TITLE:

The interaction between wheat (*Triticum aestivum*) establishment timing and pre-emergent herbicides mixture choice on annual ryegrass (*Lolium rigidum*) seed production.

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Key Words; wheat, *Triticum aestivum* L., weeds, annual ryegrass, *Lolium rigidum* Gaudin, Pre-emergent herbicides, Time of seeding, crop competition, herbicide degradation.

INTRODUCTION

With the development of more residual pre-emergent herbicides for use in no till farming systems, early seeding is now the optimum strategy to increase crop yields. However, earlier crop seeding into warm soils can also make weed control more problematic as the residual capabilities of the pre-emergent herbicides become a very important attribute. Past studies have demonstrated that pre-emergent herbicide residual time and crop competition through increased seeding rates provide excellent ryegrass control when seeding dry without glyphosate. Further refinement of this message is however needed as growers use herbicide mixes. This project assesses the herbicide bioavailability of singular and mixed chemistries for a total of 18 herbicide treatments when applied across two different times of sowing (TOS). These TOS treatments include dry seeded before weed emergence, negating the need for a glyphosate knockdown and delayed seeding following a 5-week delay, into moisture and following a weed germination.

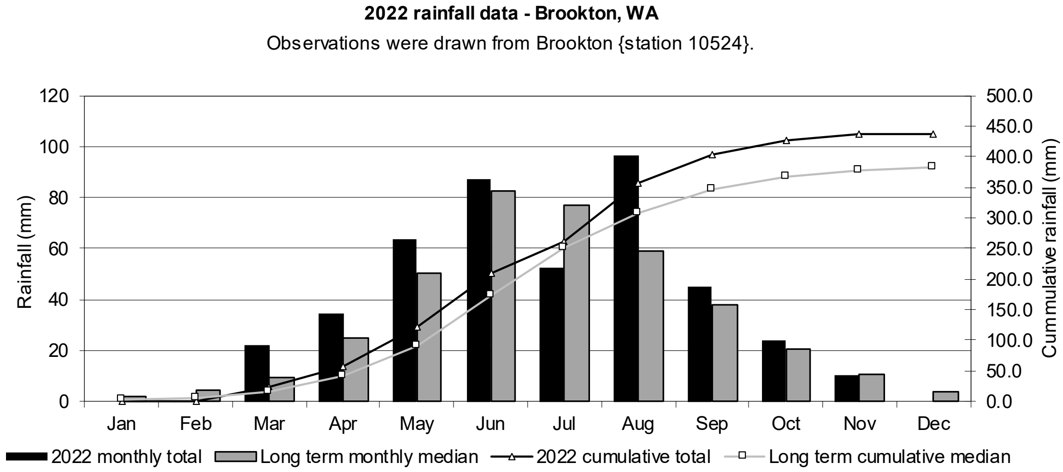
MATERIALS AND METHODS

Locations

This study was conducted over three sites in the Western Australian grainbelt during the 2022 growing season. Site 1 – Brookton (-32.245361S, 116.958917E), Site 2 – Beverley (-32.045413S, 116.968702E), Site 3 – Muresk (-31.733248S, 116.671147E). The soil characterization can be found in Table 1 with rainfall outlined in Figure 1.

Table 1 Soil description at Brookton and Beverley 2022 experimental sites.

		Site 1 – Brookton	Site 2 – Beverley	Site 3 – Muresk
	Depth (cm)	0-10	0-10	0-10
MIR - Aus Soil Texture		Loamy sand	Sandy loam	Loamy sand
Gravel % Visual Assessment	%	0	5	0
Colour		dark reddish brown	brown	Brown
Nitrate - N (2M KCl)	mg/kg	15	37	34
Ammonium - N (2M KCl)	mg/kg	<1	3	4.7
Colwell Phosphorus	mg/kg	18	40	47
Colwell Potassium	mg/kg	95	110	63
KCl Sulfur (S)	mg/kg	5.3	11	12
Boron	mg/kg	0.31	0.49	0.3
Organic Carbon (W&B)	% (40Â°C)	1.03	3.21	1.49
Exchangeable Al	mg/kg	<1.8	<1.8	<1.8
Calcium (Ca) - AmmAc	mg/kg	800	796	1090
Copper (Cu)	mg/kg	0.69	0.67	0.23
Zinc (Zn)	mg/kg	0.81	1	0.55
Manganese (Mn)	mg/kg	14	8.8	9.1
Magnesium (Mg) - AmmAc	mg/kg	81	79	96
Iron (Fe)	mg/kg	23	66	61
Salinity EC 1:5	dS/m	0.064	0.21	0.15
pH 1:5 water	pH units	6.05	6.26	6.8
pH CaCl2 (following 4A1)	pH units	5.12	5.95	6.36
Calcium (Ca) - AmmAc	cmol/kg	3.99	3.97	5.43
Magnesium (Mg) - AmmAc	cmol/kg	0.666	0.651	0.786
Potassium (K) - AmmAc	cmol/kg	0.254	0.261	0.132
Sodium (Na) - AmmAc	cmol/kg	0.064	0.299	0.252
Exchangeable aluminium	cmol/kg	<0.02	<0.02	<0.02
Manganese (Mn)	cmol/kg	0.052	0.032	0.033
MIR - Sand (+20 micron)	%	85	81.7	80.2
MIR - Silt (2-20 micron)	%	9.1	7.8	17.5
MIR - Clay	%	5.9	10.5	2.3
Organic matter	%	2.1	6.4	3
PBI + Col P		24	74	23



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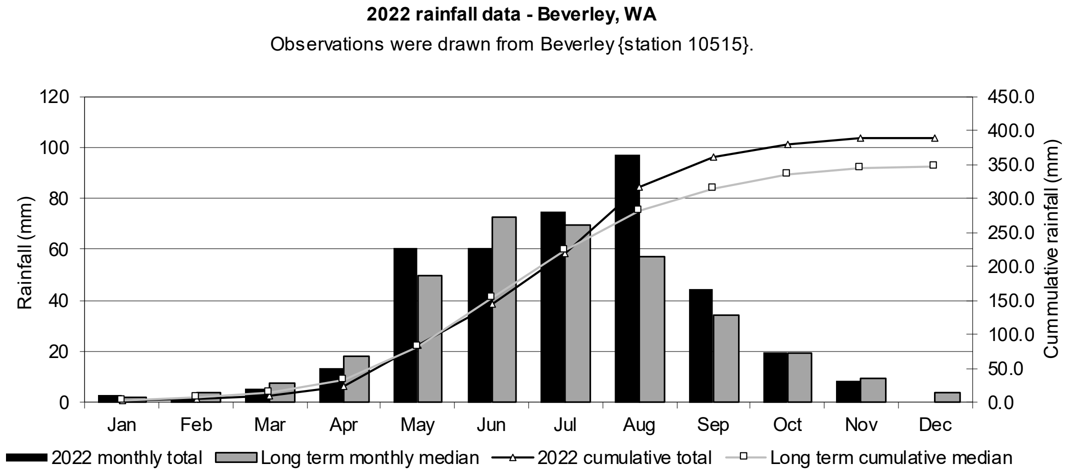

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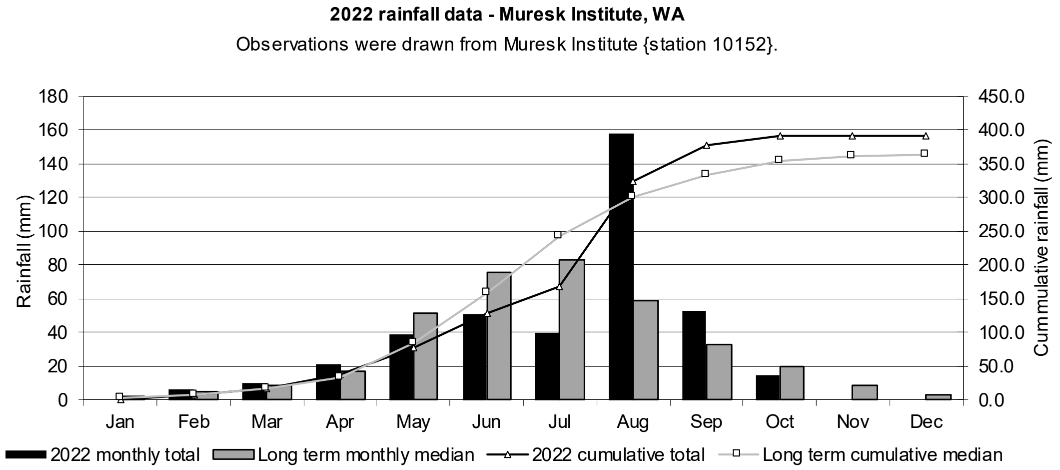


C.

Figure 1 Cumulative and Average monthly rainfall for A. Site-1 Brookton, B. Site-2 Beverley, and C. Site-3 Muresk.

Trial establishment

The first time of sowing (TOS1) took place in the first week of May and the second time of sowing (TOS2) in the second week of June. Each trial was direct seeded into cereal stubble. A factorial combination of pre-emergent herbicide chemistry and time of seeding (TOS 1 plus TOS 2 (5-week delay)) was randomized in complete blocks with four replicates (Table 2). The wheat variety used was Scepter (AGT, Australia) which is a high yielding, mid maturing variety, seeded at 25cm row spacing. The site was sown with no tillage tine openers with press wheels to provide sufficient seed soil packing and promote good weed germination. All plots were planted at one sowing depth (approx. 3cm) to minimize the confounding effects of emergence rate and seeding depth differences on biomass and grain yield. The wheat seed was treated with a fungicide/insecticide seed treatment comprising of 300ml/ha of Uniform [322 g/L Azoxystrobin + 124 g/L Metalaxyl-M, Syngenta Australia] and 500mL/ha Aviator Xpro [75 g/L bixafen + 150 g/L prothioconazole, Syngenta Australia], applied to the fertiliser to protect against foliar fungal disease. Immediately prior to seeding, the whole experimental area was treated with 1.5L ha^{-1} Roundup Ultramax (Glyphosate 540 g/L, Sinochem Australia), 100ml ha^{-1} Lontrel (Clopyralid 750g/L, DowAgrosciences Australia), to control all germinated weeds; followed by the application of each individual plot's pre-emergent herbicide treatment (Table 2).

To control dicotyledonous species such as wild radish (*Raphanus raphanistrum* L.), all plots had a post emergent application of 670 ml/ha Velocity (210 g L Bromoxynil + 37.5 g L Pyrasulfotole, Bayer Australia). For the duration of this study, no additional annual ryegrass control was applied. All herbicides were applied using a motorized sprayer calibrated to deliver a carrier volume of 120L water ha^{-1} at 275 kPa pressure. Each plot size was 2.2m wide by 10m long. To ensure optimal wheat growth, 100 kg/ha Gusto Gold (Summit Fertilisers Australia) (N – 10.2%, P- 13.1%, K- 12%, S- 7.6%, Cu- 0.07%, Zn- 0.14% and Mn- 0.01%) was drilled 3cm below the seed to minimise contact with the germinating wheat seed. To optimise crop growth supplementary nitrogen fertiliser in the form of urea (Summit fertilisers Australia) (N- 46%) was applied to all plots at Zadok's growth stage Z22 and Z39.

Table 2: Factorial combinations of pre-emergent herbicide treatment and TOS at the Brookton, Beverley and Muresk trial sites in 2022.

Trade name in text	Factor 1 - Time of sowing treatment	Herbicide timing	Comments
TOS 1	Dry seeded		8th May 2022
TOS 2	District practice (6 weeks later - Frost optimal)		16th June 2022
	Factor 2 - Pre-emergent herbicide treatment and rate		
Nil	Nil (knockdown only – No pre-emergent herbicide)		Nil herbicide applied control (knockdown glyphosate only)
Trifluralin	Trifluralin 480g/L (rate 2000ml/ha)	Herbicides incorporated at seeding	Comparative control to TOS x seeding rate x pre-em trial *WARNING: Voraxor label for suppression only.
Sakura	Pyroxasulfone 850g/kg (rate 118g/ha)		
Mateno Complete	Aclonifen 400 g/L; Diflufenican 66 g/L; Pyroxasulfone 100 g/L (Mateno complete) (rate 1000ml/ha)		
Luximax	Cinmethylin 750g/L (Luximax) (rate 500mL/ha)		
Overwatch	Bixlozone 400g/L (Overwatch) (rate 1250ml/ha)		
Voraxor	250 g/L saflufenicil; 125 g/L trifludimoxazin) (Voraxor) (rate 200ml) *		
Avadex	Triallate 500g/L (Avadex extra) (rate 2000ml)		
Trifluralin+Luximax	Trifluralin (2000ml) - Luximax (500ml)		
Trifluralin+Sakura	Trifluralin (2000ml) – Sakura (118g)		
Trifluralin+Overwatch	Trifluralin (2000ml) – Overwatch (1250ml)		
Trifluralin+Mateno-complete	Trifluralin (2000ml) – Mateno complete (1000ml)		
Triallate+Luximax	Triallate (2000ml) – Luximax (500ml)		
Triallate+Sakura	Triallate (2000ml) - Sakura (118g)		
Triallate+Overwatch	Triallate (2000ml)- Overwatch (1250ml)		
Voraxor+Luximax	Voraxor (200ml) – Luximax (500ml)		
Voraxor+Sakura	Voraxor (200ml) – Sakura (118g)		
Voraxor+Overwatch	Voraxor (200ml) – Overwatch (1250ml)		
Overwatch+Sakura	Overwatch (1250ml)– Sakura (118g)		Highly residual mix (Control)–

Annual ryegrass density was assessed at 10 WAE by counting the number of plants present in four 33 x 33cm quadrants (0.11 m^{-2}) per plot. Above ground biomass samples of annual ryegrass were removed 27 WAE in three 0.25m^2 quadrats per plot. Biomass samples were dried at 60^oC and weighed; followed by threshing and vacuum cleaning to obtain the total number of ARG seeds per plot. The seeds extracted were counted using an S-25 optical seed counter (Data Technologies, Kibbutz Tzora, Israel) and converted to the number of seeds produced per square meter. At 28WAS, the whole plot (10 m length with 6 by 25-cm rows) was machine harvested to determine grain yield.

Herbicide bioassay

Starting at the time of pre-emergent herbicide application (week 0) soil were collected from each plot at 3-week intervals. Soil samples were collected by sampling six 30mm diameter cores per plot to a depth of 10cm. Soil samples were immediately transferred into sealed plastic trays of dimensions 340 mm by 285 mm by 50 mm, with no holes and stored at <15°C for no more than 24 hours. Upon receipt at the University of Western Australia, all soil samples were moistened within the sampling trays using 75ml deionised water containing TWEEN 20 ionic surfactant (Polyethylene glycol sorbitan monolaurate, Sigma Adrich Australia). Fifty seeds from the known herbicide susceptible annual ryegrass biotype (VLR1) were seeded at 1 cm depth of the moistened soil in each tray before being placed in a temperature controlled naturally lit glasshouse (15°C night 25°C day). To ensure adequate seed germination, the containers were sealed for 48 hours before lids were removed for the remainder of the growth period. All trays were watered daily to maintain soil field capacity (Figure 2). The above ground shoot length was measured 21 days after sowing, with the percentage shoot length inhibition calculated as per (Khalil et al., 2018b) using the following formula.

$$\text{Inhibition (\%)} = 1-(Lt/Lo) \times 100\% \qquad [1]$$

where: Lt is the shoot length measured in the herbicide-treated soil or crop residue and L0 is the shoot length in the untreated soil or crop residue as per (Khalil et al., 2018a; Khalil et al., 2019b, Khalil et al., 2019a).

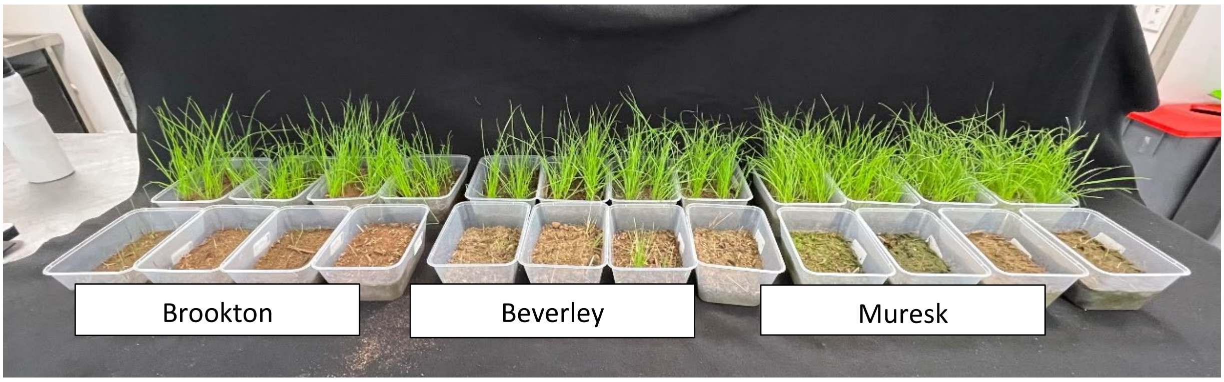


Figure 2 Experiment set up example for pre-emergent herbicide bioassay activity of Sakura (Pyroxasulfone 850 gai/kg) and Overwatch ® (Bixlozone (Isoxazolidinone) 400 g/L) mix 4 weeks after application. There are four replicates per site, the NIL herbicide treatments are located at the back of the photo.

Statistical Analysis

Experimental Design

As described above, the randomized complete block design (RCBD) accommodated two factorial experiments (time of seeding and pre-emergent herbicide). For each time of seeding, the treatment levels comprise factorial combinations of 19 levels. The 19 treatment combinations were replicated 4 times.

Statistical Models

The data is fitted with general linear mixed model [1]. The model of a response variable, such as *Wheat Yield (t/ha)*, is in the following form

$$Yield_{ijk} \sim N(\mu_{ik}, \sigma^2), [1]$$

where $\mu_{ik} = TOS_i \times Treatment_k$ is the observation that pre-emergent herbicide treatment k $(k = 1, \ldots, 21)$ were applied with TOSi $(i = 1,2)$. A two-dimensional separable autoregressive spatial structure AR1 x AR1 is assumed for the errors in the analysis, due to the plots of two times of seeding being conducted separately in this experiment. Hence, the replicates are nested within TOS and in the random term in the model. The model is fitted maximizing the restricted maximum likelihood (REML) using the R-package Asreml-R (Butler et al., 2009; Gilmour et al., 1995). The test for the fixed effect is performed using the Wald-test.

Due to similar annual ryegrass and wheat yield trends to herbicide application occurring amongst the Beverley, Brookton and Muresk sites, the results in this study have been analysed and reported as pooled data. The individual site data for each site is included as an appendix.

RESULTS:

Herbicide bioassay results for the pooled site data

The data attached below (Figure 3) is the predicted curves of the pooled herbicide bioavailability data for the Brookton, Beverley and Muresk sites in 2022. Immediately following seeding and at two weekly intervals thereafter, soil samples were taken of the top 10cm of soil from the inter-row region where herbicide would be concentrated following seeding. Using the herbicide susceptible annual ryegrass population (VLR1), herbicide bioavailability was measured as a percent of the untreated control. The lower the shoot length compared to the %UTC, the assumption is made that the herbicide is more bioavailable.

When assessing the bioavailability of the Luximax based treatments over time (Figure 3-A) it was found that the Voraxor+Luximax, Trifluralin+Luximax and Triallate+Luximax mix treatments all degraded at similar rates but was more residual than when Luximax was applied solely (TOS 1). When applied in the delayed seeding treatments (TOS 2), it was found that Trifluralin+Luximax mixture was initially the most effective treatment but degraded quickly. The Triallate+Luximax and Voraxor+Luximax mix treatments were more persistent when the crop was delayed seeded.

For the Mateno Complete treatments (Figure 3–B), it was found that the sole application of Mateno Complete was the most residual treatment, however there were no significant differences in persistence when mixed with Trifluralin (Trifluralin+Mateno Complete, TOS 1). When seeding was delayed (TOS 2), there was also a minimum difference between the Mateno Complete and Trifluralin+Mateno Complete mix.

For the Overwatch based treatments (Figure 3–C), the Sakura+Overwatch treatment was highly residual, followed by Triallate+Overwatch and Voraxor+Overwatch mixtures. The Overwatch treatment without a mixing partner was the least residual treatment (TOS 1). A similar response was demonstrated when the crop seeding time was delayed (TOS 2).

For the Voraxor based treatment (Figure 3-D) it can be seed that the straight Voraxor treatment provided limited residual effectiveness as Voraxor is registered for ARG suppression only. However, combining Voraxor with Sakura (Voraxor+Sakura) or Overwatch (Voraxor+Overwatch) did increase the persistence of the mix when early sown (TOS 1). This change in persistence was also demonstrated when seeding was delayed (TOS 2).

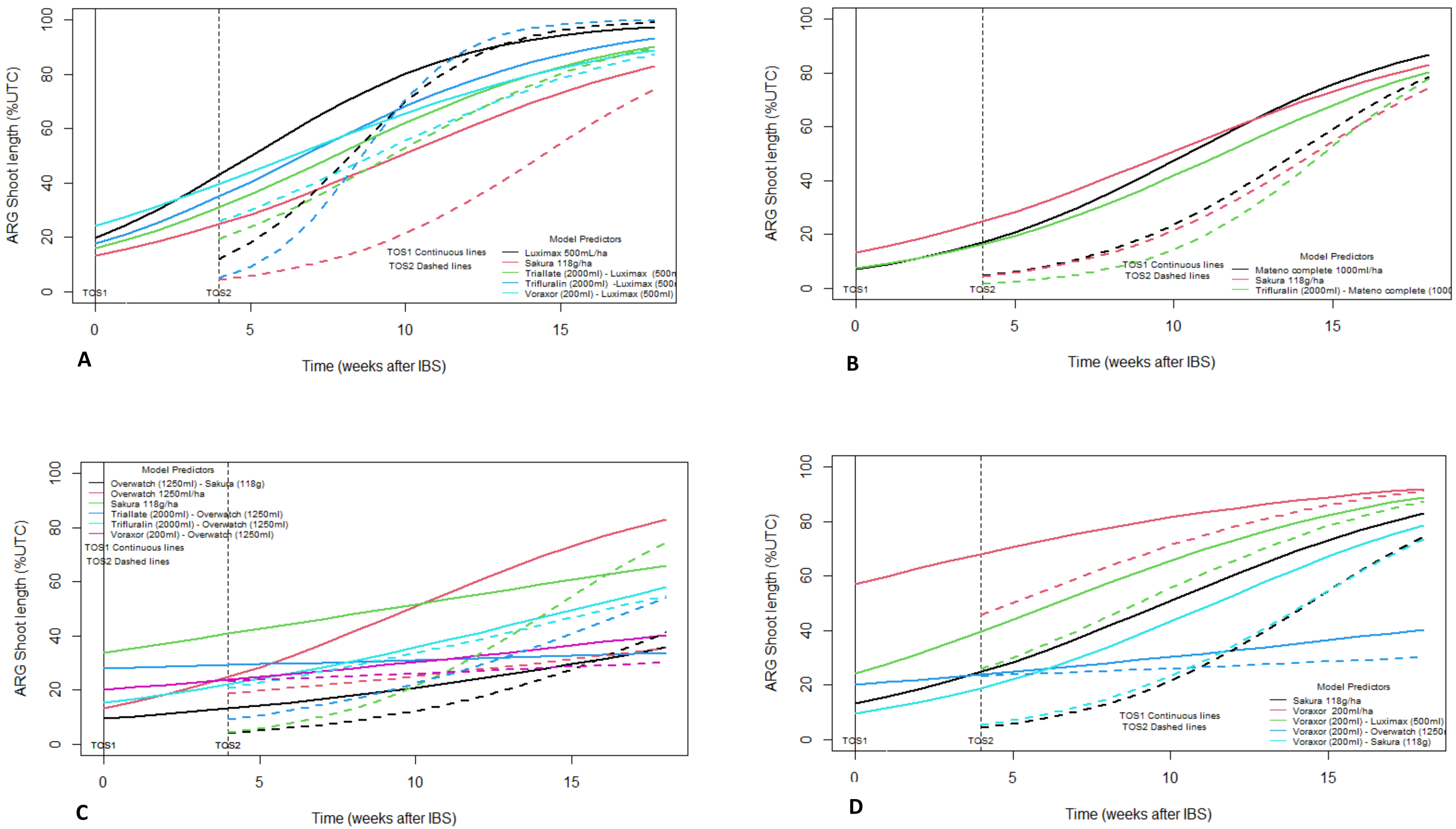
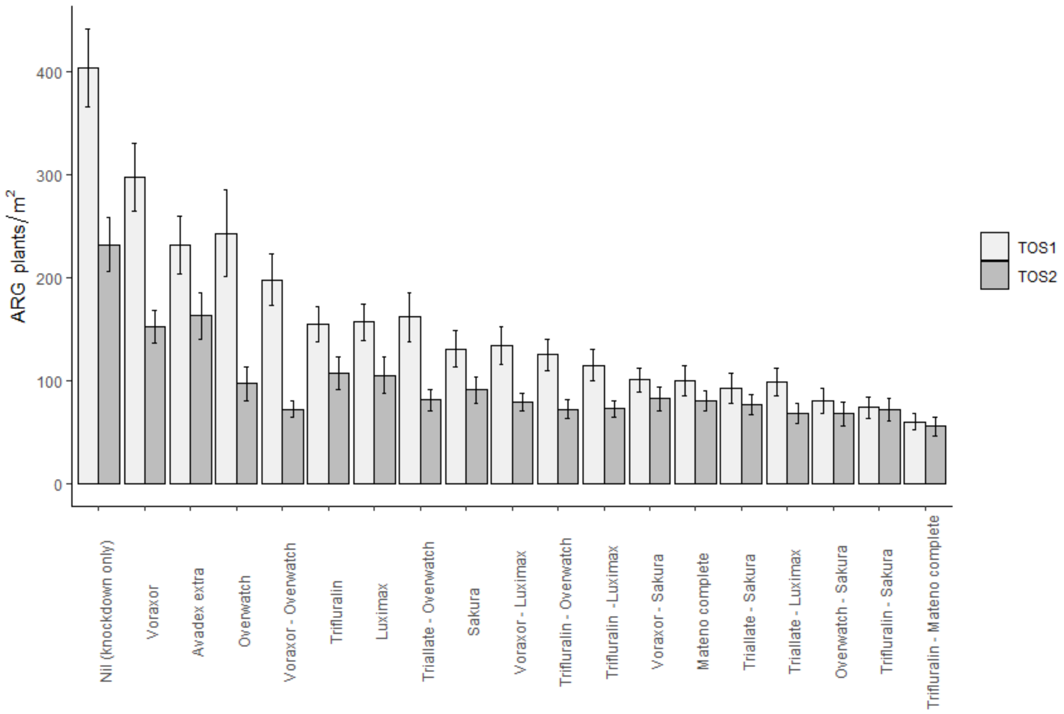


Figure 3 Pooled bioassay results for two times of sowing from the data from the Brookton, Beverley and Muresk sites in 2022. Results (TOS1- continuous lines, and TOS2- Dashed lines) are for ARG shoot length as % of untreated control (UTC) ***A****. shows Luximax treatments,* ***B****. shows Mateno complete treatments,* ***C****. shows Overwatch treatments, and* ***D****. shows Voraxor based treatments. For reference and comparison, all graphs contain the Sakura 118g/ha treatment*

Annual ryegrass establishment density and final biomass measured at harvest

The application of herbicide mixes was consistently found to reduce the establishment density of ARG and final ARG biomass when the wheat was dry seeded (TOS 1). When dry seeding, herbicides such as Voraxor and Avadex were found to be ineffective resulting in large ARG emergence densities and high ryegrass biomass (Figure 4). Interestingly, despite having a highly residual capability in previous studies, Overwatch was found to be an ineffective treatment in 2022 with treatments including straight Overwatch and the Voraxor+Overwatch mixture resulting in high ARG establishment and biomass at harvest (Figure 4). The failure of Overwatch to control ARG in this study requires further investigation. When dry seeding (TOS 1), herbicide treatments including Trifluralin+Sakura, Trifluralin+Mateno Complete, Overwatch+Sakura, Triallate+Sakura and straight Sakura provided excellent early control with ARG establishment and final ARG biomass when measured at harvest (Figure 4).

When seeding was delayed following an effective knockdown herbicide application (glyphosate), it was found that the ARG emergence was greatly reduced in the nil control as a large proportion of the germinable ARG seedbank was killed prior to crop establishment. Whist herbicide treatment consistently reduced the establishment of ARG in this study (Figure 4A), this reduction in the germinable seedbank limited the scale of the herbicide effect on ARG establishment ($P>0.05$). Herbicide mixes however influenced ARG biomass in TOS 2 at harvest (Figure 4B). Standalone herbicide treatments such as Voraxor, Avadex, all resulted in a larger ARG biomass compared to the more residual herbicides (outlined in Figure 3) used in this study, as once the herbicide became less bioactive, the ARG growth was no longer suppressed leading to higher biomass (Figure 4B). Herbicide treatments including Mateno Complete, Sakura, Trifluralin+Mateno Complete and the Triallate+Sakura mixture were found to be highly residual (Figure 3) resulting in reduced ARG biomass at harvest (Figure 4B).



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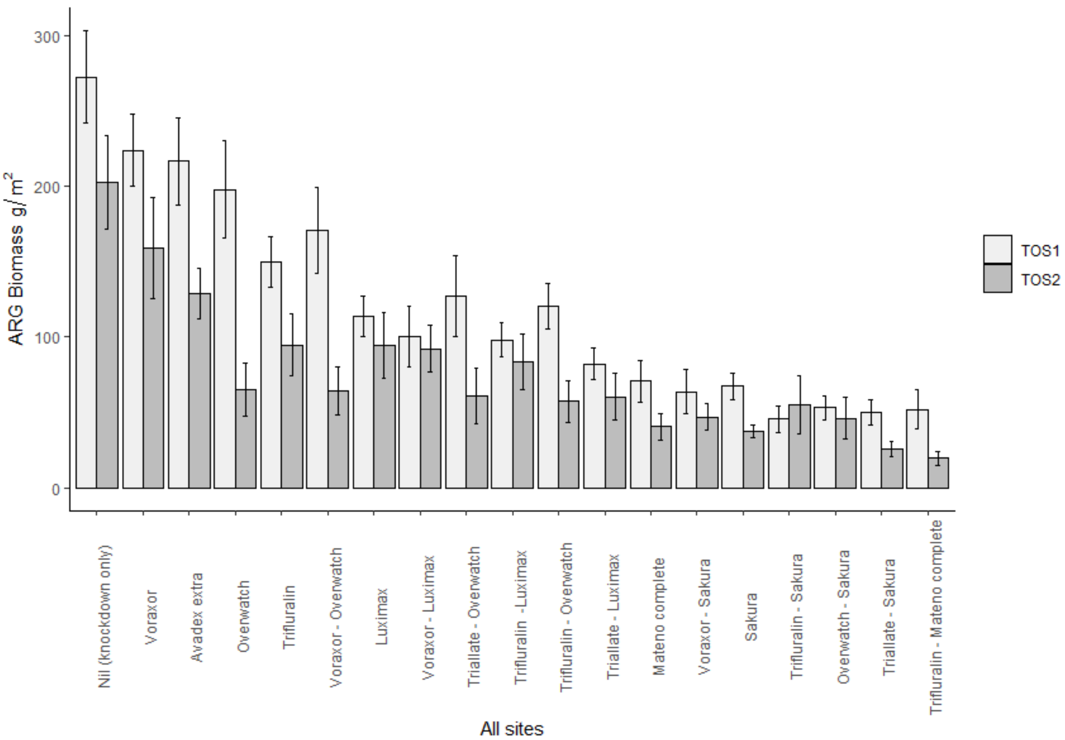


B.

Figure 4 Pooled A. ARG density 10 WAS and B. ARG Biomass at harvest time at two times of sowing (TOS) from the Brookton, Beverley and Muresk sites in 2022.

Annual ryegrass seed production

When dry seeding (TOS 1), herbicides such as Voraxor, Trifluralin, Avadex, Overwatch and the mixture of Overwatch+Avadex were found to be ineffective at reducing ARG seed production. However, herbicide treatments including Trifluralin+Mateno Complete, Triallate+Sakura, straight Sakura, Overwatch+Sakura, Trifluralin+ Sakura and Mateno Complete were found to be the most effective at reducing ARG seed production across the Brookton, Beverley and Muresk sites in 2022 (Figure 5).

When seeding is delayed following an effective knockdown herbicide application (glyphosate), it was found that the ARG seed production was greatly reduced in the nil control. The application of pre-emergent herbicides further reduced ARG seed production as compared to the knockdown treatment alone. In TOS 2, a wider range of herbicide treatments were effective in reducing ARG seed production. Herbicide treatments such as Trifluralin+Mateno Complete, Triallate+Sakura, Overwatch+Sakura, Voraxor+Sakura, Triallate+Overwatch, Triallate+Luximax, Trifluralin+Overwatch and straight Sakura or Mateno Complete all provided effective control of ARG seed production. The standalone herbicide treatment of Voraxor, Avadex, and Trifluralin, which are all shown in Figure 3 to provide limited residual efficacy, all resulted in larger ARG seed production (Figure 5).

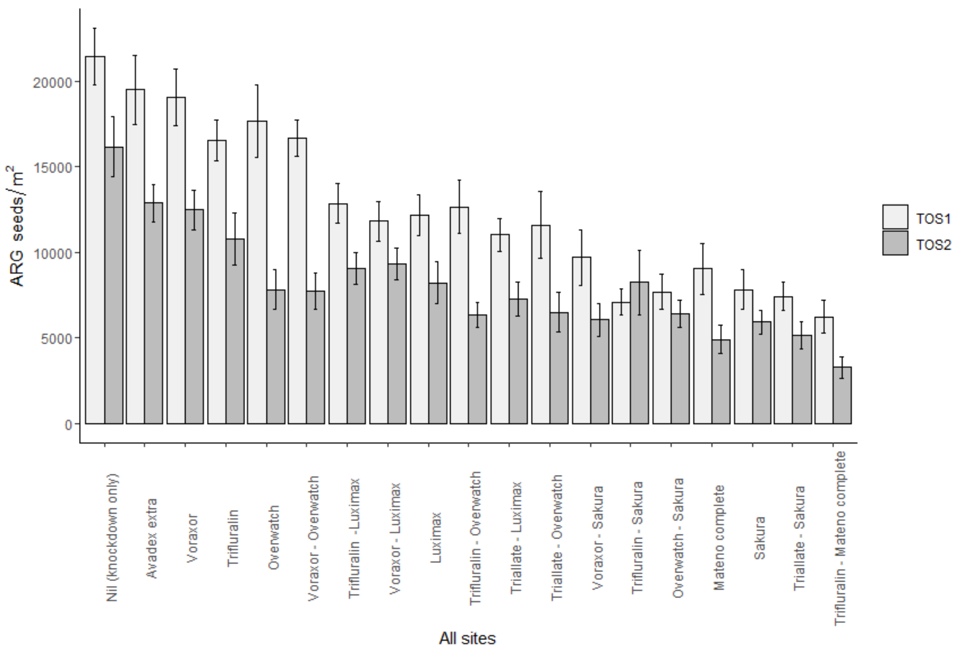


Figure 5: Pooled ARG seed production results measured at harvest time at two times of sowing from the Brookton, Beverley and Muresk sites in 2022..

Wheat Yield

Wheat yields both with TOS1 and TOS2 were found to be reduced with increases in ARG emergence and biomass (Figure 4), with the largest yields occurring within treatments with the greatest suppression of ARG biomass (Figure 6). In the dry seeded treatments (TOS1), the application of all herbicide treatments increased wheat yield compared to the untreated (nil) control. However, among the herbicide treatments, Voraxor, Avadex and Overwatch had the lowest yield (Figure 6). Herbicide treatments including Trifluralin+Mateno Complete, Triallate+Sakura and Voraxor+Sakura had the highest wheat yield across the Brookton, Beverley and Muresk sites in 2022 (Figure 6).

When the data was pooled across the Brookton, Beverley and Muresk sites in 2022, it was found that there was no significant difference in the yield of the nil herbicide applied treatments between the dry (TOS 1) and delayed (TOS 2) seeding timings (Figure 6). This lack of yield difference is expected to be due to the yield limiting effect of high ARG biomass in the TOS1 treatment and the effects of the lower yield potential of delayed seeding in theTOS2 treatment and lower resultant ryegrass density (Figure 4). While specific herbicide treatments increased wheat yield over the nil control (P<0.05), it was found that the scale of yield increase was modest compared to the dry seeded treatment (TOS 1) (Figure 6). When delayed seeding (TOS 2), treatments with high ARG biomass including Voraxor, Avadex, Luximax and the mixture of Voraxor+Luximax (Figure 4) also resulted in the lowest wheat yields that were not significantly greater than the nil control (Figure 6; P>0.05). The greatest wheat yields were achieved following the application of Trifluralin+Mateno Complete, Triallate+Sakura, Trifluralin+Overwatch, Triallate+Overwatch, Voraxor+Overwatch and straight Sakura.

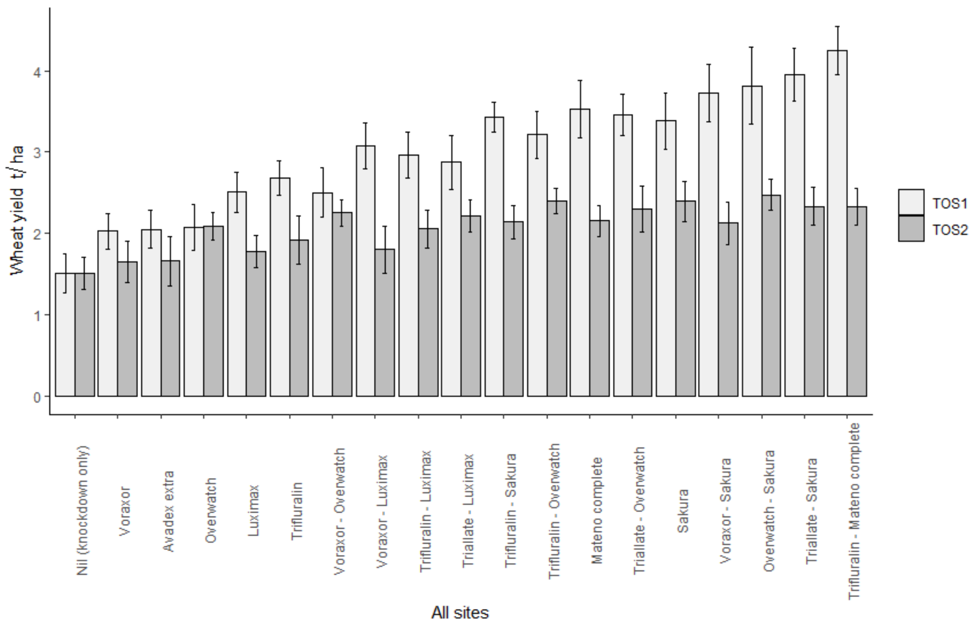


Figure 6 Pooled wheat yield results at two times of sowing. From the Brookton, Beverley and Muresk sites in 2022.

Appendix – Trial results from the Brookton, Beverly, and Muresk sites in 2022.

HERBICIDE BIOASSAY RESULTS FOR THE BROOKTON, BEVERLEY AND MURESK SITES IN WESTERN AUSTRALIA IN 2022

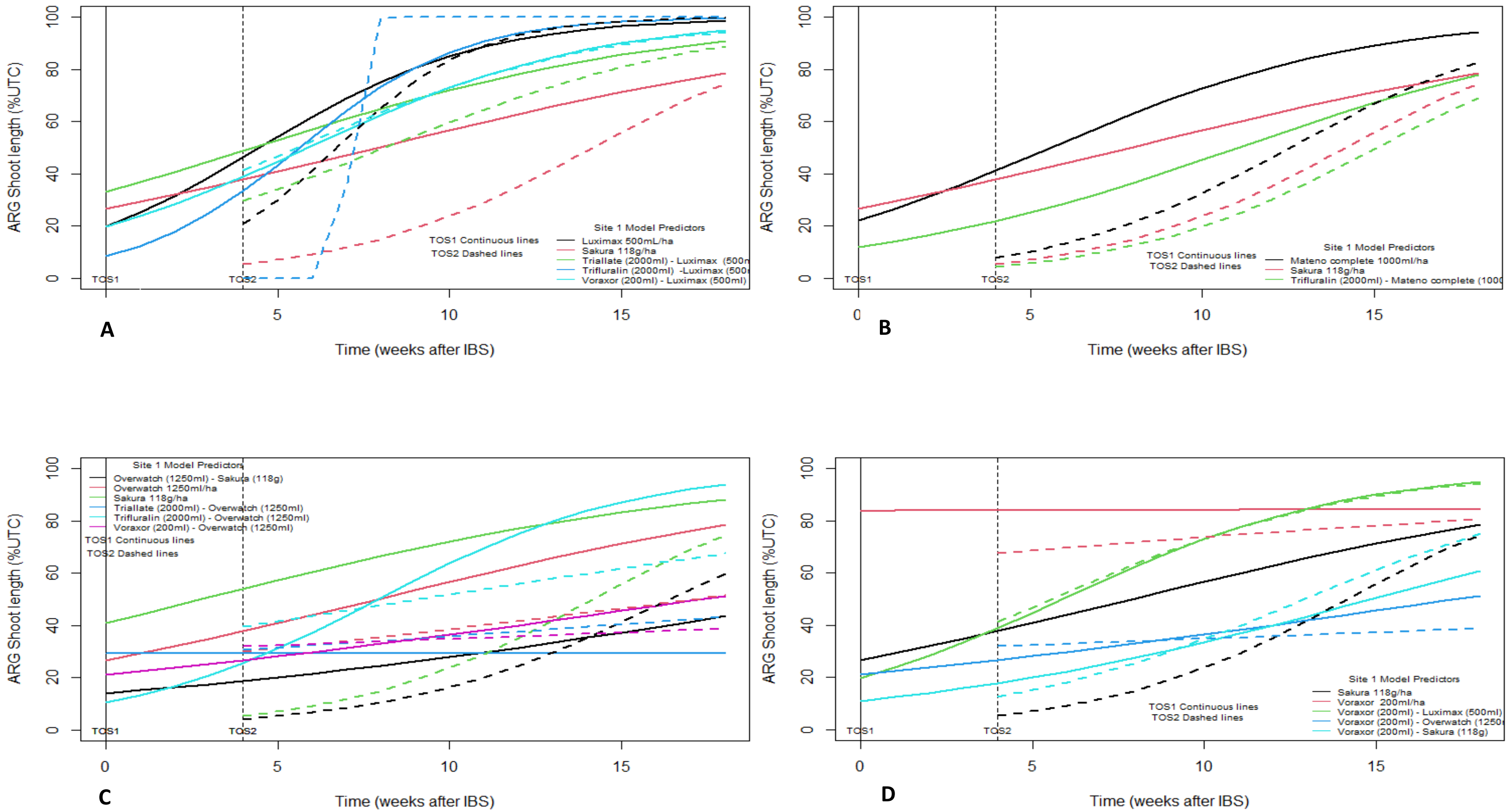


Figure 7 Bioassay results for two times of sowing for the Brookton site in 2022. Results (TOS1- continuous lines, and TOS2- Dashed lines) are for ARG shoot length as % of untreated control (UTC) ***A****. shows Luximax treatments,* ***B****. shows Mateno complete treatments,* ***C****. shows Overwatch treatments, and* ***D****. shows Voraxor based treatments. For reference and comparison, all graphs contain the Sakura 118g/ha treatment.*

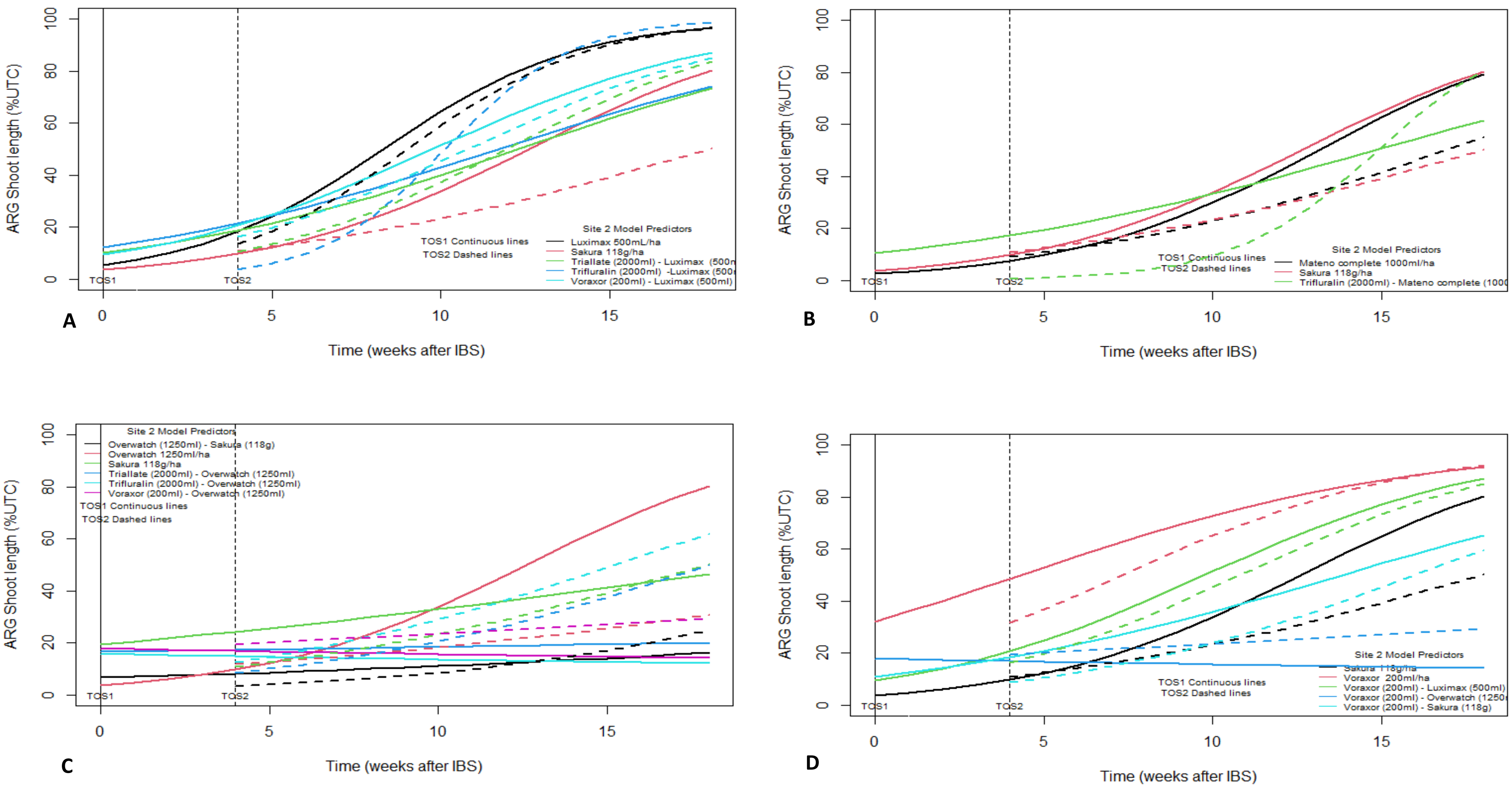


Figure 8 Bioassay results for two times of sowing for the Beverley site in 2022. Results (TOS1- continuous lines, and TOS2- Dashed lines) are for ARG shoot length as % of untreated control (UTC) A. shows Luximax treatments, B. shows Mateno complete treatments, C. shows Overwatch treatments, and D. shows Voraxor based treatments. For reference and comparison, all graphs contain the Sakura 118g/ha treatment

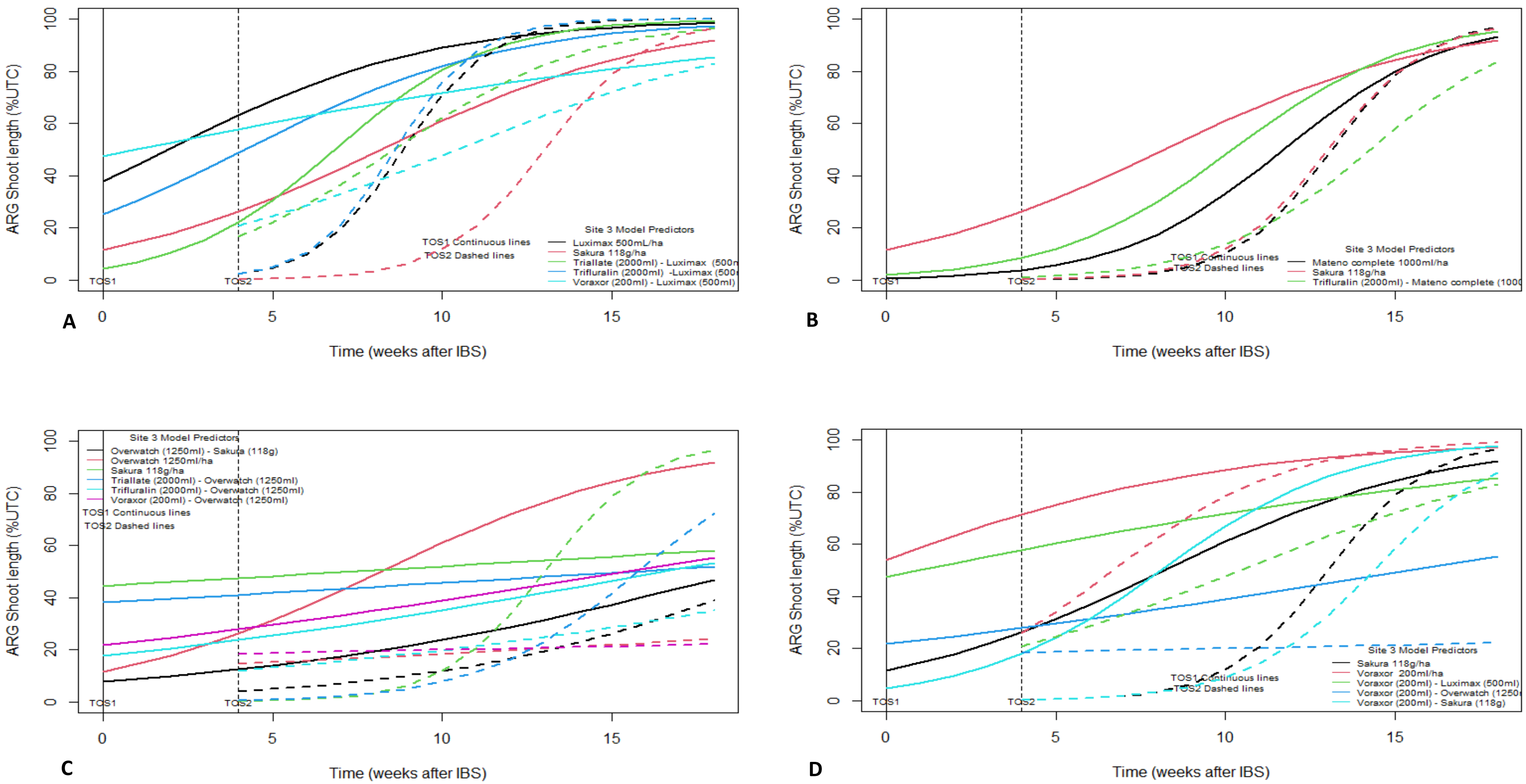
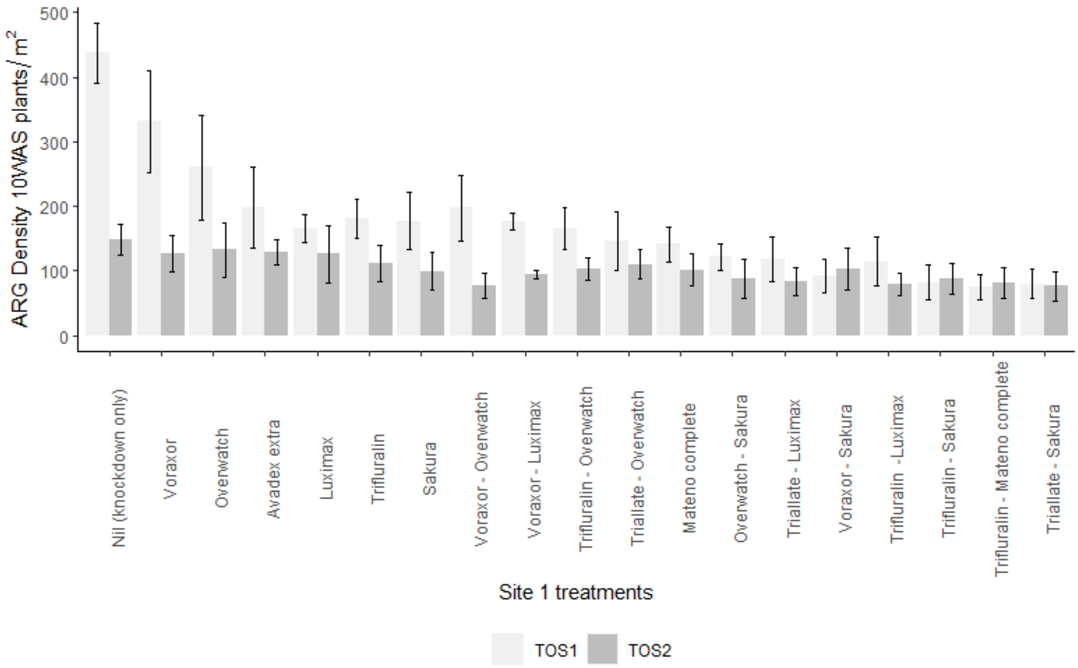


Figure 9 Bioassay results for two times of sowing for the Muresk site in 2022. Results (TOS1- continuous lines, and TOS2- Dashed lines) are for ARG shoot length as % of untreated control (UTC) A. shows Luximax treatments, B. shows Mateno complete treatments, C. shows Overwatch treatments, and D. shows Voraxor based treatments. For reference and comparison, all graphs contain the Sakura 118g/ha treatment.

ANNUAL RYEGRASS ESTABLISHMENT AND ANNUAL RYEGRASS BIOMASS (BROOKTON)



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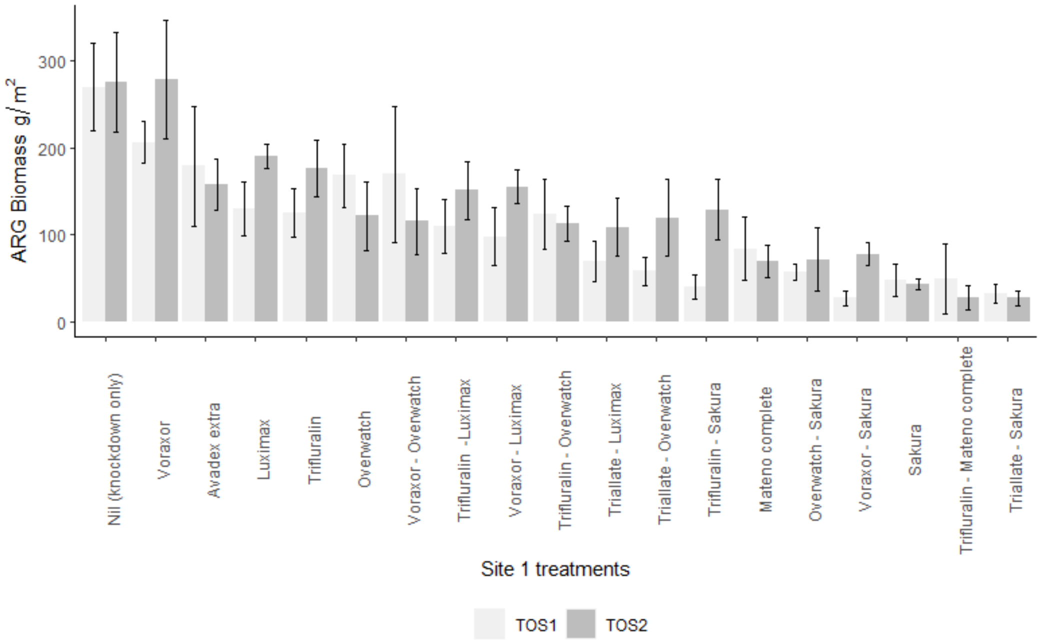
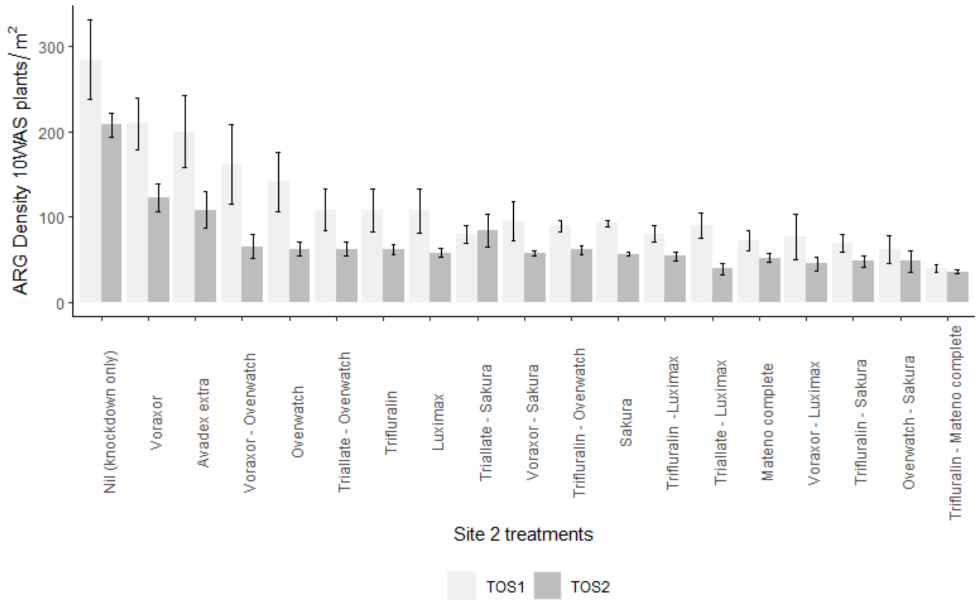


B.

Figure 10 A. ARG density 10 WAS, B. ARG Biomass at harvest time for the Brookton site at two times of sowing (TOS) where TOS2 was established 5 weeks after TOS1.

ANNUAL RYEGRASS ESTABLISHMENT AND ANNUAL RYEGRASS BIOMASS (BEVERLEY)



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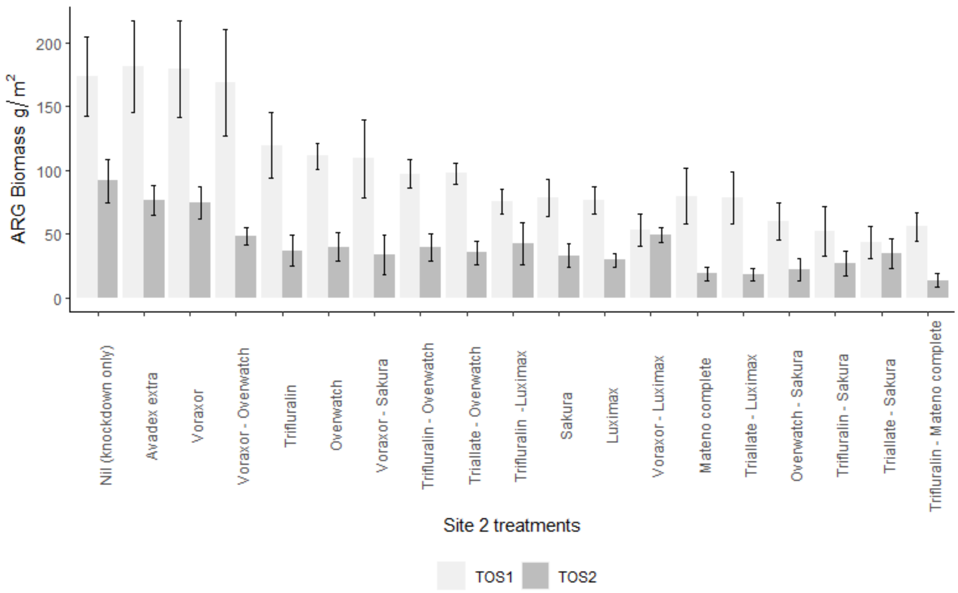


B.

Figure 11 A. ARG density at 10 WAS, B. ARG Biomass at harvest time for the Beverley site at two times of sowing (TOS) where TOS2 was established 5 weeks after TOS1.

ANNUAL RYEGRASS ESTABLISHMENT AND ANNUAL RYEGRASS BIOMASS (MURESK)

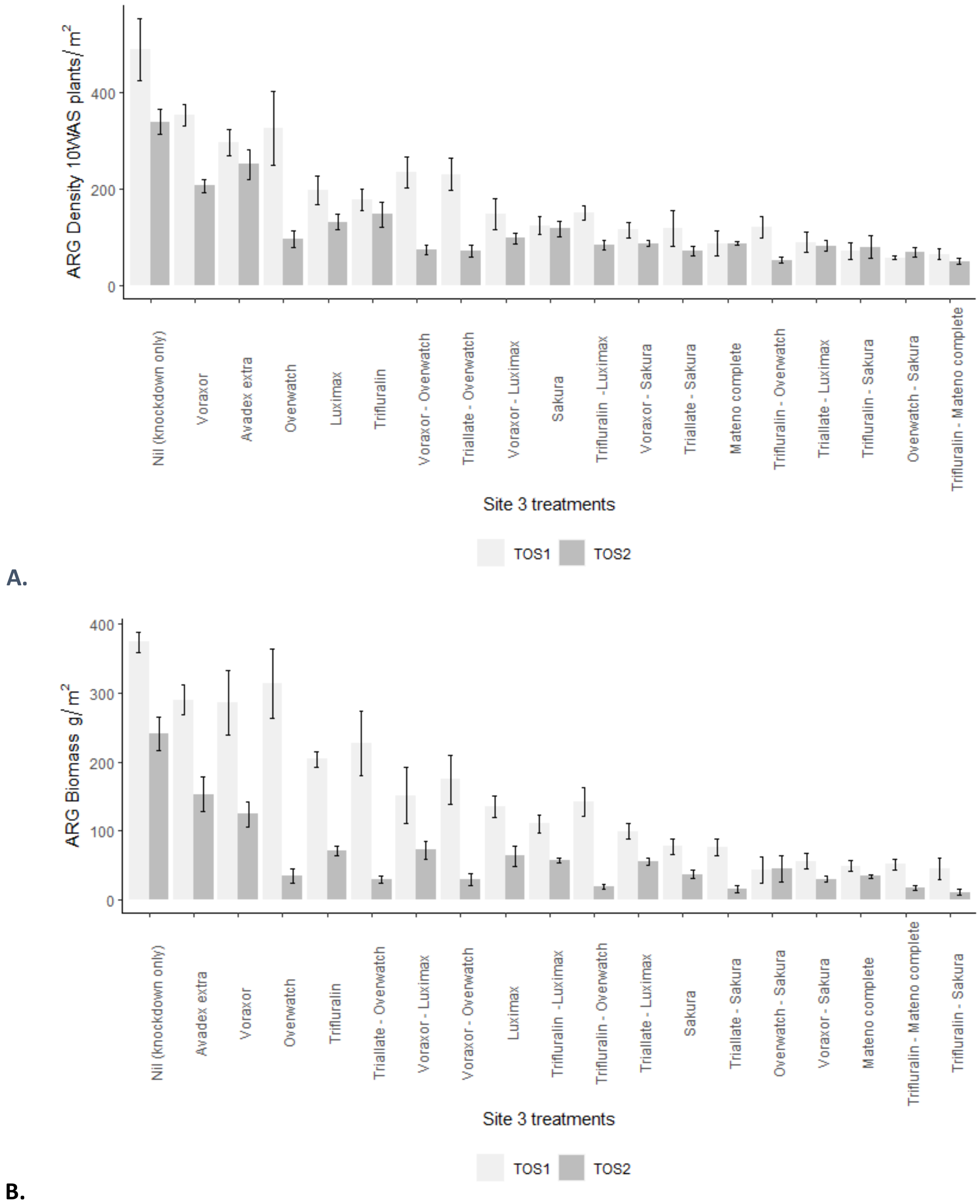
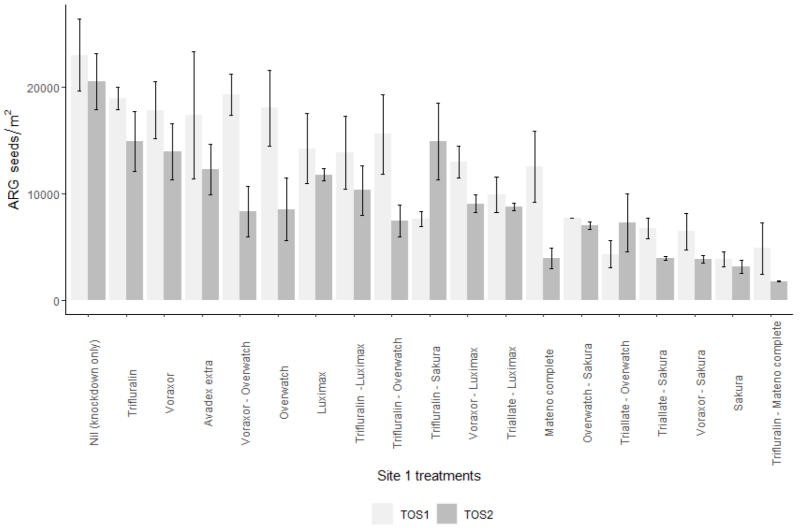
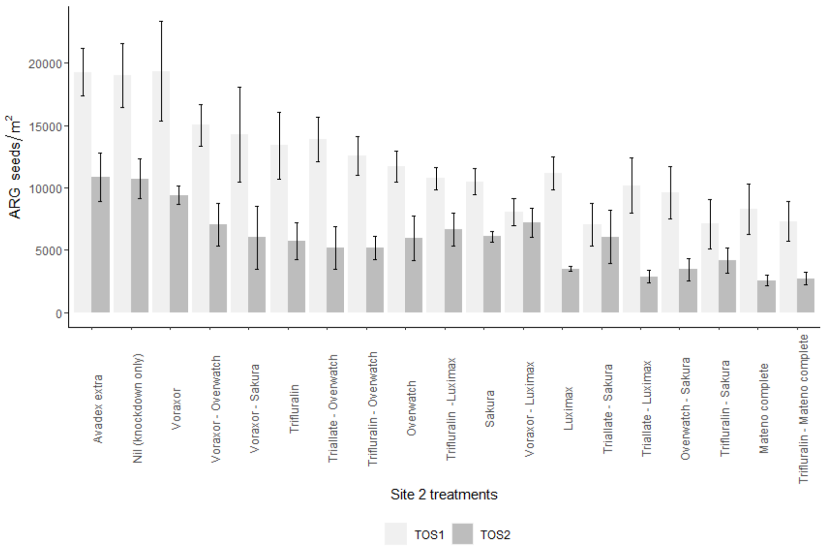


Figure 12 A. ARG density 10 WAS, B. ARG Biomass at harvest time for the Muresk site at two times of sowing (TOS) where TOS2 was established 5 weeks after TOS1.

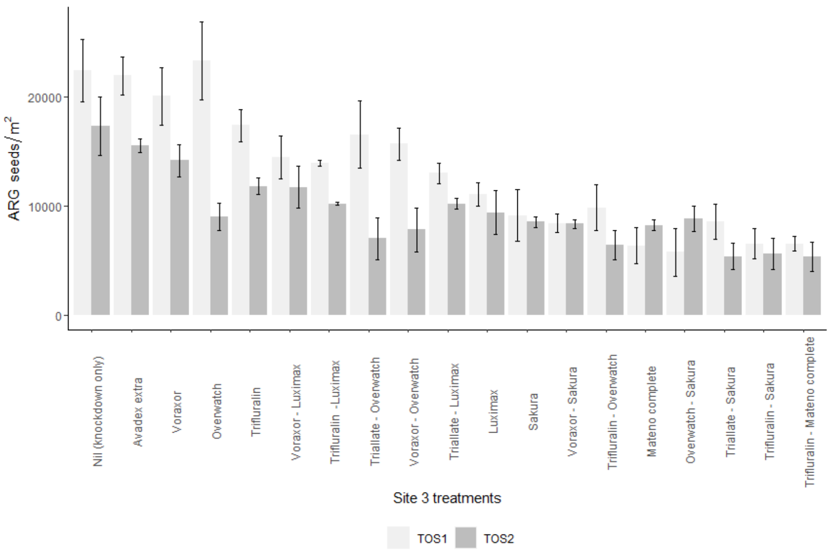
ANNUAL RYEGRASS SEED PRODUCTION FOR THE BROOKTON, BEVERLEY AND MURESK SITES IN 2022



A.



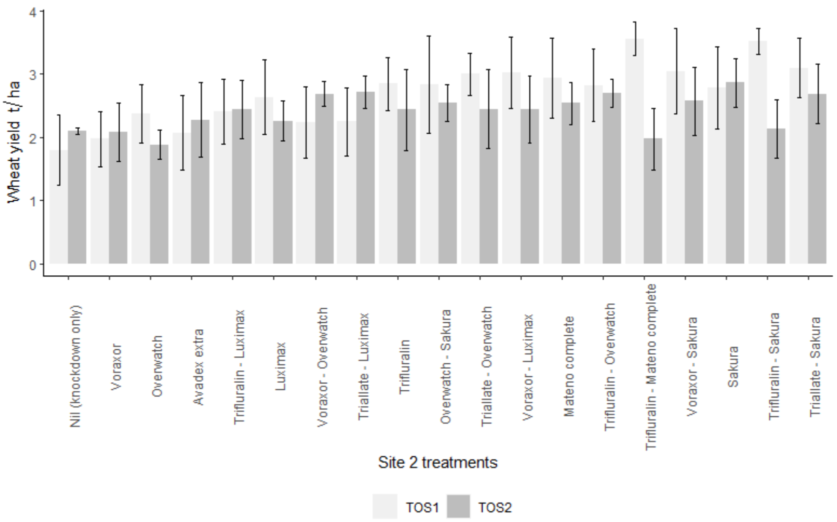
B.



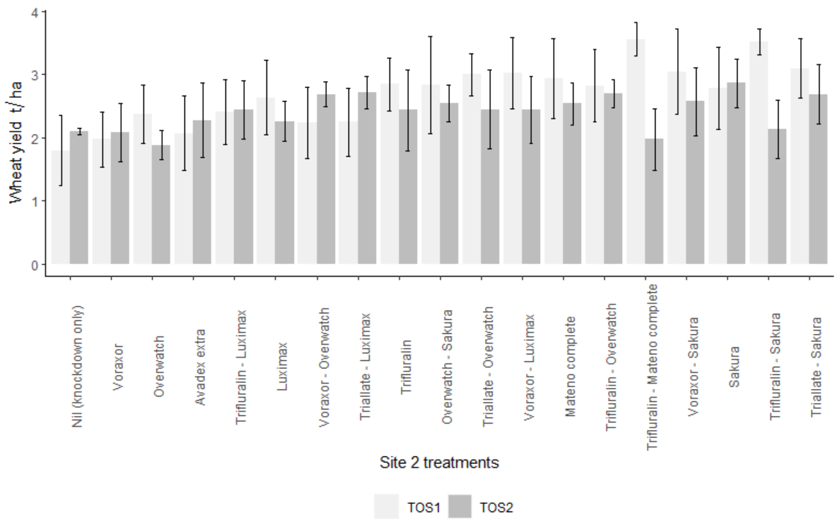
C.

Figure 13: ARG seed production for A. Brookton, B. Beverley and C Muresk sites measured at harvest time at two times of sowing (TOS) where TOS2 was established 5 weeks after TOS1.

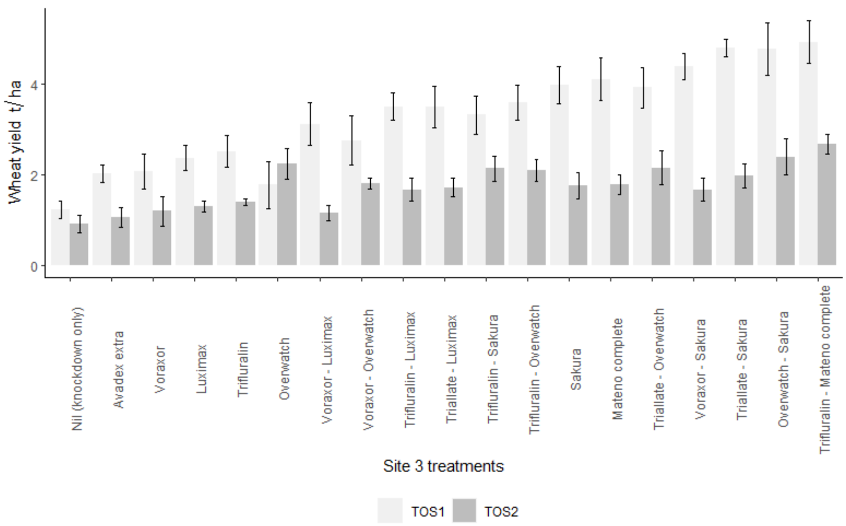
WHEAT YIELD FOR THE BROOKTON, BEVERLEY AND MURESK SITES IN 2022



A.



B. .



C.

Figure 14 Wheat yield for A. Brookton, B. Beverley and C. Muresk sites at two Times Of Sowing (TOS) where TOS2 was established 5 weeks after TOS1.