & Graham, I. A. (2006). A spatiotemporal analysis of enzymatic activities associated with carbon metabolism in wild-type and mutant embryos of Arabidopsis using in situ histochemistry. *Plant Journal*, *46*, 155–169. https://doi.org/10.1111/j.1365-313X.2006.02682.x

Bellaloui, N., Abbas, H. K., Gillen, A. M., & Abel, C. A. (2009a). Effect of glyphosate-boron application on seed composition and nitrogen metabolism in glyphosate-resistant soybean. *Journal of Agricultural Food Chemistry*, *57*, 9050–9056. https://doi.org/10.1021/jf901801z

Bellaloui, N., Bruns, H. A., Abbas, H. K., Fisher, D. K., & Mengistu, A. (2020). Effects of Harvest-Aids on Seed Nutrition in Soybean under Midsouth USA Conditions. *Plants*, *9*, pages, 1–16. 1007; https://doi.org/10.3390/plants9081007

Bellaloui, N., & Mengistu, A. (2008). Seed composition is influenced by irrigation regimes and cultivar differences in soybean. *Irrigation Science*, *26*, 261–268. https://doi.org/10.1007/s00271-007-0091-y

Bellaloui, N., Mengistu, A., & Paris, R. L. (2008). Soybean seed composition in cultivars differing in resistance to charcoal rot (*Macrophomina phaseolina*). *Journal of Agriculture Sciences*, *146*, 667–675.

Bellaloui, N., Mengistu, A., Zobiole, L. H. S., & Shier, W. T. (2012). Resistance to toxin-mediated fungal infection: Role of lignins, isoflavones, other seed phenolics, sugars, and boron in the mechanism of resistance to charcoal rot disease in soybean. *Toxin Reviews*, *31*, 16–26. https://doi.org/10.3109/15569543.2012.691150

Bellaloui, N., Mengistu, A., Walker, E. R., & Young, L. D. (2014). Soybean seed composition as affected by seeding rates and row spacing. *Crop Science*, *54*, 1782–1795. https://doi.org/10.2135/cropsci2013.07.0463

Bellaloui, N., Smith, J. R., Gillen, A. M., & Ray, J. D. (2010). Effect of maturity on seed sugars as measured on near-isogenic soybean (*Glycine max*) lines. *Crop Science*, *50*, 1978–1987. https://doi.org/10.2135/cropsci2009.10.0596

Bellaloui, N., Smith, J. R., & Mengistu, A. (2017). Seed nutrition and quality, seed coat boron and lignin are influenced by delayed harvest in exotically-derived soybean breeding lines under high heat. *Frontiers in Plant Science*, *8*, 1–16. https://doi.org/10.3389/fpls.2017.01563

Bellaloui, N., Smith, J. R., Ray, J. D., & Gillen, A. M. (2009b). Effect of maturity on seed composition in the early soybean production system as measured on near-isogenic soybean lines. *Crop Science*, *49*, 608–620. https://doi.org/10.2135/cropsci2008.04.0192

Blackman, S. A., Obendorf, R. L., & Leopold, A. C. (1992). Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. *Plant Physiology*, *100*, 225–230. https://doi.org/10.1104/pp.100.1.225

Borras, L., Slafer, G. A., & Otegui, M. (2004). Seed dry weight response to source-sink manipulations in wheat, maize and soybean: A quantitative reappraisal. *Field Crops Research*, *86*, 131–146. https://doi.org/10.1016/j.fcr.2003.08.002

Boudreaux, J. M., & Griffin, J. L. (2008). Harvest aids in indeterminate and determinate soybeans-application timing and value. *Louisiana Agriculture*, *51*, 26–27.

Boudreaux, J. M., & Griffin, J. L. (2011). Application timing of harvest aid herbicides affects soybean harvest and yield. *Weed Technology*, *25*, 38–43. https://doi.org/10.1614/WT-D-10-00045.1

Boydak, E., Alpaslan, M., Hayta, M., Gerçek, S., & Simsek, M. (2002). Seed composition of soybeans grown in the Harran region of Turkey as affected by row spacing and irrigation. *Journal of Agricultural Food Chemistry*, *50*, 4718–4720. https://doi.org/10.1021/jf0255331

Burton, J. W. (1985). Breeding soybean for improved protein quantity and quality. In R. Shibles (Ed.), *World Soybean Research Conference* III: *Proceedings*, Boulder, CO (pp. 361–367). Westview Press.

Carrera, C. S., Salvagiotti, F., & Ciampitti, I. A. (2021). Benchmarking Nutraceutical Soybean Composition Relative to Protein and Oil. *Frontiers in Nutrition*, *8*, Article 663434, pages 1–11. https://doi.org/10.3389/fnut.2021.663434

Clay, P. A., & Griffin, J. L. (2000). Weed seed production and seedling emergence responses to late-season glyphosate applications. *Weed Science*, *48*, 481–486. https://doi.org/10.1614/0043-1745(2000)0480481:WSPASE2.0.CO;2

Collakova, E., Aghamirzaie, D., Fang, Y., Klumas, C., Tabataba, F., Kakumanu, A., Myers, E., Heath, L. S., & Grene, R. (2013). Metabolic and transcriptional reprogramming in developing soybean (*Glycine max*). *Embryo Metabolites*, *3*, 347–372. https://doi.org/10.3390/metabo3020347

Dardanelli, J. L., Balzarini, M., Martinez, M. J., Cuniberti, M., Resnik, S., Ramunda, S. F., Herrero, R., & Baigorri, H. (2006). Soybean maturity groups, environments, and their interaction define mega-environments for seed composition in Argentina. *Crop Science*, *46*, 1939–1947. https://doi.org/10.2135/cropsci2005.12-0480

Ellis, J. M., Shaw, D. R., & Barrentine, W. L. (1998). Herbicide combinations for preharvest desiccation in early-maturing soybean (Glycine max). *Weed Technology*, *12*, 57–165. https://doi.org/10.1017/S0890037X00042731

ElSayed, A. I., Rafudeen, M. S., & Golldack, D. (2014). Physiological aspects of raffinose family oligosaccharides in plants: Protection against abiotic stress. *Plant Biology*, *16*, 1–8. https://doi.org/10.1111/plb.12053

Federal Grain Inspection Service. (2013). Grain inspection handbook book: II. Soybean. https://www.gipsa.usda.gov/fgis/handbook/grain-insp/grbook2/corn.pdf

Fehr, W. R., & Caviness, C. E. (1977). Stages of soybean development. Iowa Agric. Exp. Station. *Special Report 80*. *Iowa State University Cooperative Extension Service*. Ames, IA, USA.

Gramoxone SL 2.0. (2016). *Group 22 herbicides*. Syngenta.

Grichar, W. J., Dotray, P. A., & Langham, D. R. (2020). Effects of harvest aids on sesame (*Sesamum indicum* L.) drydown and maturity. *IntechOpen*, Pages 1–27. DOI: https://doi.org/10.5772/intechopen.91011

Griffin, J. L., Boudreaux, J. M., & Miller, D. K. (2010). Herbicides as harvest aids. *Weed Science*, *58*, 355–358. https://doi.org/10.1614/WS-09-108.1

Heatherly, L. G. (1999). Early Soybean Production System (ESPS). In L. G. Heatherly & H. F. Hodges (Eds.) *Soybean Production in the Midsouth*. Boca Raton, FL, USA: CRC Press. pp. 103–118.

Heatherly, L. G. (2018). Harvest aids and soybean seed quality. *Mississippi Soybean Promotion Board (MSPB)*, https://www.mssoy.org/article/harvest-aids-and-soybean-seed-quality Verified on July 19, 2021

Helms, T. C., & Orf, J. H. (1998). Protein, oil, and yield of soybean lines selected for increased protein. *Crop Science*, *38*, 707–711. https://doi.org/10.2135/cropsci1998.0011183X003800030015x

Hou, A., Chen, P., Alloatti, J., Li, D., Mozzoni, L., Zhang, B., & Shi, A. (2009). Genetic variability of seed sugar content in worldwide soybean germplasm collections. *Crop Science*, *49*, 903–912. https://doi.org/10.2135/cropsci2008.05.0256

Hymowitz, T., Collins, F. I., Panczar, J., & Walker, W. M. (1972). Relationship between the content of oil, protein and sugar in soybean seed. *Agronomy Journal*, *64*, 613–616.

IndexMundi. (2022). Commodity prices, soybeans. https://www.indexmundi.com/commodities/?commodity=soybeans Verified 21, 2022

Jeffery, L. S., English, J. R., & Connell, J. (1981). The effects of fall application of glyphosate on corn (*Zea mays*), soybeans (*Glycine max*), and johnsongrass (*Sorghum halepense*). *Weed Science*, *29*, 190–195. https://doi.org/10.1017/S0043174500061786

Jensen, R. A. (1985). The shikimate/arogenate pathway: Link between carbohydrate metabolism and secondary metabolism. *Physiologia Plantarum*, *66*, 164–168. https://doi.org/10.1111/j.1399-3054.1986.tb01251.x

Kambhampati, S., Aznar-Moreno, J. A., Bailey, S. R., Arp, J. J., Chu, K. L., Bilyeu, K. D., Durrett, T. P., & Allen, D. K. (2021). Temporal changes in metabolism late in seed development affect biomass composition. *Plant Physiology*, *186*, 874–890. https://doi.org/10.1093/plphys/kiab116

Keith, B. C., & Delouche, J. C. (1999). Seed quality, production, and treatment. In L. G. Heatherly L.G., & H. G. Hodges (Ed.). *Soybean Production in the Midsouth*. Boca Raton, FL: CRC Press. pp. 197–230.

Koster, K. L., & Leopold, A. C. (1988). Sugars and desiccation tolerance in seeds. *Plant Physiology*, *88*, 829–832. https://doi.org/10.1104/pp.88.3.829

Li, L., Hur, M., Lee, J.-Y., Zhou, W., Song, Z., Ransom, N., Demirkale, C. Y., Nettleton, D., Westgate, M., Arendsee, Z., Iyer, V., Shanks, J., Nikolau, B., & Wurtele, E. S. (2015). A systems biology approach toward understanding seed composition in soybean. *Bmc Genomics [Electronic Resource]*, *16*, 9. https://doi.org/10.1186/1471-2164-16-S3-S9

Liu, K. (1997). *Soybeans chemistry, technology, and utilization*. New York: Chapman & Hall.

Maestri, D. M., Labuckas, D. O., Meriles, J. M., Lamarques, A. L., Zygadlo, J. A., & Guzman, C. A. (1998). Seed composition of soybean cultivars evaluated in different environmental regions. *Journal of the Science of Food and Agriculture*, *77*, 494–498. https://doi.org/10.1002/(SICI)1097-0010(199808)77:4⟨494::AID-JSFA69⟩3.0.CO;2-B

Mourtzinis, S., Gaspar, A. P., Naeve, S. L., & Conley, S. P. (2017). Planting date, maturity, and temperature effects on soybean seed yield and composition. *Agronomy Journal*, *109*, 2040–2049. https://doi.org/10.2134/agronj2017.05.0247

Nishizawa, A., Yabuta, Y., & Shigeoka, S. (2008). Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiology*, *147*, 1251–1263. https://doi.org/10.1104/pp.108.122465

Obendorf, R. L., Horbowicz, M., Dickerman, A. M., Brenac, P., & Smith, M. E. (1998). Soluble oligosaccharides and galactosyl cyclitols in maturing soybean seeds in planta and in vitro. *Crop Science*, *38*, 78–84. https://doi.org/10.2135/cropsci1998.0011183X003800010014x

Pereira, I. S., Soares, L. H., Cabral, E. M. A., Fontana, D. C., Umburanas, R. C., Santos, L. L. S., & Fagan, E. B. (2020). Soybean harvest-aid herbicides management can alter seed yield, quality, and oxidative metabolism. *Revista Brasileira de Ciências Agrárias*, Recife, v.15, n.2, e7022, 7 DOI: 10.5039/agraria.v15i2a7022. https://doi.org/10.5039/agraria.v15i2a7022

Piper, E. L., & Boote, K. J. (1999). Temperature and cultivar effects on soybean seed oil and protein concentrations. *Journal of the American Oil Chemists' Society*, *76*, 1233–1242. https://doi.org/10.1007/s11746-999-0099-y

Ratnayake, S., & Shaw, D. R. (1992a). Effects of harvest-aid herbicides on soybean (Glycine max) seed yield and quality. *Weed Technology*, *6*, 339–344. https://doi.org/10.1017/S0890037X00034837

Ratnayake, S., & Shaw, D. R. (1992b). Effects of harvest aid herbicides on sicklepod (*Cassia obtusifolia*) seed yield and quality. *Weed Technology*, *6*, 985–989. https://doi.org/10.1017/S0890037X00036587

Ross, J., & Barber, T. (2018). *Arkansas soybeans: Harvest aid products and timing*, University of Arkansas Extension Specialists. Available online: https://agfax.com/2018/08/27/arkansas-soybeans-harvest-aid-products-and-timing/ (accessed on June 29, 2021)

Rotundo, J. L., & Westgate, M. E. (2009). Meta-analysis of environmental effects on soybean seed composition. *Field Crops Research*, *110*, 147–156. https://doi.org/10.1016/j.fcr.2008.07.012

Sengupta, S., Mukherjee, S., Basak, P., & Majumder, A. L. (2017). Significance of galactinol and raffinose family oligosaccharide synthesis in plants. *Frontiers of Plant Science*, *6*, 656. https://doi.org/10.3389/fpls.2015.00656

Smith, J. R., Mengistu, A., Nelson, R. L., & Paris, R. L. (2008). Identification of soybean accessions with high germinability in high-temperature environments. *Crop Science*, *48*, 2279–2288. https://doi.org/10.2135/cropsci2008.01.0026

Soltani, N., Blackshaw, R. E., Gulden, R. H., Gillard, C. L., Shropshire, C., & Sikkema, P. H. (2013). Desiccation in dry edible beans with various herbicides. *Canadian Journal of Plant Science*, *93*, 871–877. https://doi.org/10.4141/cjps2013-061

Stichler, C., & Livingston, S. (2021). Harvest Aids in Sorghum. https://agrilifeextension.tamu.edu/library/farming/harvest-aids-in-sorghum/ (accessed on August 25, 2021)

Toledo, M. Z., Sayuri Ishizuka, M., Cavariani, C., de Barros França-Neto, J., & Bilia Picoli, L. (2014). Pre-harvest desiccation with glyphosate and quality of stored soybean seeds. *Semina: Ciências Agrárias*, v.35, n.2. https://doi.org/10.5433/1679-0359.2014v35n2p765

Valentine, M. F., De Tar, J. R., Mookkan, M., Firman, J. D., & Zhang, Z. J. (2017). Silencing of soybean raffinose synthase gene reduced raffinose family oligosaccharides and increased true metabolizable energy of poultry feed. *Frontiers in Plant Sciences*, *8*, Article 692, pages 1–11. https://doi.org/10.3389/fpls.2017.00692

Wang, Z., Zhu, Y., Wang, L., Liu, X., Liu, Y., Phillips, J., & Deng, X. (2009). A WRKY transcription factor participates in dehydration tolerance in *Boea hygrometrica* by binding to the W-box elements of the galactinol synthase (BhGolS1) promoter. *Planta*, *230*, 1155–1166. https://doi.org/10.1007/s00425-009-1014-3

Whigham, D. K., & Stoller, E. W. (1979). Soybean desiccation by paraquat, glyphosate, and ametryn to accelerate harvest. *Agronomy Journal*, *71*, 630–633. https://doi.org/10.2134/agronj1979.00021962007100040027x

Whiting, K. R., Crookston, R. K., & Brun, W. A. (1988). An indicator of the R6.5 stage of development for indeterminate soybean. *Crop Science*, *28*, 866–867. https://doi.org/10.2135/cropsci1988.0011183X002800050034x

Wilcox, J. R., & Shibles, R. M. (2001). Interrelationships among seed quality attributes in soybean. *Crop Science*, *41*, 11–14. https://doi.org/10.2135/cropsci2001.41111x

Wolf, R. B., Cavins, J. F., Kleiman, R., & Black, L. T. (1982). Effect of temperature on soybean seed constituents: Oil, protein, moisture, fatty acids, amino acid, and sugars. *Journal of the American Oil Chemists' Society*, *59*, 230–232. https://doi.org/10.1007/BF02582182

Zhang, M., Kang, M. S., Reese, P. F., & Bhardwaj, H. L. (2005). Soybean cultivar evaluation via GGE biplot analysis. *Journal of New Seeds*, *7*, 37–50. https://doi.org/10.1300/J153v07n04_03

Zobiole, L. H. S., Oliveira, R. S. D. Jr., Visentainer, J. V., Kremer, R. J., Bellaloui, N., & Yamada, T. (2010). Glyphosate affects seed composition in glyphosate-resistant soybean. *Journal of Agriculture and Food Chemistry*, *58*, 4517–4522. https://doi.org/10.1021/jf904342t

Zuther, E., Büchel, K., Hundertmark, M., Stitt, M., Hincha, D. K., & Heyer, A. G. (2004). The role of raffinose in the cold acclimation response of *Arabidopsis thaliana*. *FEBS Letters*, *576*, 169–173. https://doi.org/10.1016/j.febslet.2004.09.006

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