Effect of glyphosate application time on yield parameters of South African glyphosate-resistant maize cultivars

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Glyphosate is the most used herbicide in South Africa. Due to observations by some South African maize producers that the application of glyphosate to glyphosate-resistant (GR) maize cultivars resulted in reduced yield, we conducted an in-depth study under local conditions. Through field trials, over two seasons (2017/2018 and 2018/2019), we investigated whether the application time of glyphosate would impact maize yields negatively. Various yield parameters were measured subsequent to glyphosate application to the local GR maize cultivars DKC74-74BR, DKC78-79BR, KKS4581, KKS8408, BG5785BR, PAN6R-710BR, P1814R and P2880WBR. Four glyphosate products were included (Roundup PowerMax®; Slash Plus 540 SL; TouchdownForte® and Mamba™ DMA 480 SL), resulting in 32 cultivar x glyphosate product combinations. Each product was applied at V4, V4+V6, V6 and V8 growth stages together with an untreated control. Yield parameters measured (ears per plant, rows per ear, kernels per row, thousand kernel mass and yield) were expressed as a percentage of the control. The trials were planted as randomised complete block designs with three replicates. Limited response was observed with all the parameters investigated, with a significant negative yield response, greater than the untreated control, observed in only 3.1% of the cultivar x glyphosate product combinations evaluated. No clear trends or discernible and consistent impacts on yield and yield parameters could be established based on the application time of glyphosate (within label recommendations) across seasons. The findings contribute significantly to the knowledge base and current understanding of the international community and local producers alike regarding the effective use of glyphosate and generic variations thereof in crops of diverse genetic backgrounds.

Significance:

- Limited response in the yield parameters evaluated were obtained in response to the application time (V4, V4+V6, V6 and V8) of the four glyphosate products on eight GR maize cultivars tested ($p=0.1$).
- Inconsistent patterns or trends were detected in cases where significance was obtained, implying that it would not be possible to draw accurate conclusions or formulate recommendations.
- Application time of glyphosate did not result in a significant reduction in yield compared to the untreated control, in the majority of the cultivar x glyphosate product combinations investigated, confirming that glyphosate application conducted within label specifications would not reduce yield, irrespective of the glyphosate product or genetic background of maize.

Introduction

Glyphosate, developed in 1964, was introduced to crop production during the mid-1970s as a broad-spectrum, non-selective, post-emergence herbicide.[1] Genetically modified (GM) crops, resistant to glyphosate and glufosinate, subsequently followed, with the first GM crops commercially cultivated in the 1990s.[2] Due to its rapid environmental degradation, minimal contamination of ground water, low costs as well as effective systematic action on most plants[2], glyphosate has since become one of the most widely used agrochemicals in modern agriculture[3]. Similar to the international trend, glyphosate is the most used herbicide in South Africa. Glyphosate is, however, aside from being a broad-spectrum herbicide, also a broad-spectrum chelator of macro- and micronutrients.[4] Due to this characteristic, glyphosate has been found to facilitate nutrient availability, access and/or absorption of some nutrients (Ca, Cu, Fe, K, Mg, Mn and Zn).[5] The immobility of nutrients would reduce their availability for processes such as photosynthesis, disease resistance and other essential functions in plants that in turn could potentially result in reduced yields.

The majority of herbicide-related research studies focus on weed control and the resultant maize grain yields, with few studies reporting on how herbicide applications may affect growth and development of herbicide-resistant maize plants in weed-free environments. Similarly, studies on the potential effects of glyphosate on plant health of glyphosate-resistant (GR) crops has mostly been focused on GR soybean.[6] Thelen and Penner[7], nonetheless, reported slight yield reductions in maize grain yields with the application of glyphosate under certain temporal high-yield environments. The authors speculated that under high-yielding conditions, injurious effects of glyphosate or glyphosate metabolites on GR maize may become measurable in terms of yield loss. In low-yield environments, yield limiting factors such as water stress may mask the less significant, subtler phytotoxic effects of glyphosate or glyphosate metabolites. Elmore et al.[8] concluded that, in a weed-free environment, glyphosate application had no effect on GR soybean yield and that yield suppression in GR soybean rather appears to be associated with the GR gene or its insertion process.[8,9]

Due to the expiration of the patent on glyphosate, producers have access to more glyphosate-containing herbicides.[10] All glyphosate products contain the same parent acid, but are sold as different salts of glyphosate with each formulation containing proprietary adjuvants that could influence product performance.[11] Studies to confirm the efficacy of such products concluded that there is little difference in product performance relating to glyphosate formulation and that a grower's choice among glyphosate products should be based on product cost, guarantees and other incentives from manufacturers.[11]

As glyphosate translocates from source tissue to sink tissue[12], developing sinks such as floral organs can be especially sensitive to glyphosate damage. Although glyphosate can influence reproductive development in GR and non-GR species, the impact on yield and fruit set varies greatly by crop, environment and timing of glyphosate applications. In most cases, the potential harmful effect of glyphosate is emphasised at growth stages that would be detrimental to the reproduction of the plant. Glasshouse and field studies have described several morphological abnormalities in affected flowers and balls of GR cotton.[13,14] Thomas et al.[15] demonstrated through glasshouse and field trials that maize pollen viability and overall quantity of pollen production were reduced when glyphosate was applied at growth stage V6 or later. Although both quantity and quality of pollen were compromised by glyphosate applied beyond the V6 stage, there was no significant effect on kernel set or yield using controlled pollinations. Pline-Srnic[16] speculated that sufficient pollen is produced by maize to ensure successful pollination even with reductions in viability and quantity, therefore preventing the manifestation of glyphosate effects on seed set. Glyphosate applications made near the time of pollen development in GR crops generally result in greater reproductive damage than early applications.[16] Locally, glyphosate application is recommended up until V8 leaf stage. Although several international studies have accordingly reported on the optimal time of glyphosate application[17], few investigated whether all applications applied before V8 would have an equal effect on crop yield in various genetic backgrounds of GM maize in weed-free environments.

Based on reduced yields reported by some local producers after glyphosate application, South African producers requested a study to establish whether the growth stage of glyphosate application could have a negative influence on yields of GM South African maize cultivars in weed-free environments. To achieve this objective, we conducted field trials over two consecutive seasons to establish whether yield parameters are affected by the growth stage of glyphosate application in various genetic backgrounds of South African GM maize.

Materials and methods

Eight randomised block design trials were planted during the 2017/2018 and 2018/2019 growing seasons, respectively, at the Agricultural Research Council's Grain Crops division in Potchefstroom, North West Province, South Africa (-26.743200°; 27.070775°). All eight trials were planted on 30 September 2017 for the 2017/2018 growing season and again on 23 October 2018 for the 2018/2019 growing season. Soil was prepared using standard seedbed preparations for a clay loam soil site (35% clay, 59% sand and 5% silt). Fertiliser with the formulation 3:2:1 was applied at 150 kg/ha, with LAN applied at V6 stage as top dressing at 150 kg/ha based on soil analyses. Each trial was planted to GR-maize cultivars that included DKC74-74BR, DKC78-79BR, KKS4581, KKS8408, BG5785BR, PAN6R-710BR, P1814R and P2880WBR. Plots consisted of eight 5-m rows with inter-row spacing of 0.9 m. The middle two rows of each plot were harvested. Plant density was approximately 34 000 plants/ha. Pre-emergence herbicides (Frontier® Optima and Gesaprim Super) were applied before onset of the trials after which weeds were removed from all plots by hand hoeing to prevent confounding effects of differential weed control. The treatment design was a strip-split with application at V4, V4+V6, V6 and V8 growth stages[18], respectively, as main plot factor and the four glyphosate products applied as sub-plot factor that were replicated in three blocks. The glyphosate products applied were (1) Roundup PowerMax® (540 g ae/L Monstanto, hereafter referred to as PowerMax), (2) Slash Plus 540 SL (540 g ae/L Villa Crop Protection, hereafter referred to as Slash), (3) TouchdownForte® (500 g ae/L Syngenta, hereafter referred to as Touchdown) and (4) Mamba™ DMA 480 SL (480 g ae/L Dow AgroScience, hereafter referred to as Mamba). An untreated control was included as the fifth treatment. Each product was applied at 2 L/ha, which is a common reference for glyphosate application amongst producers. PowerMax and Slash were accordingly applied at 1080 g ae/ha; Touchdown at 1000 g ae/ha and Mamba at 960 g ae/ha. The V4, V6 and V8 applications were conducted on 1, 12 and 20 December 2017 and on 21 and 28 November and 11 December in 2018. Ammonium sulfate (2%) was added as per label instruction. Herbicides were applied with a tractor sprayer, calibrated to deliver 200 L water/ha using flat fan nozzles. Supplementary irrigation was provided to all trials on a weekly basis as needed using overhead sprinklers. Daily weather data (maximum/minimum temperature and rainfall) were captured on site.

Parameters measured included ears per plant (EpP), rows per ear (RpE), kernels per row (KpR), thousand kernel mass (TKS) and yield (t/ha; 12.5% moisture). An average of five cobs per plot were randomly selected to determine RpE and KpR. From each cob selected, the number of kernels within two randomly selected rows were counted and the average number of kernels calculated to obtain KpR. All parameters were expressed as percentage of control.

For each cultivar x glyphosate product combination, the data over the two seasons were combined. Analysis of variance (ANOVA) was used to establish whether season and application time significantly impacted yield parameters in the respective combinations. Means were separated using Fisher's protected least significant difference (LSD) if the F probability from the ANOVA was significant at the 10% ($p=0.1$) level of significance. The lower significance level used was due to limited significance in treatments in general, as well as to compensate for the variation in data generated. All the analyses were conducted using GenStat for Windows 18th edition.

Results

Weather data captured for the 2017/2018 and 2018/2019 planting seasons is presented in Table 1. The average temperatures achieved during October, November and December were 2.2 ºC, 1.6 ºC and 3.6 ºC, respectively, higher during 2018/2019 than during 2017/2018. Total rainfalls recorded during the two seasons were similar. Higher early season rainfall (October to December 2017) was recorded for 2017/2018, compared to the corresponding period in 2018/2019. However, as all trials received supplementary irrigation, water was not a limiting factor. As all values were expressed as a percentage of the control, any value greater than the LSD of the relevant parameter assessed will also indicate that a specific treatment resulted in a significantly greater or lower measured effect than that of the untreated control. It was considered important to record such cases as an indication of whether the application of glyphosate would have a significant impact on the yield compared to that where no glyphosate was applied.

Ears per plant

Seasonal variation (year as main effect) significantly affected EpP with the application of PowerMax, to DKC74-74BR (Table 2), with an average reduction of 5.17% observed during 2018/2019 compared to the general increase of 1.67% noted in 2017/2018 (data not shown).

Application time (as main effect) significantly affected three cultivar x glyphosate product combinations (Table 2). Touchdown increased EpP by 19.5% in BG5785BR at V4, and by 23.1% in P2880WBR at V4+V6. Reductions in EpP detected at the various application times were never below 8% in either cultivar, and did not differ significantly from the untreated control (BG5785BR – $LSD_{(p=0.1)} = 15.1$; P2880WBR – $LSD_{(p=0.1)} = 13.5$). Slash applied at V4 reduced EpP by 13.3% in KKS8408, whilst an 11.5% increase was observed when applied at V8 ($LSD_{(P=0.1)} = 15.6$) (Table 2).

Seven cultivar x glyphosate product combinations were significantly affected by the application time x year interaction. DKC74-74BR was significantly affected by Mamba and Touchdown with a significant increase in EpP observed (Mamba – 35.4%; Touchdown – 30.9%) when applied at V8 in 2017/2018. During the following season, reductions of 11.8% (Mamba) and 12.7% (Touchdown), respectively, were observed for the same application time. A similar effect is observed for KKS4851 (PowerMax). When applied to P1814R, PowerMax reduced EpP during the first season (2017/2018) by 35%, with an 11.1% increase observed for the same application time in the following season. Touchdown applied at V4+V6 reduced EpP of BG5785BR by 21.1% in the first season, but increased EpP by 13.4% in the second season at the same application time. Touchdown similarly resulted in a 10.5% reduction in EpP when applied to P2880WBR at V4 in the first season.

Table 1: Temperature and rainfall data at Potchefstroom during the 2017/2018 and 2018/2019 planting seasons

		Temperature (ºC)					Rainfall (mm)	
		Maximum		Minimum				
		2017/2018	2018/2019	2017/2018	2018/2019		2017/2018	2018/2019
October	Average	26.4	28.6	11.4	11.4	Total	56.13	22.1
	Highest	32.4	34.4	17.1	18.7	Highest	23.88	11.68
	Lowest	18.0	17.2	5.1	5.8			
November	Average	29.1	30.7	12.7	14.0	Total	69.34	17.02
	Highest	34.5	36.8	17.3	23.4	Highest	18.54	10.16
	Lowest	17.1	23.4	4.5	4.3			
December	Average	29.3	32.9	15.7	16.2	Total	62.48	42.42
	Highest	33.4	38.8	19.1	20.7	Highest	13.72	12.19
	Lowest	15.8	21.9	10.3	11.2			
January	Average	31.0	31.5	16.1	16.7	Total	47.24	70.87
	Highest	36.6	36.2	20.3	19.5	Highest	12.45	25.65
	Lowest	24.4	21.2	9.3	13.5			
February	Average	27.7	28.7	15.6	15.6	Total	68.33	49.02
	Highest	31.5	33.3	17.7	19.0	Highest	14.99	8.64
	Lowest	20.5	22.0	11.8	11.4			
March	Average	27.5	30.2	14.6	15.1	Total	58.93	34.54
	Highest	31.1	33.9	19.2	19.0	Highest	21.84	22.35
	Lowest	17.6	20.4	10.2	11.3			
April	Average	25.3	23.8	11.1	11.7	Total	35.56	145.8
	Highest	29.0	28.7	16.1	17.0	Highest	10.67	35.31
	Lowest	19.7	14.4	5.6	5.4			
May	Average	22.8	24.3	4.9	5.8	Total	11.18	0.00
	Highest	26.4	28.1	12.1	11.2	Highest	9.91	0.00
	Lowest	16.4	20.7	1.3	0.6			
June	Average	21.6	21.5	1.5	1.1	Total	0.00	0.00
	Highest	25.6	25.0	4.6	7.5	Highest	0.00	0.00
	Lowest	17.5	17.7	-2.0	-3.6			
July	Average	19.3	21.8	1.1	-0.1	Total	5.08	0.40
	Highest	26.3	27.2	7.8	5.9	Highest	2.03	0.40
	Lowest	14.2	14.7	-6.0	-4.6			
						Total seasonal rainfall (mm)	414.27	382.17

In two of the seven cases in which the highest order interaction (application time x year) resulted in significant differences, the reduction observed was greater than the untreated control, suggesting that a significantly lower EpP was observed compared to when no product was applied. In both cases, the effect was observed for PowerMax (KKS8408 and P1814R) when applied at V8, but the seasons in which the effect was observed differed for the two cultivars. Based on these findings, it can be stated with 90% certainty ($p=0.1$) that the application time across two seasons (application x year interaction) affected EpP in 21.8% of the 32 cultivar x glyphosate product combinations investigated, but, that in only 6.3% of the cases, a significant decrease greater than the untreated control was achieved. The general effect observed was furthermore inconsistent between product, cultivar and season.

Kernels per row

Year as main effect significantly affected KpR in five of the cultivar x glyphosate product combinations tested. Lower KpR was achieved in 2018/2019 by DKC74-74BR (Slash and Touchdown), KKS4851 (PowerMax) and PAN6R-710BR (Touchdown) (data not shown). BG5785BR realised lower KpR in 2017/2018.

Application time (main effect) significantly affected KpR for Mamba applied to DKC78-79BR and P2880WBR, as well as PowerMax applied to KKS4851 (Table 3). Although Mamba reduced KpR of DKC78-79BR by 3% and 7.3% at V6 and V8, respectively, the reductions observed were not significantly greater than those for the untreated control. The 14.5% reduction observed in P2880WBR with the application of Mamba at V6, was significantly greater than the untreated control. PowerMax applied at V6 to KKS4851 significantly increased KpR by 14.1%.

The application time x year interaction was only significant for the P1814R x Slash combination, in which a very variable response regarding application time across seasons was observed. A 16.1% increase in KpR was achieved at V4+V6 application during the first season (2017/2018), whilst no reductions significantly greater than the untreated control were observed for the remaining application times across seasons.

Seasonal differences subsequently resulted in significant differences in 15.6% of the cultivar x glyphosate product combinations evaluated, application time in 9.3%, and the application time x year interaction in 3.1% of the cultivar x glyphosate product combinations tested. The effect observed, similar to EpP, remains unpredictable regarding cultivar x glyphosate product combinations across seasons.

Rows per ear

Year as main effect significantly affected RpE in KKS4851 (PowerMax), PAN6R-740BR (Mamba) and P1814R (Touchdown) (data not shown).

Table 2: The effect of glyphosate application time on the number of ears produced per plant (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	8.6	-0.7	4.0	-0.4bc	-20.6c	-10.5	0.5	6.1	3.3	2.3ab	9.6a	5.9	-1.1	-11.7	-6.4	0.3	10.8	5.6	21.7	4.0	12.9	20.4	12.5	16.5
	V4+V6	-18.3	11.9	-3.2	-6.1bc	18.6ab	6.3	-16.0	10.2	-2.9	13.2a	-4.5ab	4.3	-5.1	4.5	-0.3	7.0	4.9	6.0	26.7	11.7	19.2	12.2	-0.8	5.7
	V6	11.5	4.2	7.9	-13.8bc	-13.5bc	-13.6	-17.5	-11.3	-14.4	-19.5b	-0.7ab	-10.1	-11.7	5.3	-3.2	5.4	-35.0	-14.8	-14.9	4.4	-5.3	8.1	-7.0	0.6
	V8	-0.9	-24.3	-12.6	35.4a	-11.8bc	11.8	16.1	-2.0	7.0	21.8a	-23.1b	-0.7	14.7	-6.3	4.2	-3.9	-16.2	-10.1	-10.9	-13.6	-12.3	0.2	-9.3	-4.6
LSD		*ns*			*AppxYr = 40.5*			*ns*			*AppxYr = 27.0*			*ns*			*ns*			*ns*			*ns*		
Roundup PowerMax	V4	-8.2	13.0	2.4	-21.2	-2.9	-12.1	10.8	5.2	8.0	4.8	-0.5	2.2	10.4a	-13bc	-1.3	8.2	-3.6	2.3	10.8a	-7.6abc	1.6	5.3	-5.8	-0.3
	V4+V6	-10.4	-12.7	-11.6	18.2	-2.1	8.0	40.8	24.7	32.8	-5.7	-5.7	-5.7	-0.5ab	8.5a	4.0	-5.3	6.0	0.4	-6.2abc	-7.4abc	-6.8	11.1	8.5	9.8
	V6	-8.0	3.8	-2.1	-3.9	-11.0	-7.5	-3.0	9.2	3.1	-0.7	23.0	11.1	2.7ab	3a	2.9	12.9	-19.9	-3.5	-21.5bc	0.4ab	-10.5	11.1	2.0	6.6
	V8	1.7	-35.5	-16.9	13.6	-4.7	4.5	-0.7	5.0	2.1	-3.5	-15.4	-9.4	8a	-22.9c	-7.4	28.7	-5.4	11.7	-35c	11.1a	-12.0	8.5	-6.9	0.8
LSD		*ns*			*Yr = 6.7*			*ns*			*ns*			*Appxyr = 16.1*			*ns*			*AppxYr = 28.6*			*ns*		
Slash Plus	V4	2.4	-19.8	-8.7	6.5	-6.1	0.2	-6.6	2.2	-2.2	2.7	-1.0	0.9	-15.8	-10.9	-13.3b	2.1	13.6	7.9	22.4	13.8	18.1	1.5	-11.4	-5.0
	V4+V6	-9.3	14.2	2.5	-5.6	-4.7	-5.2	49.9	-1.6	24.2	15.9	-14.6	0.6	-7.6	3.1	-2.3ab	31.4	15.1	23.3	-3.6	-17.6	-10.6	8.4	-7.2	0.6
	V6	10.0	-4.9	2.6	5.5	-4.8	0.4	23.1	-3.2	9.9	1.0	-4.1	-1.5	0.5	14.0	7.2a	0.0	1.1	0.6	-27.3	21.2	-3.0	5.0	13.2	9.1
	V8	-4.3	-22.2	-13.3	-10.0	-0.4	-5.2	-7.8	11.0	1.6	10.3	-11.0	-0.3	7.4	15.7	11.5a	23.0	13.2	18.1	-18.8	-10.6	-14.7	19.5	11.3	15.4
LSD		*ns*			*ns*			*ns*			*ns*			*App = 15.6*			*ns*			*ns*			*ns*		
Touchdown Forte	V4	23.0a	16ab	19.5a	-20.4b	-9.3b	-14.8	-15.6	5.6	-5.0	21.9	-1.1	10.4	-12.7	-8.7	-10.7	-5.7	-23.9	-14.8	15.2	8.7	11.9	-10.5c	-2.4bc	-6.5c
	V4+V6	-21.1d	13.4ab	-3.8ab	7.2ab	6.8ab	7.0	26.8	-9.2	8.8	12.7	-2.6	5.0	-0.7	5.4	2.3	-4.8	23.4	9.3	-5.3	-3.3	-4.3	24.1a	22.2a	23.1a
	V6	5.2abc	-9.1cd	-1.9cd	-11b	-2.1b	-6.5	-9.7	-1.6	-5.6	-12.5	0.8	-5.9	-7.4	3.2	-2.1	13.1	-27.8	-7.4	-10.5	-2.3	-6.4	20.2ab	-3.3bc	8.5b
	V8	-3.9bcd	-11.2cd	-7.5cd	30.9a	-12.7b	9.1	-5.3	8.0	1.3	4.8	3.0	3.9	6.2	20.1	13.2	-9.4	14.5	2.6	-16.7	-0.2	-8.4	7.6abc	26.4a	17ab
LSD		*App = 15.1* *AppxYr = 23.2*			*AppxYr = 30.5*			*ns*			*ns*			*ns*			*ns*			*ns*			*App = 13.5* *AppxYr = 26.1*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

Table 3: The effect of glyphosate application time on the number of kernels produced per row (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	-14.5	-9.3	-11.9	-7.6	-6.9	-7.2	15.0	1.4	8.2a	6.3	-7.0	-0.4	4.1	-1.0	1.5	3.1	2.7	2.9	3.2	4.0	3.6	1.9	4.7	3.3b
	V4+V6	-16.2	-16.2	-16.2	0.4	-3.7	-1.6	8.7	4.1	6.4ab	5.5	-0.7	2.4	12.6	1.3	6.9	7.0	1.1	4.1	-8.7	8.3	-0.2	9.5	0.6	5.0b
	V6	-24.0	-1.2	-12.6	-4.4	-7.9	-6.2	-6.1	0.2	-3.0bc	-7.2	7.4	0.1	-5.1	-0.5	-2.8	-5.9	1.0	-2.5	-0.2	-13.2	-6.7	-24.1	-4.9	-14.5a
	V8	-20.3	-0.5	-10.4	23.9	-9.8	7.0	-1.0	-13.6	-7.3c	7.7	-4.7	1.5	2.1	-5.7	-1.8	-4.6	-3.1	-3.9	-1.3	2.7	0.7	-3.8	2.0	-0.9b
LSD		*ns*			*ns*			*App = 9.8*			*ns*			*ns*			*ns*			*ns*			*App = 12.0*		
Roundup PowerMax	V4	-19.1	-2.5	-10.8	-4.8	-1.8	-3.3	10.7	-2.9	3.9	1.6	-13.3	-5.8b	2.0	-1.3	0.3	16.5	0.2	8.4	1.9	0.9	1.4	-0.7	-0.1	-0.4
	V4+V6	-12.5	-9.2	-10.9	-2.7	3.4	0.3	-5.4	0.4	-2.5	10.0	-11.3	-0.6b	7.0	31.4	19.2	-14.6	3.4	-5.6	-14.9	1.2	-6.8	2.0	3.8	2.9
	V6	-20.3	-0.4	-10.4	5.8	-8.2	-1.2	2.5	3.1	2.8	16.3	12.0	14.1a	-13.2	-4.4	-8.8	-3.3	-1.0	-2.2	-1.9	1.5	-0.2	-0.9	0.6	-0.2
	V8	2.4	3.8	3.1	9.5	-1.8	3.8	14.8	-6.5	4.1	-1.1	-10.3	-5.7b	-8.7	-6.0	-7.3	-13.8	1.5	-6.2	5.1	2.2	3.6	-14.3	7.9	-3.2
LSD		*ns*			*ns*			*ns*			*Yr = 12.0* *App = 11.8*			*ns*			*ns*			*ns*			*ns*		
Slash Plus	V4	-10.4	-9.3	-9.9	19.9	-11.6	4.1	-11.8	-10.1	-11.0	-7.3	4.9	-1.2	-10.6	-9.7	-10.1	10.2	3.7	7.0	7.6abc	-5.4bc	1.1	14.7	8.7	11.7
	V4+V6	-8.8	-15.1	-12.0	6.3	-12.0	-2.8	-11.5	-4.6	-8.0	6.7	-0.8	2.9	6.0	-2.3	1.8	10.2	2.2	6.2	16.0a	4.7abc	10.3	11.2	4.8	8.0
	V6	-18.7	-1.3	-10.0	-10.8	-1.1	-5.9	-19.5	-10.1	-14.8	-4.1	3.3	-0.4	0.9	-0.7	0.1	0.3	0.6	0.5	-5.1bc	9.0ab	1.9	-6.8	4.3	-1.3
	V8	-15.8	-6.7	-11.3	2.0	-8.6	-3.3	8.1	-3.5	2.3	-4.5	0.6	-1.9	-5.1	-1.1	-3.1	-7.0	-2.8	-4.9	-6.3c	0.8bc	-2.7	-9.0	-0.3	-4.7
LSD		*ns*			*Yr = 11.0*			*ns*			*ns*			*ns*			*ns*			*AppxYr=14.3*			*ns*		
Touchdown Forte	V4	-5.9	-2.4	-4.2	1.2	3.1	2.1	5.9	5.5	5.7	6.8	0.3	3.6	6.9	-3.9	1.5	6.2	-3.4	1.4	7.2	3.4	5.3	-7.8	5.1	-1.4
	V4+V6	-10.8	-5.1	-8.0	-1.0	-2.8	-1.9	12.4	4.2	8.3	1.3	-2.3	-0.5	-6.0	1.3	-2.4	6.2	-2.9	1.7	-13.7	4.6	-4.6	10.5	7.4	9.0
	V6	-19.5	-3.3	-11.4	8.2	-14.4	-3.1	-12.7	-5.7	-9.2	7.2	0.7	3.9	-4.1	2.7	-0.7	-8.4	-3.5	-6.0	-3.6	1.2	-1.2	-4.2	9.7	2.8
	V8	-19.5	7.1	-6.2	26.9	-5.8	10.6	3.0	2.2	2.6	7.6	-4.0	1.8	-10.3	-1.1	-5.7	-2.2	-1.1	-1.7	-6.2	4.3	-0.9	-7.3	3.0	-2.2
LSD		*Yr = 8.5*			*Yr = 13.1*			*ns*			*ns*			*ns*			*Yr = 2.8*			*ns*			*ns*		

p = *0.1*
Yr1 = 2017/2018; Yr2 = 2018/2019
LSD, least significant difference; ns, not significant

For both KKS4851 and P1814R, lower RpE was achieved in 2018/2019, whereas PAN6R-740BR had lower RpE in the first season (2017/2018).

Only the KKS4851 x Slash combination was significantly affected by the application time as main effect, with a 6.3% increase observed when glyphosate was applied at V6 (Table 4). None of the reductions observed in the remaining treatments were significantly lower than that of the untreated control ($LSD_{(p=0.1)}$ = 7.4).

The application time x year interaction significantly affected BG5785BR (Mamba), P1814R (Mamba) and DKC78-79BR (Touchdown). Of these, only Touchdown applied at V8 to DKC78-79BR during 2017/2018 resulted in a reduction in RpE (10.5%) which was significantly greater than the untreated control ($LSD_{(p=0.1)}$ = 7.7). A similar effect was not observed during the following season.

Seasonal variation was accordingly evident in 9.3% of the cultivar x glyphosate product combinations evaluated, application time in 3.1%, and the application time x year interaction in 9.3% of the cultivar x glyphosate product combinations tested. The observed effect of application time was furthermore unpredictable pertaining to the cultivar x glyphosate product combinations tested across seasons.

Thousand kernel mass

Seasonal variation (year as main effect) significantly influenced TKM obtained by PAN6R-710BR after the application of PowerMax and Slash, with lower TKM generally recorded for 2018/2019 (data not shown).

With application time as the main effect, significant differences were observed in 8 of the 32 cultivar x glyphosate product combinations (Table 5). PAN1814R generally yielded lower TKM with the application of Mamba, PowerMax and Slash at the later application times (V6 and/or V8). However, the reductions were not greater than that observed in the untreated control in any of these cases. A similar effect was observed in PAN6R-710BR, with the application of Touchdown at V8, reducing TKM by 6.1% (also not significantly different from the untreated control). KKS4851 was negatively affected with the application of Slash at V4, whilst the same product negatively affected TKM of KKS8408 at both V4 and V6 (not significantly different from the untreated control). Touchdown applied at V8 to DKC74-74BR resulted in a significant increase in TKM of 8.8% (Table 5). Of the eight cultivar x glyphosate product combinations which were significantly affected by application time, only the P2880BR x Slash combination resulted in significant differences in TKM which were greater than the untreated control ($LSD_{(p=0.1)}$=6.2%), with an 11%

Table 4: The effect of glyphosate application time on the number of rows produced per ear (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	9.7a	-0.3abcd	4.7	-4.2	2.7	-0.7	-5.5	1.0	-2.3	-6.3	-4.9	-5.6	3.5	-0.3	1.6	3.3	-1.9	0.7	-4.2bc	-2.4bc	-3.3	5.3	-0.1	2.6
	V4+V6	2.5ab	-6.1bd	-1.8	-7.0	0.9	-3.0	-5.6	-4.0	-4.8	-1.7	1.6	0.0	-0.8	7.6	3.4	-5.9	0.6	-2.7	9.6a	-1.6bc	4.0	0.4	8.2	4.3
	V6	-2.2bcd	2.3abc	0.0	-2.9	0.0	-1.4	-0.6	2.0	0.7	-10.9	-1.2	-6.0	-1.0	6.9	2.9	-5.4	-0.2	-2.8	-8.7c	0.1bc	-4.3	-13.7	3.8	-5.0
	V8	-2.2bcd	-0.3abce	-1.3	-2.6	0.9	-0.8	-5.6	0.0	-2.8	-0.6	-4.0	-2.3	3.8	2.7	3.2	-14.5	-1.9	-8.2	0.6b	-3.2bc	-1.3	-4.0	-1.3	-2.7
LSD		*AppxYr = 15.6*			*ns*			*ns*			*ns*			*ns*			*Yr = 3.91*			*AppxYr = 10.0*			*ns*		
Roundup PowerMax	V4	11.7	-1.2	5.3	2.5	-4.5	-1.0	-8.0	-4.0	-6.0	-1.2	-3.0	-2.1	8.4	-0.4	4.0	-5.5	-3.6	-4.6	0.2	-1.6	-0.7	0.6	2.9	1.8
	V4+V6	-7.0	9.8	1.4	-2.6	5.5	1.4	-5.6	-3.0	-4.3	-1.7	-0.2	-0.9	-1.0	0.6	-0.2	-5.9	0.7	-2.6	-4.0	0.9	-1.6	0.6	3.4	2.0
	V6	-7.0	0.1	-3.5	-2.6	-2.7	-2.6	4.4	-1.0	1.7	18.8	-4.9	7.0	4.0	-0.5	1.7	-1.0	1.5	0.3	-4.2	1.0	-1.6	0.6	-0.3	0.2
	V8	-2.5	2.3	-0.1	6.3	3.6	5.0	-0.6	-2.0	-1.3	4.0	-9.6	-2.8	-5.9	-0.3	-3.1	-5.9	-3.7	-4.8	5.4	-5.8	-0.2	-4.0	1.9	-1.1
LSD		*ns*			*ns*			*ns*			*Yr = 5.7*			*ns*			*ns*			*ns*			*ns*		
Slash Plus	V4	14.1	1.4	7.8	6.3	4.6	5.5	-3.1	5.0	1.0	-1.7	0.7	-0.5ab	-1.3	-2.5	-1.9	-2.6	-1.0	-1.8	7.0	0.1	3.5	5.3	3.0	4.2
	V4+V6	-1.9	-4.7	-3.3	11.9	0.9	6.4	-0.7	1.0	0.2	-6.3	-3.5	-4.9b	-1.2	5.6	2.2	-5.9	-0.2	-3.1	0.9	-2.4	-0.7	0.3	1.8	1.1
	V6	2.5	-2.1	0.2	2.5	1.8	2.1	-0.7	-3.0	-1.8	13.7	-1.2	6.3a	-3.2	6.8	1.8	3.8	-0.2	1.8	47.5	-2.4	22.6	-4.3	0.6	-1.9
	V8	2.2	-0.3	1.0	6.3	0.9	3.6	-0.6	-1.0	-0.8	-5.7	-3.1	-4.4b	3.8	2.6	3.2	-10.4	-4.5	-7.5	-3.7	2.6	-0.5	5.4	3.4	4.4
LSD		*ns*			*ns*			*ns*			*App = 7.4*			*ns*			*ns*			*ns*			*ns*		
Touchdown Forte	V4	12.1	-8.3	1.9	-7.0	4.5	-1.2	4.4a	-2ab	1.2	-6.3	2.5	-1.9	3.9	-3.7	0.1	-5.6	0.7	-2.5	9.6	-0.7	4.5	0.6	2.1	1.4
	V4+V6	7.3	-2.1	2.6	9.4	2.8	6.1	-5.6bc	1ab	-2.3	3.4	3.6	3.5	-1.0	5.9	2.5	-1.1	-0.2	-0.7	-1.5	-3.2	-2.4	5.3	3.0	4.2
	V6	2.2	1.4	1.8	-7.6	-5.5	-6.5	-5.5bc	-2ab	-3.7	9.1	-0.2	4.4	3.9	4.8	4.4	-5.4	1.6	-1.9	0.2	1.8	1.0	0.6	0.6	0.6
	V8	2.5	-3.8	-0.7	-0.3	-0.9	-0.6	-10.5c	2ab	-4.3	-1.2	-0.2	-0.7	-1.0	-0.6	-0.8	-5.9	1.5	-2.2	9.8	-0.7	4.5	0.9	1.7	1.3
LSD		*ns*			*ns*			*AppxYr = 7.7*			*ns*			*ns*			*ns*			*Yr = 4.5*			*ns*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

reduction recorded at V6. As the application time x year interaction was also significant for this specific cultivar x glyphosate product combination (Table 5), the interpretation of this result should also take seasonal variation into account.

The application time x year interaction was significant in 4 of the 32 cultivar x glyphosate product combinations (Table 5). TKM of P2880WBR was significantly affected with the application of Mamba, Slash and Touchdown (Table 5). The application of Slash to P2880WBR at V6 was, however, the only treatment combination which resulted in a reduction in TKM (20.3%; 2017/2018), which was significantly greater than the untreated control ($LSD_{(p=0.1)}$=9.29). The effect was not evident in the following season. Of the four cultivar x glyphosate product combinations affected by the highest order interaction, the DKC78-79BR x Mamba combination demonstrated the greatest variation in resultant TKM. Similar to what was observed for the aforementioned parameters, the effect of application time on TKM achieved was inconsistent across application times and seasons. The greatest reduction in TKM for the DKC78-79BR x Mamba combination was achieved at V4 of 2017/2018 (30.3%), followed by a 21.9% reduction at V8 of 2018/2019. The latter treatment indicated a 25% increase for the previous season (V8, 2017/2018).

Seasonal differences were subsequently evident in 6.3% of the cultivar x glyphosate product combinations evaluated. Although application time (as main effect) significantly affected 25% of the cultivar x glyphosate product combinations, a negative impact, which was greater than the untreated control, was observed in only 3.1% of the 32 combinations tested. A total of 12.5% of the cultivar x glyphosate product combinations tested were significantly affected by the application time x year interaction, with only 6.3% resulting in significantly reduced TKM compared to the untreated control. The effect observed was unpredictable regarding cultivar x glyphosate product combinations across seasons.

Yield

Of the five yield-related parameters investigated (EpP, KpR, RpE, TKM and yield), yield was characterised the most by large variations in yield response recorded within the same application time, and across the respective seasons. This contributed to a lower frequency of significant differences observed, regardless of apparent large percentage increases or decreases recorded at various application times, compared to the untreated control (Table 6). This observation is noteworthy and emphasises the need for a greater number of treatment replications for future field trials on glyphosate. Despite the large variation observed, significant differences were obtained, which, with regard to frequency, were somewhat consistent with the frequency of significance observed in the previous four yield-related parameters evaluated (EpP, KpR, RpE and TKM).

Seasonal differences were evident in 1 of the 32 cultivar x glyphosate product combinations (BG5785BR x Mamba), with greater yield reduction observed in 2017/2018 than in the following season.

Table 5: The effect of glyphosate application time on thousand kernel mass (% of control) in eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	-7.0	6.5	-0.3	1.9	3.4	2.7	-30.3d	-2.1bc	-16.2	3.5	4.0	3.7	-6.8	2.0	-2.4	2.4	1.5	2.0	0.9	13.3	7.1a	-3.8b	-0.3ab	-2.0
	V4+V6	-8.1	7.1	-0.5	-3.6	-8.1	-5.9	-14.3bcd	2.5b	-5.9	2.0	7.7	4.8	-1.9	-9.1	-5.5	7.5	-1.6	3.0	22.8	11.2	17a	12.9a	-8.7b	2.1
	V6	1.9	-11.5	-4.8	1.1	2.2	1.7	0.6b	0.2bc	0.4	5.1	8.6	6.9	-0.4	-2.1	-1.3	3.8	4.3	4.1	-20.7	4.3	-8.2b	-8.5b	5ab	-1.7
	V8	-2.3	-11.0	-6.7	-0.2	-1.1	-0.6	25a	-21.9cd	1.5	-7.5	10.7	1.6	-9.6	-0.7	-5.2	-4.5	-3.5	-4.0	1.7	9.9	5.8ab	3.9ab	-1.4ab	1.2
LSD		*ns*			*ns*			*AppxYr = 21.8*			*ns*			*ns*			*ns*			*App = 14.25*			*AppxYr = 20.69*		
Roundup PowerMax	V4	7.0	14.5	10.5	2.6	0.5	1.5	14.7	0.1	7.4	5.2	6.0	5.6	-4.3	-0.5	-2.4	9.9	1.5	5.7	13.1	14.8	14a	7.1	6.0	6.6
	V4+V6	-4.0	2.0	-1.0	7.1	-0.5	3.3	-8.6	1.8	-3.4	1.7	2.3	2.0	-6.6	-1.8	-4.2	3.4	-12.8	-4.7	2.7	6.9	4.8ab	-11.6	6.0	-2.8
	V6	-3.0	-10.0	-6.5	8.2	1.9	5.1	-1.8	-8.0	-4.9	-3.5	3.9	0.2	-3.3	1.6	-0.9	0.6	-12.6	-6.0	-13.0	6.3	-3.4b	-2.4	2.9	0.3
	V8	-10.0	31.0	10.5	6.3	-3.2	1.6	7.3	-3.8	1.7	5.1	-4.1	0.5	-2.6	3.6	0.5	4.8	-1.0	1.9	-14.9	9.2	-2.8b	-3.1	1.7	-0.7
LSD		*ns*			*ns*			*ns*			*ns*			*ns*			*Yr = 5.15*			*App = 10.6*			*ns*		
Slash Plus	V4	-2.7	-4.0	-3.4	-16.1	-2.1	-9.1	-8.1	0.6	-3.8	-8.9	4.5	-2.2c	-17.4	1.7	-7.9b	1.8	-5.1	-1.7	8.7	12.1	10.4a	1.2a	5.2a	3.2a
	V4+V6	-4.3	-0.7	-2.5	0.4	4.3	2.3	-3.8	-0.2	-2.0	9.4	3.8	6.6ab	6.3	7.4	6.8a	4.6	-8.6	-2.0	14.7	13.0	13.8a	5.4a	2.7a	4.1a
	V6	-5.4	1.1	-2.2	-2.2	-2.4	-2.3	-3.6	-7.1	-5.4	1.6	2.0	1.8bc	-12.7	-2.7	-7.7b	11.3	-0.6	5.4	2.5	-1.9	0.3b	-20.3b	-1.6a	-11b
	V8	-17.1	30.7	6.8	-3.1	-1.7	-2.4	11.0	-14.5	-1.7	5.1	14.6	9.9a	-4.2	5.5	0.7ab	7.1	1.0	4.1	0.5	-1.9	-0.7b	6.2a	1.8a	4a
LSD		*ns*			*ns*			*ns*			*App = 7.1*			*App = 9.72*			*Yr = 8.4*			*App = 9.87*			*App = 6 .15* *AppxYr = 9.29*		
Touchdown Forte	V4	0.2	5.3	2.8	0.7	3.1	1.9b	-4.7	-4.0	-4.3	4.2	9.8	7.0	-3.4	0.9	-1.2	5.6	2.2	3.9a	4.4	1.6	3.0	0.53abc	3.49abc	2.0
	V4+V6	1.7	4.1	2.9	2.0	-0.9	0.6b	0.3	4.1	2.2	-2.7	0.5	-1.1	-13.1	-1.5	-7.3	5.5	2.2	3.9a	-7.4	6.3	-0.5	-0.78ac	7.91a	3.6
	V6	-13.0	-0.1	-6.6	1.4	-0.1	0.6b	1.9	2.5	2.2	-12.5	7.9	-2.3	-6.1	0.0	-3.0	16.8	-0.1	8.3a	10.6	4.9	7.8	5.64ab	-2.35bc	1.6
	V8	-4.3	4.9	0.3	5.4	12.1	8.8a	3.0	1.8	2.4	1.6	3.5	2.5	-5.3	-2.1	-3.7	-8.6	-3.6	-6.1b	2.7	-2.8	-0.1	1.53abc	1.26bc	1.4
LSD		*ns*			*App = 5.8*			*ns*			*ns*			*ns*			*App = 8.33*			*ns*			*AppxYr = 11.5*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

Three glyphosate product combinations were affected by application time as the main effect. PAN6R-710BR showed 20% and 8.5% yield reductions with the application of Mamba at V8 and V6, respectively ($LSD_{(p=0.1)}=16.4$). KKS4851 recorded 19% and 17.3% yield reductions with the application of PowerMax at V4+V6 and V8, respectively, whilst P2880WBR demonstrated a 12.3% yield reduction with the application of Touchdown at V4. The yields of both KKS4851 and P2880WBR were, however, significantly influenced by the application time x year interaction, and interpretation should take seasonal variation into account.

Yield was significantly influenced by the application time x year interaction in five cultivar x glyphosate product combinations, of which only one resulted in a yield which was significantly lower than that of the untreated control. The application of PowerMax to KKS4851 reduced yields by 28.1% and 27.1% when applied at V4+V6 and V8, respectively, during 2018/2019, which in both instances was greater than that of the untreated control ($LSD_{(p=0.1)}=18.6$). Yield reductions of 23.4% (V6) and 26% (V8) were observed in 2017/2018 and 2018/2019, respectively, with the application of Mamba to KKS4851 (but were not significantly greater than that of the untreated control). A 22.7% reduction was observed in KKS8408 with the same product, applied at V4 (but was not significantly greater than that of the untreated control). In P1814R, PowerMax reduced yield by 38.2% and 31.3% when applied at V6 and V8, respectively, during 2017/2018 (not greater than the untreated control). A similar effect was not observed in the following season.

Yield was accordingly significantly impacted by seasonal variation in 3.1% of the cultivar x glyphosate product combinations evaluated. A total of 9.3% of the cultivar x glyphosate product combinations were influenced by glyphosate application time alone and 15.6% by the application time x year interaction. However, significant yield reduction, which was greater than that of the untreated control, occurred in only 3.1% of the cultivar x glyphosate product combinations evaluated.

Discussion

Many research studies on maize and the effects of herbicides have focused on weed control and grain yields, with little focus on how herbicide applications may possibly affect growth and development of maize plants that have resistance to a particular herbicide (as tested in the absence of weeds).[6] Our objective in this study was to determine if label rate glyphosate, applied at different growth stages and in the absence of weed competition, alters the development of South African GR maize cultivars during the growing season in such a way that it would cause a reduction in yield.

The optimum growth in a maize crop occurs in climates with mid-summer temperatures of between 21 °C and 27 °C.[19] The average temperature ranges experienced in both seasons during this study – especially those in the months of November, December and January – were up to 5 °C above the documented and accepted norm of 27 °C. According to Thelen and Penner[7], sub-optimum growth conditions might result in additional

Table 6: The effect of glyphosate application time on yield (% of control) of eight glyphosate-resistant maize cultivars as evaluated over two seasons (2017/2018 and 2018/2019)

		BG5785BR			DKC74-74BR			DKC78-79BR			KKS4851			KKS8408			PAN6R-710BR			P1814R			P2880WBR		
		Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG	Yr1	Yr2	AVG
Mamba	V4	-0.1	15.0	7.5	-9.9	-14.1	-12.0	5.2	11.8	8.5	-1.9ab	12.6a	5.3	14.7a	-22.7b	-4.0	12.3	7.3	9.8a	43.9	-2.1	20.9	13.8	2.8	8.3
	V4+V6	-40.9	26.5	-7.2	-33.8	14.6	-9.6	-3.9	4.0	0.0	17.8a	-5.6ab	6.1	0.1ab	2.7a	1.4	9.2	13.4	11.3a	42.5	24.8	33.7	3.3	-9.5	-3.1
	V6	0.4	3.1	1.8	-34.3	-4.9	-19.6	-14.6	-12.8	-13.7	-23.4b	10.1a	-6.7	-8.4ab	4.9a	-1.8	9.7	-26.7	-8.5b	-19.8	3.1	-8.4	8.6	0.7	4.7
	V8	-13.5	5.5	-4.0	45.3	-16.9	14.2	41.3	7.3	24.3	8.3a	-26b	-8.9	11a	-10.9ab	0.0	-21.5	-18.5	-20b	-6.9	-13.1	-10.0	-14.6	-18.3	-16.5
LSD		*Yr = 22.7*			*ns*			*ns*			*AppxYr = 32.6*			*AppxYr = 27.7*			*App = 16.4*			*ns*			*ns*		
Roundup PowerMax	V4	-9.0	30.2	10.6	-22.0	6.8	-7.6	-1.7	7.2	2.8	-2b	2.1b	0.1b	13.3	-13.2	0.0	31.1	-8.7	11.2	37.2a	-0.5abcd	18.3	-3.1	-9.8	-6.5
	V4+V6	-22.0	-23.2	-22.6	22.9	2.4	12.7	65.5	12.0	38.8	-9.8bc	-28.1c	-19.0c	15.7	3.3	9.5	-6.4	-1.4	-3.9	-6.3bcd	4.1abc	-1.1	-4.9	-8.0	-6.5
	V6	-35.5	6.4	-14.6	22.5	-3.8	9.3	17.4	0.9	9.2	0.0b	28.3a	14.1a	-8.4	4.8	-1.8	7.8	-23.1	-7.7	-31.3cd	12.4ab	-9.4	-5.2	-10.4	-7.8
	V8	-20.8	-2.7	-11.8	45.0	-2.5	21.2	1.0	-2.5	-0.8	-7.5b	-27.1c	-17.3c	-2.0	-16.0	-9.0	11.0	-3.5	3.8	-38.2d	8.8abc	-14.7	-7.6	-4.3	-6.0
LSD		*ns*			*ns*			*ns*			*App = 11.7* *AppxYr = 18.6*			*ns*			*ns*			*AppxYr = 40.3*			*ns*		
Slash Plus	V4	-1.6	-40.0	-20.8	13.3	-8.3	2.5	-30.1	-8.4	-19.3	5.2	10.4	7.8	2.0	-13.6	-5.8	4.0	14.0	9.0	43.2	-4.1	19.6	-20.1	-18.0	-19.1
	V4+V6	-23.4	27.1	1.9	10.1	-2.3	3.9	71.7	3.2	37.5	7.3	-16.9	-4.8	-24.1	3.4	-10.3	6.0	8.4	7.2	12.9	11.5	12.2	4.9	-13.5	-4.3
	V6	-11.9	14.5	1.3	-3.3	-3.3	-3.3	13.3	-6.6	3.3	-16.2	5.5	-5.4	0.7	8.9	4.8	0.8	-4.1	-1.7	-14.7	17.5	1.4	-1.2	0.8	-0.2
	V8	-14.4	17.0	1.3	-4.9	6.9	1.0	-0.5	5.5	2.5	-2.4	-2.5	-2.5	2.6	11.8	7.2	8.5	9.2	8.9	-11.9	-16.9	-14.4	-8.9	-4.6	-6.8
LSD		*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*ns*		
Touchdown Forte	V4	5.2	11.0	8.1	-25.8	-3.2	-14.5	-26.0	3.4	-11.3	8.8	4.2	6.5	1.9	-20.7	-9.4	0.9	-27.6	-13.4	45.7	10.5	28.1	-22.8bd	-1.8abcd	-12.3c
	V4+V6	-26.2	11.1	-7.6	10.7	5.4	8.0	51.4	-14.3	18.6	-6.6	-4.2	-5.4	14.0	7.4	10.7	-1.0	15.3	7.2	-32.0	-1.7	-16.8	31.4a	7.1ab	19.3a
	V6	-35.7	-7.2	-21.5	-0.9	6.5	2.8	-14.5	-0.6	-7.5	-21.0	13.2	-3.9	-12.6	4.6	-4.0	7.9	-27.5	-9.8	-10.1	20.5	5.2	32.3a	-9.2abcd	11.6ab
	V8	-29.5	-5.7	-17.6	71.6	-0.7	35.4	4.0	19.2	11.6	-10.7	11.1	0.2	0.4	22.9	11.6	-17.9	9.0	-4.5	-12.5	6.4	-3.1	-1.2bc	6.8ab	2.8b
LSD		*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*ns*			*App = 12.6* *AppxYr = 43.3*		

p = *0.1*

Yr1 = 2017/2018; Yr2 = 2018/2019

LSD, least significant difference; ns, not significant

stressors on the plant and mask the possible effect that glyphosate might have on maize yield. Pline et al.[20] similarly reported greater sensitivity to glyphosate injury in GR soybean at higher temperatures, as warmer temperatures resulted in greater translocation of glyphosate to new meristematic areas within the plant. Despite the current study being conducted under conditions considered above the international norm for maize production[19], supplementary irrigation provided throughout the duration of the trials would have lessened the level of drought/heat stress experienced by the plants to some extent. Large variation in yield data generated within the same application time of various cultivar x product combinations nevertheless occurred, despite supplementary irrigation and the weed-free environment maintained throughout the season, suggesting that additional unknown external factors were at play. Our findings in this regard concur with that of the international community in that determining yield differences between glyphosate-treated and non-treated GR cultivars remains a challenge due to the influence of other environmental factors.[20] Future glyphosate yield related field studies would benefit greatly from more treatment replicates.

Investigating the possibility that glyphosate application at specific application times or growth stages would result in a more favourable yield response in GR cultivars (in the absence of weeds), it was necessary to assess whether any significant patterns became evident amongst the 32 cultivar x product combinations tested. Whether the glyphosate application time resulted in a response significantly greater or smaller than that of the untreated control, is accordingly not of importance here. A digestible manner in which the data could be approached is by first establishing the frequency at which significant differences occurred amongst the 32 cultivar x glyphosate product combinations. In this regard, application time as main effect resulted in significant differences observed in between 3.1% (RpE) and 25% (TKM) of the cultivar x glyphosate product combinations tested, depending on the yield-related parameter in question, whilst the application time x year interaction resulted in significant differences in 3.1% (KpR) to 21.8% (EpP) of the cultivar x glyphosate product combinations tested. From this result, it is evident that less than a quarter of the cultivar x glyphosate product combinations tested were affected in one way or the other by the application time of glyphosate, whilst in some cultivars, the effect, where present, was season dependent. Yield, being the most relevant parameter, was significantly affected by application time as main effect in 9.3% of the combinations tested, and by the application time x year interaction in 15.6% of the combinations tested. Focussing on the highest order interaction, no consistent pattern was evident across cultivar or product. A unique response was accordingly obtained by each of the cultivar x product combinations, which demonstrated significant differences, suggesting that it will be impossible to predict how any cultivar x product combination would react to glyphosate application at various growth stages.

The main concern of local producers is, however, whether the application of glyphosate itself, in the absence of weed pressure, results in a yield loss due to some form of genetic predisposition. In a recent local study, Odendaal[21] concluded that glyphosate application resulted in a reduction in plant height, dry mass and yield when applied at different growth stages of GR maize. The study, which evaluated two glyphosate products and five GR maize cultivars, also indicated that different GR maize cultivars showed significant variation in reaction to glyphosate applications, suggesting that some cultivars are more sensitive/tolerant to glyphosate than others. Seasonal variation was, similar to the current study, prominent within the field trials. In the current study, negative or positive values greater than the LSD represent instances in which a specific treatment resulted in a response which was either greater or poorer than that of the untreated control. In less than 6% of all cases where significance was observed in EpR, KpR, RpE, TKM or yield, a significant negative effect could be observed which was greater than that for the untreated control. For yield, only one cultivar x glyphosate product combination (KKS4851 x PowerMax) suffered yield losses which could be attributed to the application time of glyphosate. In this case, the effect was season dependent and was evident at the V4+V6 and V8 growth stages. Based on this observation, our findings from the current study concur with international research findings that the yield of GR maize cultivars is not significantly affected by the application of glyphosate or the application time thereof[8,13], under growing conditions in which water is not a limiting factor.

Conclusions

Whether the application of herbicides to transgenic, herbicide-resistance crops has a negative effect on crops, either directly or indirectly, has always been a controversial topic, with most of the controversy focussed on GR crops. Limited response was observed with the application of glyphosate at V4, V4+V6, V6 and V8 stages on eight South African GR maize hybrids evaluated with four different glyphosate products over two seasons. Only 3.1% of the cultivar x glyphosate product combinations evaluated showed a significant negative yield response which was greater than that of the untreated control. Based on the findings of the current study, we conclude that the application time of glyphosate (within label recommendations) did not affect yield consistently or sufficiently enough for it to be considered a threat to maize yields as evaluated with four glyphosate products and eight GM maize cultivars.

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Competing interests

We have no competing interests to declare.

Authors' contributions

M.C.: Project management, methodology, writing the first draft. P.T.M.: Data collection, writing revisions. L.M.: Data analyses, validation, writing revisions. A.E.J.S.d.T.: Project leadership, writing revisions, mentorship to M.C.

References

1. Monaco TJ, Weller SC, Ashton FM, editors. Herbicide resistance in crops and weeds. In: Weed science: Principles and practices. 4th ed. New York: John Wiley & Sons, Inc. 2002. p. 379–396.

2. Green JM, Castle AL. Transitioning from single to multiple herbicide-resistant crops. In: Nandula VK, editor. Glyphosate resistance in crops and weeds: History, development, and management. Hoboken, NJ: Wiley; 2010. p. 67–91. https://doi.org/10.1002/9780470634394.ch4

3. Baylis AD. Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. Pest Manag Sci. 2000;56:299–308. https://doi.org/10.1002/(sici)1526-4998(200004)56:4%3C299::aid-ps144%3E3.3.co;2-b

4. Huber DM. Agricultural chemical and crop nutrient interactions – current update. In: Proceedings of Fluid Fertilizer Forum; 2010 February 14–16; Scottsdale, AZ, USA. Manhattan, KS: Fluid Fertilizer Foundation; 2010.

5. Bott S, Tesfamariam T, Candan H, Cakmak I, Roemheld V, Neumann, G. Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (*Glycine max* L.). Plant Soil. 2008;312:185–194. https://doi.org/10.1007/s11104-008-9760-86

6. Costa FR, Rech R, Duke SO, Carvalho LB. Lack of effect of glyphosate and glufosinate on growth, mineral content, and yield of glyphosate and glufosinate resistant maize. GM Crops Food. 2018;9:189–198. https://doi.org/10.1080/21645698.2018.1511204

7. Thelen KD, Penner D. Yield environment affects glyphosate-resistant cultivar response to glyphosate. Crop Sci. 2007;47(5):2098–2107. https://doi.org/10.2135/cropsci2007.02.0062

8. Elmore RW, Roeth FW, Nelson LA, Shapiro CA, Klein NR, Knezevic SZ, et al. Glyphosate resistant soybean cultivar yields compared to sister lines. Agron J. 2001;93(2):408–412. https://doi.org/10.2134/agronj2001.932408x

9. Elmore RW, Roeth FW, Klein RN, Knezevic SZ, Martin A, Nelson LA, et al. Glyphosate resistant soybean cultivar response to glyphosate. Agron J. 2001;93(2):404–407. https://doi.org/10.2134/agronj2001.932404x

10. Mueller TC, Main CL, Thompson MA, Steckel LE. Comparison of glyphosate salts (isopropylamine, diammonium, and potassium) and calcium and magnesium concentration on the control of various weeds. Weed Technol. 2006;20:164–171. http://dx.doi.org/10.1614/wt-05-038r.1

11. Parker RG, York AC, Jordan DL. Comparison of glyphosate products in glyphosate-resistant cotton (*Gossypium hirsutum*) and corn (*Zea mays*). Weed Technol. 2005;19:796–802. https://doi.org/10.1614/wt-040271r2.1

12. Wyrill JB, Burnside OC. Absorption, translocation, and metabolism of 2,4-D and glyphosate in common milkweed and hemp dogbane. Weed Sci. 1976;24:557–566. https://doi.org/10.1017/s0043174500062949

13. Pline WA, Viator R, Wilcut JW, Edmisten KL, Thomas JF, Wells R. Reproductive abnormalities in glyphosate-resistant cotton due to lower cp4-epsps levels in male reproductive tissue. Weed Sci. 2002;50:438–447. https://doi.org/10.1614/0043-1745(2002)050[0438:raigrc]2.0.co;2

14. Yasuor H, Abu-Abied M, Belausov E, Madmony A, Sadot E, Riov J, et al. Glyphosate-induced anther indehiscence in cotton is partially temperature dependent and involves cytoskeleton and secondary wall modifications and auxin accumulation. Plant Physiol. 2006;141:1306–1315. https://doi.org/10.1104/pp.106.081943

15. Thomas WE, Pline-Srnic WA, Thomas JF, Edmisten KL, Wells R, Wilcut JW. Glyphosate negatively affects pollen viability but not pollination and seed set in glyphosate resistant corn. Weed Sci. 2004;52:725–734. https://doi.org/10.1614/ws-03-134r

16. Pline-Srnic W. Technical performance of some commercial glyphosate-resistant crops. Pest Manag Sci. 2005;61:225–235. https://doi.org/10.1002/ps.1009

17. Purba E, Desmarwansyah N. Growth and yield of glyphosate-resistant corn under different timing of glyphosate application. Asian J Plant Sci. 2008;7(7):692–695. https://doi.org/10.3923/ajps.2008.692.695

18. Ritchie SW, Hanway JJ, Benson GO. How a corn plant develops. Special report 48. Ames, IA: Iowa State University of Science and Technology, Cooperative Extension Service; 1997.

19. Shaw RH. Climatic requirement. In: Sprague GF, editor. Corn and corn improvement. 2nd ed. Madison, WI: American Society of Agronomy, Inc., 1977. p. 591–623.

20. Pline WA, Wu J, Hatzios KK. Effects of temperature and chemical additives on the response of transgenic herbicide-resistant soybeans to glufosinate and glyphosate applications. Pestic Biochem Physiol. 1999;65:119–131. https://doi.org/10.1006/pest.1999.2437

21. Odendaal HB. Effect of glyphosate applications on growth responses of various glyphosate tolerant maize cultivars [MSc dissertation]. Potchefstroom: North-West University; 2016.