RUNNING TITLE:

Nitrogen placement and wheat competitiveness against annual ryegrass

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TRIAL 4: The contribution of nitrogen fertilizer timing and placement in reducing the growth and seed production of ryegrass (*Lolium rigidum* Gaudin) in competition with wheat (*Triticum aestivum* L.).

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INTRODUCTION

Managing fertilizer inputs in a dryland cropping system is an important yet underutilized component of integrated weed managements (Gill & Holmes, 1997; Liebman & Janke, 1990). Of all nutrients, the greatest competition between plants and weeds is for nitrogen (N) which is routinely applied by growers to optimize crop yield and grain quality (Patterson, 1995; Raun & Johnson, 1999) However the application of additional N can greatly alter the competitive balance between crops and weeds. In many situations, this is because increased available N in the form of applied fertiliser can greatly increases the weeds growth rate relative to the surrounding crop species, resulting in an increased capacity for weeds to compete with the crop (Ampong-Nyarko & De Datta, 1993; Robert E. Blackshaw et al., 2003; Carlson & Hill, 1985; Dhima & Eleftherohorinos, 2001; Morales-Payan, Santos, Stall, & Bewick, 1998; Peterson & Nalewaja, 1992a, 1992b; Supasilapa, Steer, & Milroy, 1992) However with modern sprayer technology and liquid fertilisers, N can accurately be placed on the row (known as streaming). As a result, the timing, rate and placement of N fertilisers can be now manipulated to maximise uptake of N by the crop and potentially reduce the availability of N to the weeds, therefore maximising crop competitiveness (Di Tomaso, 1995).

Past research has demonstrated that the placement of the N fertilizer influences the competitiveness of crops and weeds. Fertilizer placed in narrow bands below the soil surface, favoring crop growth compared to being surface broad-cast evenly across the site increasing weed growth and reducing the ability of crops to compete against wild oat (*Avena fatua* L.) (Kirkland & Beckie, 1998; Reinertsen, Elliott, Cochran, & Campbell, 1984), foxtail barley (*Hordeum jubatum* L.) (R. E. Blackshaw, Semach, Li, O'Donovan, & Harker, 2000) and downy brome (*Bromus tectorum* L.) (Rasmussen, 1995). This study determines the effect of N fertilizer placement, N fertiliser rate and N application timing on the competitive ability of wheat *(Triticum aestivum* L.*)* in dryland no-tillage cropping system in the Western Australian grainbelt.

MATERIALS AND METHODS

Locations

In 2018, an experiment was conducted east of York (-31.87S, 116.76E) in the Western Australian grainbelt. Soil at the site was a sandy loam over a medium calcareous clay subsoil with a pH of 5.9 with the long-term average growing season (April to October) rainfall at York being 367 mm (presented in Figure 1). Prior to this study, the York site had been under no-till production for 10 years. Soil sampling across the site indicated that the soil had a total N concentration of 32mg/kg (Ammonium nitrate 30 mg/kg, Nitrate nitrogen 2 mg/kg) and a soil bulk density of 1.4 g/cm^3, resulting in 44.8kg N ha^{-1} available to the crop in the top 10cm of soil.

Prior to seeding

In March 2018, before weed seed bank germinating rains, the baseline seedbank of both sites were estimated by collecting 4 replicate intact soil core samples (8 cm in diameter by 10 cm deep) up the center of each plot location (total of 384 soil samples per site). Soil samples from each plot (n=4) were combined to estimate the annual ryegrass seedbank in each plot. Samples were placed in shallow seedling trays that had been partially filled with weed free potting mix to ensure drainage. The soil samples from the field were then spread in a 2cm thick layer and watered using micro-jet irrigation. Samples were then maintained outside from March to August each year. Germinated seedlings were recorded and removed at regular intervals. The census for annual ryegrass was ceased in August when no new seedlings emerged over a 4 week period. The number of seedlings to germinate in each tray represented the germinable weed seedbank and was converted to seeds per square meter for each plot.

Trial establishment

In May 2018, the York experiment was direct-seeded into cereal stubble. A factorial combination of Urea and ammonium Nitrate (UAN) liquid nitrogen fertiliser placement (streaming over the crop row vs broadcast spray), UAN rate, and UAN application timing was randomized in complete blocks with four replicates (Table 1). The wheat variety used was Magenta (Intergrain Australia) which is a high yielding, mid-late maturing variety, seeded at 25cm row spacing at 75kg ha^{-1}. The site was sown with no tillage tine openers with press wheels to provide sufficient seed soil packing promote good weed germination. All plots were planted at only one sowing depth (approx. 2cm) in an effort to minimise the confounding effects of emergence rate and seeding depth differences on biomass and grain yield. 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), 250g ha^{-1} Diuron (Diuron, Syngenta Australia) and 2.5L ha^{-1} of Boxer Gold (800 g/L Prosulfocarb + 120 g L S-metolachlor, Syngenta Australia) to control all germinated weeds and provide modest post emergent annual ryegrass control to reduce weed densities within the trial.

To control wild radish (*Raphanus raphanistrum* L.) germinations, all plots had a post emergent application of 670 ml/ha Velocity (210 g L Bromoxynil + 37.5 g L Pyrasulfotole). No additional annual ryegrass weed control was applied. All herbicides were applied using a motorized sprayer calibrated to deliver a carrier volume of 120 L water ha^{-1} at 275 kPa pressure. Each subplot size was 4m 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. Depending on the treatment, urea and ammonium nitrate liquid fertiliser (UAN) (Summit fertilisers Australia) (N- 32%) was applied as per Table 1.

Table 1: Factorial combinations of Urea and ammonium Nitrate (UAN) fertiliser placement (streaming over the crop row vs broadcast spray), UAN rate, and UAN application timing applied to wheat in 2018.

Tmt	Basal treatment	Treatment description	Total N applied
1	-10 kg N ha^{-1} with establishment fertilizer	Nil N applied post emergent	10 kg N ha^{-1}
2	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN sprayed evenly post sowing	10 kg N ha^{-1} UAN sprayed evenly at GS21,	30 kg N ha^{-1}
3	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN banded to the crop row post sowing	10 kg N ha^{-1} UAN banded to the crop row at GS21	30 kg N ha^{-1}
4	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN sprayed evenly post sowing	10 kg N ha^{-1} UAN sprayed evenly at GS31	30 kg N ha^{-1}
5	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN banded to the crop row post sowing	10 kg N ha^{-1} UAN banded to the crop row at GS31	30 kg N ha^{-1}
6	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN sprayed evenly post sowing	40 kg N ha^{-1} UAN sprayed evenly at GS21,	60 kg N ha^{-1}
7	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN banded to the crop row post sowing	40 kg N ha^{-1} UAN banded to the crop row at GS21	60 kg N ha^{-1}
8	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN sprayed evenly post sowing	40 kg N ha^{-1} UAN sprayed evenly at GS31	60 kg N ha^{-1}
9	-10 kg N ha^{-1} with establishment fertilizer -10 kg N ha^{-1} UAN banded to the crop row post sowing	40 kg N ha^{-1} UAN banded to the crop row at GS31	60 kg N ha^{-1}

At ten weeks after emergence (WAE), wheat establishment was assessed by counting two adjacent 50cm rows over 4 replicate locations per plot. Annual ryegrass density was assessed at 10 and 14 WAE by counting the number of plant present in four replicate a 33 x 33cm quadrants (0.11 m^{-2}) per plot. To compare the growth of the wheat between treatments, Normalized Difference Vegetation Index (NDVI) was measured at 7, 10, 13 and 16 WAE using a Crop Circle™ Handheld Optical Sensor Unit (Holland Scientific, Lincoln, NE, USA) oriented 0.8m off the ground, perpendicular to the center row of the plot. NDVI quantifies vegetation by measuring the difference between near-infrared in which vegetation strongly reflects and red light which vegetation absorbs. In each plot three replicate NDVI measurements were made and reported as a plot average.

Measurements of the fractional green canopy cover (FGCC) were done using the Canopeo™ android application (www.canopeoapp.com) to estimate canopy development and light interception (Patrignani & Ochsner, 2015). Canopeo™ is an image analysis tool (Mathworks, Inc., Natick, MA) that uses color values in the red–green–blue (RGB) system to measure the green canopy cover percentage. Canopeo™ images were assessed for all weed free crop treatments at 13 WAE using a 14 megapixel camera that was oriented 0.8m above the top of the crop canopy, perpendicular to the center row of the plot.

Both incoming and outgoing photosynthetically active radiation (PAR) values were measured 14WAS at the top and bottom of the wheat canopy using line quantum sensor LI-191SA (LICOR Inc., Lincoln, NE, USA). The fraction intercepted (PAR) was calculated as per Monteith (1981)

$$PAR = \frac{(Io - I)}{I} \qquad [1]$$

: where Io is incident PAR at the top of canopy and I is the transmitted PAR at the bottom of the canopy.

Above ground biomass samples of annual ryegrass were removed 27 WAE in three 0.25m^2 quadrants per plot. Biomass samples were dried at 60^{o}C and weighed. From these samples, the number of ryegrass panicles were counted. In order to estimate annual ryegrass seed production, a representative sample of 50 panicles were collected from each plot and thrashed to extract seed. The number of seeds extracted was counted using an S-25 optical seed counter (Data Technologies, Kibbutz Tzora, Israel) to calculate the mean number of seeds produced per panicle. Total seed produced was estimated by multiplying the average seed yield per panicle by the number of panicles produced.

At 28WAS, the whole plot (10 m length with 6 by 25-cm rows) was machine harvested to determine grain yield. Grain samples (400 g) were analysed for moisture and protein using an Infratec™ Sofia Near Infrared Spectroscope (NIR) (FOSS analytics, VIC, Australia). To calculate the mean wheat seed weight, approximately 1500 seeds were counted using a S-25 optical seed counter (Data Technologies, Kibbutz Tzora, Israel) and weighed to calculate the mean wheat grain weight.

Statistical Analysis

Experimental Design

As described above, the randomised complete block design (RCBD) accommodated three factorial experiment (UAN placement, UAN timing and UAN rate) and additional control (Figure 1). The treatment levels comprise all factorial combinations of eight levels plus the control. The eight treatment combinations were replicated 4 times and the control 8 times and were allocated in 4 columns x 10 rows, where each column represents a replicate.

Statistical Models

The data were analysed using the classical techniques for the analysis of factorial experiment. The blocking structure accounted in the analysis was Replicate (Column). The treatment structure can be defined in two ways, addressing different objectives. The first allows comparing all 8 factorial combination and the control, i.e. using just a single factor with 9 levels. The second model, adopted here, allows assessing the significance of the main effect of UAN placement (topdressing), UAN timing and UAN rate, and their interactions. In order to allow a comparison between the control and the rest of the treatments, an additional two level factor Control was created. The latter divided the data into two groups, with and without any UAN application. Therefore, the treatment structure was defined and fitted in the model as Control/(UAN placement*UAN timing*UAN rate).

The above described model was used to analyse the following traits: Wheat establishment, ARG establishment, NDVI 4/07, NDVI 19/07, NDVI 31/07, Canopeo 18/07, Canopeo 3/09, Radiation interception (µmol m^{-2} s^{-1}), Radiation interception (%), ARG tissue content (% N), Total N uptake ARG (g N/m^2), Biomass ARG (g/m^2), Biomass Wheat (g), Total ARG Seed Production (seeds/m^2), Before treatment ARG soil seed bank (seeds/m^2), After treatment ARG soil seed bank (plants/m^2), Yield (t/ha), Wheat 1000 seed weight (g), Moisture (%) and Protein

(%). There was only one trait *Yield* where a linear mixed model was used in order to accommodate some spatial variability. The analysis did not reveal any global linear trends, the local variation was not strong, only low AR1 along the rows.

The analyses were conducted using R package msanova 1.0 (VSN International Ltd, Hemel Hempstead, UK).

Figure 1: Aerial photo of the York trial site in 2018.

Results

Wheat competitiveness factors

In this study the main effect of UAN placement (placement), UAN timing (timing) and UAN rate (rate) and their interactions were assessed in a field trial in York, Western Australia in 2018. In this trial, all of the main effects of timing and rate significantly affected the sequential measurements of normalized difference vegetation index (NDVI) which was used as an indicator of biomass accumulation. UAN rate alone affected wheat biomass at anthesis and % radiation interception.

However despite the main effects only being confined to timing and rate, interactions between these main factors (UAN placement, UAN timing and UAN rate) were apparent. Whilst it was apparent that applying an optimum UAN rate early significantly increases Canopy coverage (%) and final biomass at anthesis, the non UAN rate interaction between (UAN placement x UAN timing) was also shown to increase % canopy cover, % radiation interception by the wheat and anthesis wheat biomass.

Table 2 Mean, P-values and LSD for UAN placement, UAN timing and UAN rate on the growth and competitiveness of a wheat crop

UAN Placement	**UAN Timing**	**UAN Rate**	**Wheat establishment plants/m^2 4WAS**	**NDVI 8WAS**	**NDVI 10WAS**	**NDVI 12WAS**	**Canopy cover (%) 18/07 10WAS**	**Canopy cover (%) 3/09 14WAS**	**Wheat Biomass (26WAS)**	**Radiation Interception (μmol m^{-2} s^{-1}) 14WAS**	**Radiation Interception (%) 14WAS**
Control (nil)	Nil	Nil	140.5	0.329	0.394	0.534	44.26	42.23	306.2	459.60	0.764
Band (stream)	Early	Optimum	152.8	0.350	0.453	0.628	69.31	84.67	465.0	534.70	0.876
		Low	157.9	0.350	0.393	0.550	56.02	58.37	336.2	500.60	0.818
	Late	Optimum	135.3	0.350	0.388	0.513	43.58	70.03	390.1	487.49	0.825
		Low	148.8	0.313	0.363	0.503	42.14	45.36	337.4	462.31	0.769
Broadcast	Early	Optimum	134.8	0.350	0.425	0.625	66.10	75.18	421.9	528.40	0.859
		Low	137.6	0.355	0.448	0.525	54.90	43.55	310.5	488.38	0.795
	Late	Optimum	147.4	0.348	0.383	0.500	44.62	93.02	411.4	521.20	0.871
		Low	149.3	0.323	0.388	0.450	43.62	71.34	405.6	496.18	0.827
Source of variation			**P-value (LSD 5%)**								
UAN Placement			NS	NS	NS	NS	NS	0.051 (8.76)	NS	NS	NS
UAN Timing			NS	NS	<0.001 (0.031)	<0.001 (0.055)	<0.001 (6.55)	NS	NS	0.045 (23.76)	NS
UAN Rate			NS	NS	0.023 (0.031)	0.021 (0.055)	0.006 (6.55)	<0.001 (8.76)	<0.001 (43.61)	<0.001 (23.76)	<0.001 (0.039)
UAN Placement x UAN Timing			0.002 (10.70)	NS	NS	NS	NS	<0.001 (8.76)	0.014 (43.61)	0.004 (23.76)	0.012 (0.039)
UAN Placement x UAN Rate			NS	NS	NS	NS	NS	NS	NS	NS	NS
UAN Timing x UAN Rate			NS	NS	NS	NS	0.021 (6.55)	NS	0.005 (43.61)	NS	NS
UAN Placement x UAN Timing x UAN Rate			NS	NS	NS	NS	NS	NS	NS	NS	NS

NS – Not significant

Annual ryegrass biomass and seed production

For the annual ryegrass (ARG), the main effects of placement, timing and rate were less apparent with main effects only identified in ARG plant tissue nitrogen concentration (%N). However despite the limited significant differences attributed to main effect factors, interactions between (placement x timing) were apparent for ARG establishment, ARG plant tissue nitrogen concentration with interactions between (rate x timing) and (UAN placement x UAN timing) identified in ARG plant tissue nitrogen concentration.

Despite no significant interactions, many treatments showed significant trends. When UAN was applied early and at optimum N rates, increases in ARG biomass and seed production was evident. These increases in ARG biomass and seed production occurred both in the streamed and broadcast UAN placement treatments, however the increases were greater where UAN was broadcast, indicating a modest decrease in ARG growth was evident when UAN was streamed.

Whilst this trial is not replicated and preliminary, it was found that at the York site in 2018 had a background soil N supply of approximately 44.8 kg/N/ha. At this site, the streaming of UAN to the crop rows did not result in a significant decrease in both the annual ryegrass biomass or seed production, however in subsequent trials, with lower background N supply, changes in ARG growth may be evident.

Table 3 Mean, P-values and LSD for UAN placement, UAN timing and UAN rate on the growth and seed production of annual ryegrass.

UAN Placement	**UAN Timing**	**UAN Rate**	**ARG Establishment Plants/m^2 4WAS**	**ARG Tissue Content % N**	**Total N ARG %/m^2**	**Ryegrass Biomass**	**Total ARG Seed Production Seeds/m^2**	**Before treatment ARG Soil Seed Bank Seeds/m^2**	**After treatment ARG Soil Seed Bank Plants/m^2**
Control (nil)	Nil	Nil	42.2	0.963	17.7	18.5	14072	5098.27	19170
Band (stream)	Early	Optimum	32.1	0.905	39.0	43.3	30340	6359.14	36699
		Low	41.3	0.910	28.3	31.9	25632	5098.27	30730
	Late	Optimum	48.8	1.098	43.6	40.3	26251	4659.71	30910
		Low	47.0	0.943	37.6	40.1	29145	4111.51	33256
Broadcast	Early	Optimum	40.7	0.860	52.9	62.6	44177	4330.79	48508
		Low	67.1	0.975	31.5	32.4	26234	10196.55	36431
	Late	Optimum	31.6	1.438	59.3	41.9	28040	4440.43	32481
		Low	28.7	1.050	59.7	55.9	42569	4166.33	46736
Source of variation			**P-value (LSD 5%)**						
UAN Placement			NS	0.008 (0.119)	NS	NS	NS	NS	NS
UAN Timing			NS	<0.001 (0.119)	NS	NS	NS	NS	NS
UAN Rate			NS	0.016 (0.119)	NS	NS	NS	NS	NS
UAN Placement x UAN Timing			0.021 (20.69)	0.015 (0.119)	NS	NS	NS	NS	NS
UAN Placement x UAN Rate			NS	NS	NS	NS	NS	NS	NS
UAN Timing x UAN Rate			NS	0.004 (0.119)	NS	NS	NS	NS	NS
UAN Placement x UAN Timing x UAN Rate			NS	0.047 (0.169)	NS	NS	NS	NS	NS

NS – Not significant

Wheat yield and quality

The main effects of UAN placement, UAN timing and UAN rate in the wheat crop appear to be significant for NDVI (indicator of biomass), Measured wheat biomass cuts (30cmx 30cm) % canopy cover, % radiation interception by the crop, wheat yield, wheat seed weight and wheat grain protein concentration. For the annual ryegrass, the main effects were less apparent with UAN placement, UAN timing and UAN rate main effect only identified in annual ryegrass plant tissue nitrogen concentration.

However despite the limited number of main significant effects, the interaction of these main factors (UAN placement, UAN timing and UAN rate) were apparent. Analyses revealed significant interaction between UAN placement and UAN timing for wheat and ARG establishment, % canopy cover, % radiation interception by the wheat, wheat biomass, wheat yield, wheat seed weight and wheat grain protein concentration. For the annual ryegrass, the UAN placement and UAN timing interactions were again less apparent only identified in annual ryegrass plant tissue nitrogen concentration. It appears that the interaction between UAN placement and UAN rate is significant only for wheat grain weight and wheat grain protein concentration, with the interaction between UAN Timing and UAN Rate identified in % wheat canopy cover, wheat biomass and Protein and annual ryegrass plant tissue nitrogen concentration. Finally the third order interaction (UAN placement x UAN timing x UAN rate) is significant for ARG tissue, Wheat 1000 seed weight and wheat protein. Despite no significant interactions, many treatments showed significant trends

Table 4. Mean, P values and LSD for UAN placement, UAN timing and UAN rate on the yield and quality of wheat.

UAN Placement	UAN Timing	UAN Rate	Yield (t/ha)	Wheat 1000 Seed Weight (g)	Protein (%)
Control (nil)	Nil	Nil	2.529	43.35	6.675
Band (stream)	Early	Optimum	3.285	40.85	6.975
		Low	2.715	41.50	6.750
	Late	Optimum	2.738	42.00	6.950
		Low	2.470	42.13	6.600
Broadcast	Early	Optimum	3.008	41.46	6.825
		Low	2.555	42.00	6.875
	Late	Optimum	3.424	37.84	8.325
		Low	2.890	42.28	6.725
Source of variation			**P-value (LSD 5%)**		
UAN Placement			NS	NS	<0.001 (0.168)
UAN Timing			NS	NS	<0.001 (0.168)
UAN Rate			<0.001 (0.248)	0.004 (1.329)	<0.001 (0.168)
UAN Placement x UAN Timing			<0.001 (0.248)	0.001 (1.329)	<0.001 (0.168)
UAN Placement x UAN Rate			NS	0.030 (1.329)	<0.001 (0.168)
UAN Timing x UAN Rate			NS	NS	<0.001 (0.168)
UAN Placement x UAN Timing x UAN Rate			NS	0.023 (1.879)	<0.001 (0.238)

NS – Not significant

Summary and Implications

The results of this study suggests:

1. Whilst the streaming of UAN modestly reduced annual ryegrass N percentage in the tissue, no difference in annual ryegrass biomass or seed production was evident.
2. Nitrogen placement did not affect Wheat biomass accumulation (NDVI results), however modest differences in Canopy closure percentage existed. UAN rate and timing however had a greater effect on wheat biomass (NDVI results and wheat biomass cut) and percent canopy closure.
3. UAN rate, UAN timing (plus interaction of UAN timing and placement) were found to have significantly affected Radiation interception of the crop in the absence of weeds.
4. Nitrogen placement affected the yield and protein content of the grain produced.

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