

SCAP Wheat Cluster

Test 321

Split Application Wheat Field Protocol



TABLE OF CONTENTS

Field Selection.....	1
Treatments	1
Field Layout.....	2
General Agronomy & Field Measures	3
Prior to Seeding	3
At the Time of Seeding.....	3
Growing Season	3
Pre-Harvest	4
Soil Sampling & Analysis	4
Prior to Seeding: Soil Characterization and Nitrogen Fertility (0-15 cm and 15-60 cm)	4
Growing Season: Nitrogen Transformation (0-15 cm)	4
Prior to UAN Application: Available Nitrogen (0-15 cm and 15-60 cm)	5
Post Harvest: Residual Nitrogen (0-15 cm and 15-60 cm)	5
Gas Sampling & Analysis	5
Direct Emissions – N ₂ O.....	5
Indirect Emissions – NH ₃	7
Biomass Sampling & Analysis.....	7
Growing Season: Crop Measures	7
Harvest: Yield, N Use Efficiency, and Grain Quality.....	11
Economic Analysis.....	12
Appendix – Updates to the 2024 Field Protocol	13

FIELD SELECTION

Find a field site that fits the following parameters:

- **Low nitrogen testing soil (<40 lbs N / ha) is crucial for detecting responses to addition of N*
- **Not used for this project in the prior growing season*
- Whenever possible, seed into canola stubble; if not possible, **avoid cereals as the previous crop* to reduce affecting yield and the pressure from pathogens such as fusarium. Use your best agronomic judgement
- Cropped uniformly in the previous three years
- No history of manure addition in the past 10 years
- No history of perennials in the past 10 years
- Field should be in close proximity to your location as this project requires frequent visits

Treatments

See [Table 1](#) for a summary of treatments, which are a factorial combination of the following 4R Nutrient Management practices:

1. **Timing** – fertilizer applied once at the time of seeding *or* application split between the time of seeding and in-season
 - **Once** plots will receive the totality of N addition as urea side-row banded to a depth of 8 cm at the time of seeding
 - **Split** plots will receive 60% of their N as urea side-row banded to a depth of 8 cm at the time of seeding and the remaining 40% as UAN dribble top-dressed in-season, specifically when the crop reaches Feekes Growth Stage 4
2. **Inhibitor** – untreated fertilizer *or* fertilizer treated with an inhibitor
 - **No Inhibitor** plots will be fertilized with untreated urea at the time of seeding and if also a Split Application plot, untreated UAN in-season
 - **With Inhibitor** plots will be fertilized with urea treated with a nitrification inhibitor (eNtrench) at the time of seeding and if also a Split Application plot, UAN treated with a urease inhibitor (ANVOL) in-season
3. **Rate** – 70%, 90%, or 100% of the recommended rate
 - The 100% rate will be determined for each field site based on the standard practice of local growers (e.g., fall soil N test, yield target, and past field response to N additions experience)

There will also be a Control which will receive no addition of nitrogen at the time of seeding or in season.

Table 1. Treatment summary.

Ttmt #	Timing	Inhibitor	N Source		N Rate of Total Applied		
			At-Plant	In-Season	At-Plant	In-Season	Total
1	Once	No-Inhibitor	Urea	-	70%	-	70%
2	Once	No-Inhibitor	Urea	-	90%	-	90%
3	Once	No-Inhibitor	Urea	-	100%	-	100%
4	Once	With Inhibitor	Urea + eNtrench	-	70%	-	70%
5	Once	With Inhibitor	Urea + eNtrench	-	90%	-	90%
6	Once	With Inhibitor	Urea + eNtrench	-	100%	-	100%
7	Split	No-Inhibitor	Urea	UAN	42%	28%	70%
8	Split	No-Inhibitor	Urea	UAN	54%	36%	90%
9	Split	No-Inhibitor	Urea	UAN	60%	40%	100%
10	Split	With Inhibitor	Urea + eNtrench	UAN + ANVOL	42%	28%	70%
11	Split	With Inhibitor	Urea + eNtrench	UAN + ANVOL	54%	36%	90%
12	Split	With Inhibitor	Urea + eNtrench	UAN + ANVOL	60%	40%	100%
13	None	None	None	0%	0%	0%	0%

Field Layout

Trials will be a randomized complete block design of all treatments including the Control, with four replicates of each. Figure 1 illustrates just one example of the field layout but you can use your own. The key is to include one plot of each treatment and a control and randomize their order in each replicate block. You may reuse your randomized plot layout from one year to the next provided you have moved your site to a different field. If you would like to confirm your layout and/or want assistance designing gas sampling runs, please 4R.Stewardship@umanitoba.ca.

Summary:

- 2 Timing (Once, Split) x 2 Inhibitor (No Inhibitor, With Inhibitor) x 3 Rates (70%, 90%, 100%) = 12 Treatments
- 12 Treatments + 1 Control = 13 Plots per Block
- 13 Plots x 4 Replicate Blocks = 52 Plots Total

Plot Size

The 4R IRC Lab uses an 8-row plot seeder with a drill width of 1.5 m to lay in treatments when conducting a field study of this design. Each plot for us is two passes wide, for a total of 16 seed rows, and 8 m long with a 1.5 m buffer at either end to ensure the seeder is applying the correct treatment by the time it reaches the defined plot area. The width of the plot is less important than the number of seed rows available for sampling; ensure you have at least 6-8 rows for sampling and an additional 2 rows on each side which will not be sampled to avoid edge effect.

You can leave space between plots but it will need to be seeded with the same or similar crop. Whether there is a buffer or not, reduce the chance of edge effects by avoiding the peripheral two seed rows and fertilizer bands when collecting soil, gas emissions, and biomass samples.

Because this project will side-band fertilizer and soil samples are collected from on-band, it is imperative that plots, especially those established with more than one pass, are seeded in the same direction so the band is on the same side of the row.

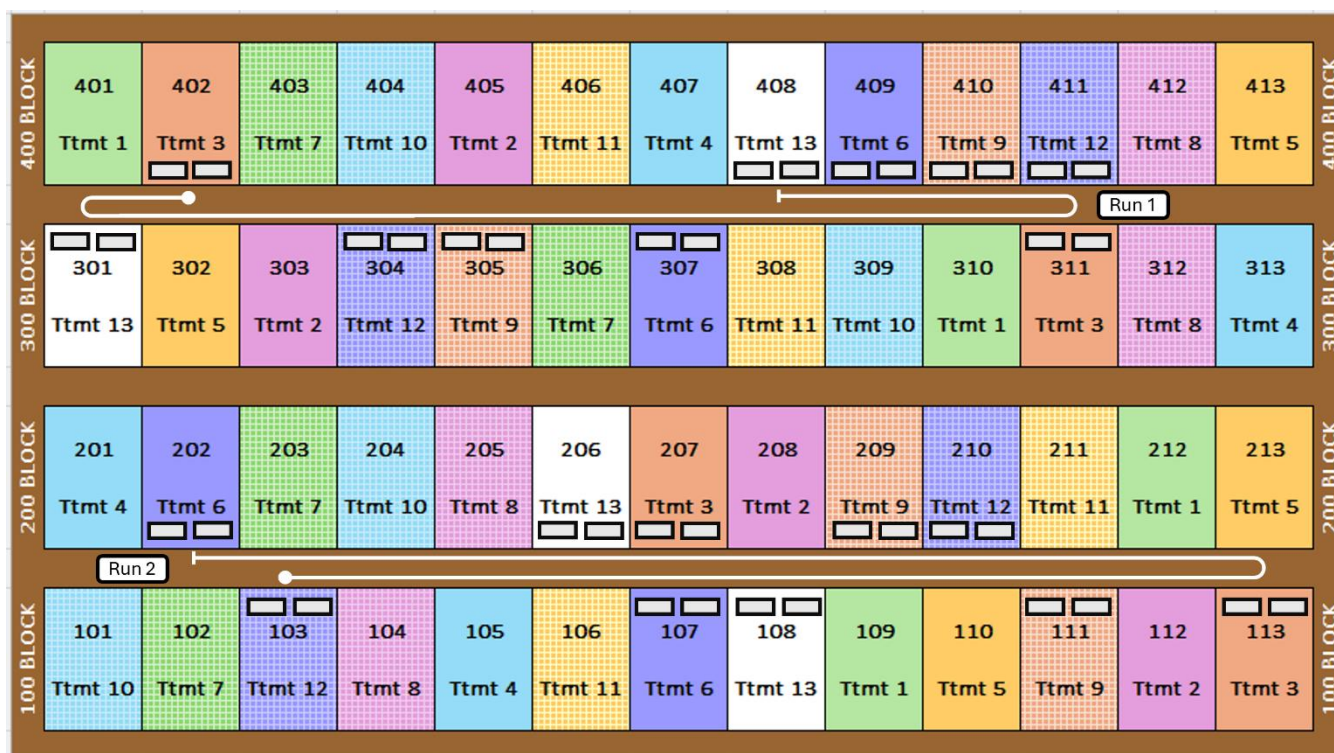


Figure 1. A randomized complete block field layout design showing four replicate blocks of 12 treatments and a Control plot in each, as well as static vented chamber placements (□) in the Control (Treatment 13) and 100% rate plots (Treatments 3, 6, 9, and 12) and potential gas sampling runs (—) based on the relative location of chambers.

GENERAL AGRONOMY & FIELD MEASURES

Prior to Seeding

Locate a nearby weather station that can provide air temperature and rainfall data. If there is none available, install the necessary equipment to monitor air temperature and rainfall at the field site.

Apply herbicide and other fertility products (e.g., TSP, K₂O, micros) to the field site as needed. Add phosphate in-furrow based on soil test recommendations but use TSP (0-45-0) so as not to add N. If ammonium sulphate is applied to the field site, calculate its N contribution and reduce the amount of urea added at the time of seeding to ensure that each treatment maintains the correct Recommended Rate. Apply other nutrients as needed.

Define 100% N Rate

Use the same standard method (fall soil N test, yield target, past field response to N additions) as *local growers* to determine the 100% N application rate. It is likely and acceptable that each field site will have a different recommended rate.

At the Time of Seeding

All fields will be direct-seed with treated AAC Brandon wheat. Seeding Density target rate is 300 to 350 plants/m².

N Treatments – Urea with and without eNtrench

Do not fertilize Control plots (i.e., Treatment 13) with *any* form of nitrogen.

Apply urea as a side-row band to a depth of 8 cm. Use untreated urea on No Inhibitor plots and urea treated with a nitrification inhibitor (eNtrench) on With Inhibitor plots.

When treating urea with eNtrench, do not follow the protocol given to researchers – follow the rates and directions provided on the commercial label. This will allow our field methods to reflect how growers handle and apply the product to their fields. You may mix urea and eNtrench by hand in large pails, but a drum mixer will be more thorough and consistent. Be sure to treat the urea in a well-ventilated area, preferably outside. Including diatomaceous earth as a drying agent when you treat the urea can prevent caking and improve flow through the drill.

If you require the 4R IRC Lab to supply you with eNtrench and/or urea, please contact 4R.Stewardship@umanitoba.ca to make the necessary arrangements. See the [Test 321 – Split Application webpage](#) for an inhibitor mix rate calculator.

Growing Season

Conduct in-crop weed control and foliar fungicide as needed.

If you have soil moisture and soil temperature sensors and data loggers to install, they can be added to a 100% N plot at a depth of 10 cm from the surface. If you do not have data loggers, your site will rely on the instantaneous soil moisture and soil temperature measurements collected with a hand probe when gas sampling. Ultimately, we need to collect sufficient data to summarize moisture and temperature changes for the field site over the growing season.

N Treatments – Split Application of UAN with and without ANVOL

Split plots will already have received 60% of the treatment rate as urea at the time of seeding. When the crop reaches Feekes growth stage 4 (see Figure 2), fertilize Split plots with the remaining 40% as UAN dribble top-dressed. The trial is considered at a stage when half the plants in split N fertilized plots have entered the growth stage. Note that the amount applied must reflect the Rate treatment (i.e., 70%, 90%, or 100% of the recommended rate, see [Table 1](#)). Application at Feekes growth stage 3 is acceptable if rain is forecasted. It is too labour intensive to monitor and fertilize on a plot-by-plot basis; fertilize all Split-Application plots at the same time. Ensure you do not fertilize Once or control plots.

Untreated UAN will be used for No Inhibitor plots; UAN plus a urease inhibitor (ANVOL) will be used for With Inhibitor plots (see [Table 1](#)).

As with eNtrench, follow ANVOL commercial label rates and directions when mixing – do not use the protocol given to researchers. This will allow our field methods to reflect how growers handle and apply the product to their fields.

If you require the 4R IRC Lab to supply you with ANVOL, please contact 4R.Stewardship@umanitoba.ca to make the necessary arrangements. You will need to obtain your own in-season UAN. See the [Test 321 – Split Application webpage](#) for an inhibitor mix rate calculator.

Pre-Harvest

Apply pre-harvest glyphosate if needed.

SOIL SAMPLING & ANALYSIS

Prior to Seeding: Soil Characterization and Nitrogen Fertility (0-15 cm and 15-60 cm)

Soil samples collected prior to seeding, either during the previous fall or current spring, are for soil characterization and determining nitrogen fertility. These samples will be used to determine the recommended N rate for each site.

Specifically, collect 0-15 cm and 15-60 cm samples from 15 random locations within a block using a Dutch auger or soil push probe. Composite the samples for a block but not between blocks.

The 4R IRC Lab uses manual Dutch Augers with a core diameter of 5 cm to collect soil cores because of clay content in our soils. Other samplers, such as Backsavers (core diameter of ~1.7 cm), are also acceptable. Ultimately, the cores collected to form one sample must supply enough soil for any and all analyses to be conducted twice, should there be an issue with the initial analysis.

Soil Characterization

Analyze samples for pH, EC, organic matter, texture, macronutrients, and micronutrients (Zn, Cu and others you normally analyze for).

Nitrogen Fertility Analysis

Analyze samples fresh for 2M KCl extractable NH_4^+ and NO_3^- . Using a third-party commercial lab is acceptable but confirm that they conduct these analyses on fresh / non-dried soil. To obtain a homogenous sample, fresh soil cores must be broken down into small pieces roughly between the size of the head of a matchstick and pencil eraser, by hand or with the aid of knives, prior to analysis.

Growing Season: Nitrogen Transformation (0-15 cm)

These soil samples are intended to capture the transformation of nitrogen over the growing season.

Conduct Nitrogen Transformation sampling on the same day or one day after seeding. Continue this sampling once per week up to and for three weeks following in-season application of UAN. Thereafter, conduct this sampling once every two weeks up to harvest. You do not need to collect soil samples each time you gas sample. Do not continue this sampling after harvest.

Collect composite soil samples from only the Control and 100% rate treatments (i.e., Treatments 3, 6, 9, 12, and 13) in each block, for a total of 20 plots.

Specifically, collect six on-band 0-15 cm cores and combine them into one composite 0-15 cm sample per plot. In the case of a seeder that alters the microtopography around the fertilizer band (e.g., Conservapak), define the top of the core (i.e., 0 cm) based on the general soil height prior to seeding. Avoid areas disturbed by foot traffic and other sampling and do not sample

from bands near an adjacent plot. Conduct soil sampling in the same end of the plot as the emissions chambers; this leaves the other end undisturbed for passive crop measures, biomass sampling, and yield determination.

Because these samples do not exceed 15 cm, a manual auger or push probe is recommended over a hydraulic auger.

Analyze samples fresh for 2M KCl extractable NH_4^+ and NO_3^- as per the [Nitrogen Fertility Analysis](#) samples collected prior to seeding. If you are unable to analyze samples as quickly as they are collected, you may freeze them *immediately* after collection but take care to *thaw them only once* when you are ready to process and analyze them.

Prior to UAN Application: Available Nitrogen (0-15 cm and 15-60 cm)

Collect composite soil samples from each of the 52 plots approximately 1-2 days prior to in-season application of UAN. Specifically, collect six on-band 0-15 cm and six on-band 15-60 cm cores and combine them into one composite 0-15 cm sample and one composite 15-60 cm sample per plot. Avoid areas disturbed by foot traffic and other sampling and do not sample from near an adjacent plot. The 0-15 cm cores can be used in place of this week's Nitrogen Transformation samples.

Analyze samples fresh for 2M KCl extractable NH_4^+ and NO_3^- as per the [Nitrogen Fertility Analysis](#) samples collected prior to seeding. If you are unable to analyze samples as quickly as they are collected, you may freeze them *immediately* after collection but take care to *thaw them only once* when you are ready to process and analyze them.

Post Harvest: Residual Nitrogen (0-15 cm and 15-60 cm)

Collect composite soil samples from 0-15 cm and 15-60 cm in all 52 plots after harvest as per the [Available Nitrogen](#) protocol described above. While gas sampling continues to freeze-up, these are the last soil samples collected for the growing season.

Analyze samples fresh for 2M KCl extractable NH_4^+ and NO_3^- as per the [Nitrogen Fertility Analysis](#) samples collected prior to seeding. If you are unable to analyze samples as quickly as they are collected, you may freeze them *immediately* after collection but take care to *thaw them only once* when you are ready to process and analyze them.

GAS SAMPLING & ANALYSIS

Endeavour to sample during the thaw period of the following spring but realize access to plots will be extremely challenging and we won't be able to sample as much as we would like or at all.

Soil volumetric moisture content and soil temperature readings at a depth of 10 cm are required each time gas samples are collected. If you have enough probes and dataloggers to collect continuous data in each plot, make note of the date/time you collected gas samples so they can be connected to the correct datalogger recording. Otherwise, use hand-held probes to obtain instantaneous readings near each set of emissions chambers *each time you collect gas samples*. This is so measurements can be related to treatment productivity and emissions.

See the [4RIRC-SOP-Static Vented Chambers](#) PDF for info on procedure and equipment used.

Direct Emissions – N_2O

Emissions of N_2O will be monitored in all Control and 100% rate treatments (i.e., Treatments 3, 6, 9, 12, and 13) in each block, for a total of 20 monitored plots. Those receiving reduced rates of fertilizer will not be monitored. Install two chambers towards one end of the plot, leaving the other for passive crop measures, biomass sampling, and yield determination. Begin monitoring gas emissions immediately after seeding.

If there are weeds in an emission chamber but not elsewhere in the plot, remove them by snipping at ground level as the discrepancy indicates that their presence is an artifact of the chamber and will bias flux data. If weeds are elsewhere in the field, simply clip all crop and weeds to the height of the chamber to allow closing of the lid with a proper seal.

The static vented chamber (SVC) method will be used to estimate N₂O fluxes on at least 20-30 days over the non-frozen portion of each cropping year (i.e., seeding to freeze-up and, if possible, during the following spring thaw). Frequency of sampling is based on expected emissions events:

- 2 to 3 times each week from seeding up to and for three weeks following in-season application of UAN, then reduce to
- 1 time a week until freeze-up

With respect to rain events, do not conduct gas sampling while it is actively raining and do not necessarily target the day after a rain event for sampling. Instead, have a set schedule that you adhere to, adjusting the days only if inclement weather or an agronomic activity prevents you from sampling on one of those days. For example, during the 3-4 weeks following seeding, sample Monday, Wednesday, and Friday; if it rains on Wednesday, sample Thursday instead.

The **4RIRC-SOP-Static Vented Chambers** PDF contains detailed instructions on chamber placement, installation, and how to conduct a gas sampling run.

Chambers are to be about 50 cm long and 15 cm high; ideally, either the length or width will be long enough to entirely cover the space between seed rows (i.e., the inter-row). The 4R IRC Lab uses rectangular Plexi-glass chambers but food serving trays are acceptable. Regardless of the type of chamber used, the size and position must be representative of the relative on-band and off-band soil area of the plot as this is critical to estimating N₂O fluxes – you *must not bias* the sampling area by covering disproportionately more or less banded area.

Each monitored plot will have two SVCs. Correct placement of a second chamber relative to the first is imperative. Follow the instructions in the *Chamber Placement and Installation - Ubiquitous Fertilizer Distribution – Broadcast* section of the **4RIRC-SOP-Static Vented Chambers** PDF.

The Sampling Interval (i.e., the amount of time between sample collection from a given chamber) for this project is flexible, though should be between 15 and 20 minutes, to ensure that enough time passes for emissions to accumulate but not so much that chambers become saturated if emissions are high. A Sampling Interval of 10 minutes is too short when emissions are low. A Sampling Interval of 12 minutes is generally not recommended as it is on the cusp of being too short.

Factors influencing Sampling Run design include the number of field technicians available and their level of experience, where the monitored plots are located relative to each other, and the ease of travel within a field (e.g., wet clay is more difficult than dry sandy soils).

The example provided in Figure 1 consists of two Sample Runs of 10 plots (i.e., 20 chambers each). These Sample Runs can be accomplished by one technician conducting them consecutively or two technicians conducting them simultaneously. The Sampling Interval for these runs could be:

- 15 min with a Chamber Time of 30 sec (i.e., 1 min per plot), for a total Sample Run time of 60 min
- 20 min with a Chamber Time of 45 sec (i.e., 90 sec per plot), for a total Sample Run time of 80 min
- Note: in both cases, sampling is completed 5 minutes before the next Sample Round begins, during which time technicians can reorganize their sample kits, make necessary notes on their field sheets, etc.

Alternatively, each replicate block *could* be its own Sample Run of 5 plots (i.e., 10 chambers), but the potential Sample Run designs have either a large amount of downtime or Sampling Intervals that are too short. These Sample Runs could be conducted by one, two, or four technicians. The Sampling Interval for these runs could be:

- 12 min with a Chamber Time of 45 sec (i.e., 90 sec per plot), for a total Sample Run time of 48 min
- 15 min with a Chamber Time of 1 min (i.e., 2 min per plot), for a total Sample Run time of 60 min

N₂O Analysis

The N₂O concentration of sample gas will be determined in the laboratory by gas chromatography. Flux rates will be determined from the progress of mass of N₂O accumulation (from chamber area, volume, gas concentration, temperature using the Ideal Gas Law) with time. The R-package *HMR* will be used to determine the flux rate using the most appropriate

linear and non-linear model fitting the data. The **4RIRC-SOP-HMR-Draft** PDF provides a preliminary outline of this process. Emissions for each Sample Site will be estimated by interpolation of between sampling dates.

Growing season emissions will be estimated from cumulative daily fluxes. Emissions will then be scaled and reported on an area (g N₂O-N/ha), the fraction of fertilizer N added (g N₂O-N/kg N fertilizer), the fraction of N supply (soil at planting plus fertilizer, g N₂O-N/kg N fertilizer + soil), and production intensity (kg N₂O-N/Mt grain) bases and statistically analyzed. As treatments may affect grain protein and not yield, N₂O emissions will also be reported on a protein intensity (g N₂O-N/Mt grain protein).

Indirect Emissions – NH₃

Emissions of N₂O and NH₃ will be monitored in all Control and 100% rate treatments (i.e., Treatments 3, 6, 9, 12, and 13) in each block, for a total of 20 monitored plots. Those receiving reduced rates of fertilizer will not be monitored. Install chambers in the same end of the plot as the N₂O static vented chambers; this will leave the other end for passive crop measures, biomass sampling, and yield determination. Begin monitoring gas emissions immediately after seeding

If there are weeds in an emission chamber but not elsewhere in the plot, remove them by snipping at ground level as the discrepancy indicates that their presence is an artifact of the chamber and will bias flux data.

The **4RIRC-SOP-Ammonia Dosimeter Chambers** PDF contains information on chamber design, placement, installation, and recording ammonia emissions. Only one ammonia chamber will be installed in each monitored plot but will be relocated after rain events and/or on a weekly basis if the soil is moist or biweekly if the soil is dry. Replace dosimeters with fresh ones when the current tube is nearly spent (i.e. almost all the reagent has changed colour) or if it has fallen off the stake. Past experience indicates that up to 2-3 dosimeters will be required per plot over the growing season. Fewer will be required when volatilization rates are low.

NH₃ Analysis

Qualitative NH₃ volatilization will be done using passive sampler dosimeter tubes at all sites for the determination of ammonia volatilization losses and the benefit of urease inhibition. The **4RIRC-SOP-Ammonia Dosimeter Chambers** PDF contains information on recording ammonia emissions.

BIOMASS SAMPLING & ANALYSIS

Growing Season: Crop Measures

Do not collect data from seed rows near an adjacent plot or areas disturbed by foot traffic and other sampling.

Phenology

Visit the field site Monday, Wednesday, and Friday, to collect the following Crop Phenology* data:

- Days to emergence
- Days to anthesis / flowering
- Anthesis duration
- Days to physiological maturity (maturity is reached when kernel moisture from the lower third of the spike is less than 40% and the seed is no longer easily severed when pinched between thumbnail and fingernail)
- Critical period in growing season (defined as the day of onset of stem elongation (Feekes growth stage 4 / Zadoks 30) to 9 days post-anthesis (Zadoks 69 + 9 days))

**For the purposes of this study, a plot is considered to have reached a specific growth stage when 50% of its plants have reached the stage in question.*

See Figure 2 and Figure 3 for an overview of Feekes and Zadoks growth stages of wheat, respectively.

Population

Determine plant population shortly after emergence, before tillers (Feekes growth stage 2) complicate counts. For each plot, stake two 1 m sections of seed row and count the plants. Do not combine the counts; record the two sections separately. Note any issue(s) with emergence and stand establishment.

Crop Vigor

Determine early vigor at Feekes growth stage 4 and late-season vigor at Feekes growth stage 8. Vigor is determined on a scale of 1 to 5, with 1 having very poor vigor and light colour, and 5 having very rigorous growth habit and bright green colouration.

Canopy Development & Architecture

Canopy development and architecture is monitored weekly in each plot from Feekes growth stage 4 to 10.5 (i.e., anthesis). Be sure to include the day of in-season UAN application. Collect the following:

- LAI using a handheld **AccuPAR LP 80 Ceptometer®*
- NDVI using a **Greenseeker Optical Sensor*
- Chlorophyll levels using a **FieldScout CM 1000 Chlorophyll Meter* for five plants in each plot

*If you do not have access to the equipment listed above, it is acceptable to collect data using similar equipment provided you adhere to the methodology to generate values similarly.

Head Count

For each plot, stake two 1 m sections of seed row and count the total number of heads (do not go to the trouble of counting heads per plant). Do not combine the counts; record the two sections separately.

Belgian Lodging Score at Maturity

Calculated as:

$$\text{Area of Surface Lodged} \times \text{Intensity of Lodging} \times 0.2$$

Where:

- Area is on a scale of 1 to 9, with 1 = no lodging and 9 = totally lodged
- Intensity is on a scale of 1 to 5, with 1 = standing upright and 5 = totally flat (see Figure 4)

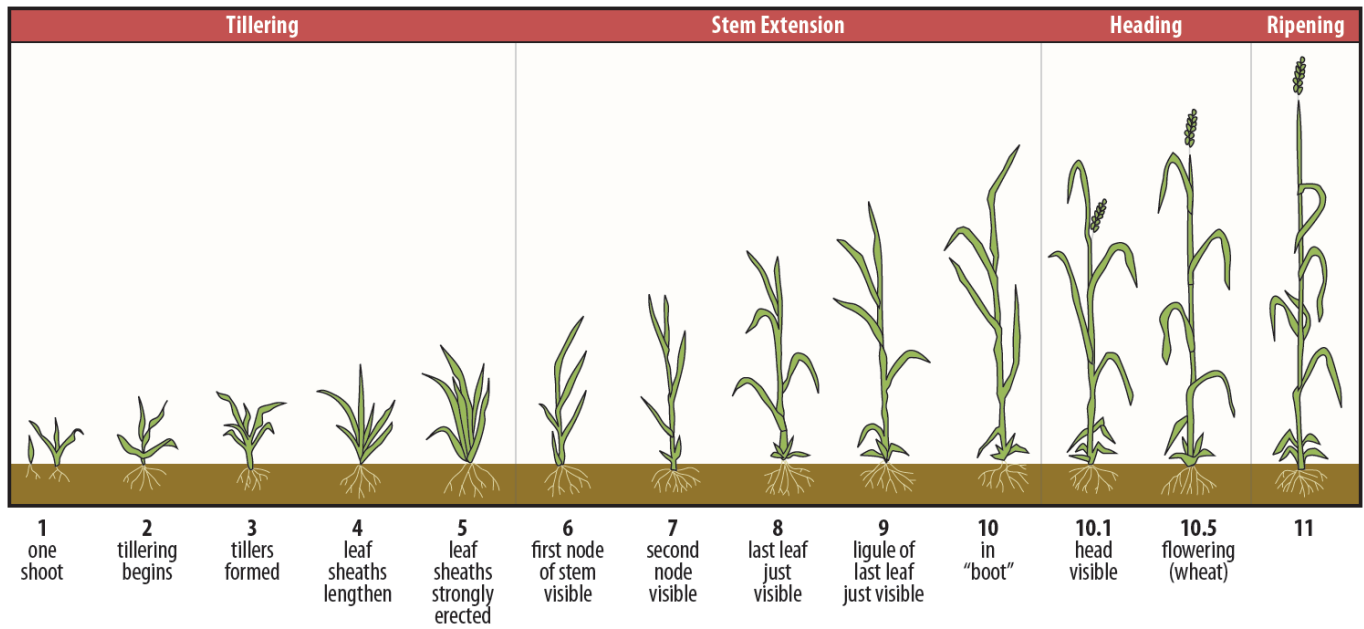


Figure 2. Feekes Growth Stages of wheat (taken from *A Comprehensive Guide to Wheat Management in Kentucky* <https://www2.ca.uky.edu/aqcomm/pubs/ID/ID125/ID125.pdf>)

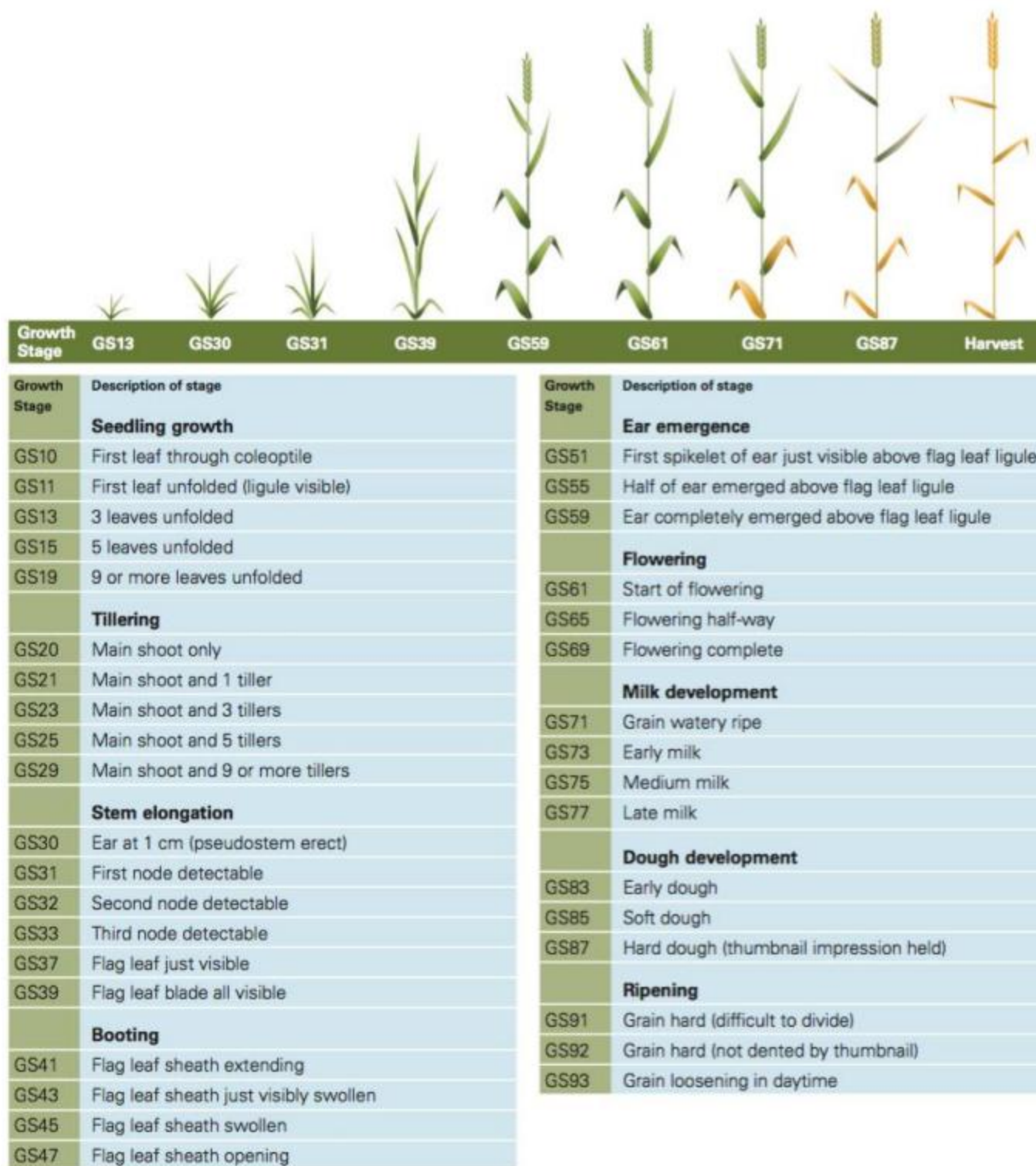


Figure 3. Zadoks Growth Stages of wheat (taken from Agriculture and Horticulture Development Board (<https://ahdb.org.uk/cereals-oilseeds>)).

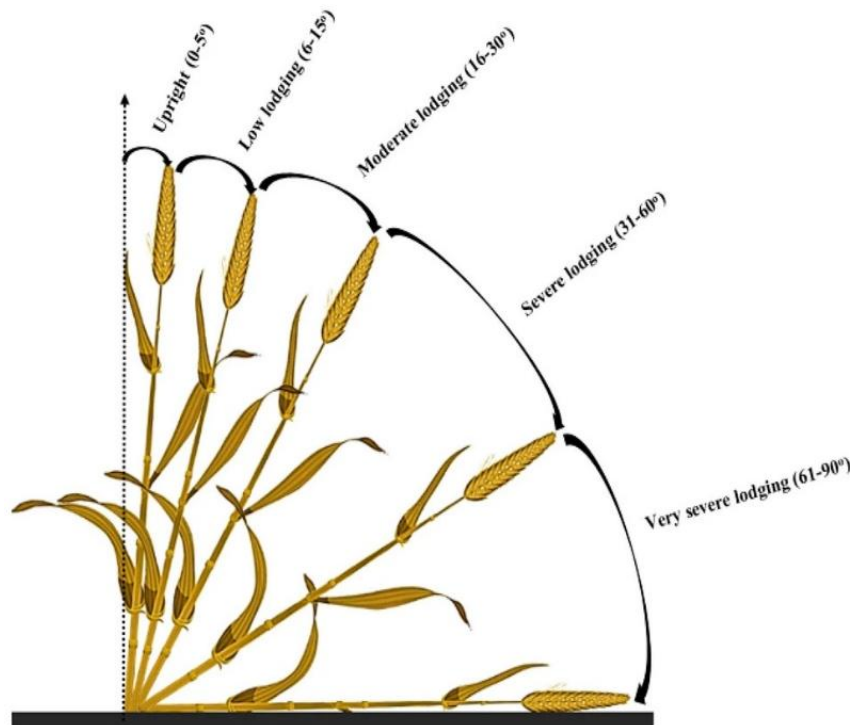


Figure 4. Schematic showing wheat lodging intensity (adapted from Rabieyan, E., Darvishzadeh, R. & Alipour, H. Identification and estimation of lodging in bread wheat genotypes using machine learning predictive algorithms. *Plant Methods* **19**, 109 (2023). <https://doi.org/10.1186/s13007-023-01088-w>).

Harvest: Yield, N Use Efficiency, and Grain Quality

Hand Harvest – Yield and NUE

After maturity and prior to combine harvest, collect aboveground biomass to determine yield and N content. Hand harvest plants by clipping stems 1-2 cm above the soil surface from either a defined row length or by square meter. If using row length, collect subsamples from four 1 m long sections of seed rows in each plot. If using a quadrat, collect subsamples from two 0.5 m² (0.71 m x 0.71 m) areas, ensuring that each covers two seed rows. Record which method you used; in the case of defining sample size by square meter, make note of the quadrat dimensions and confirm the number of seed rows included in each subsample. Do not collect from areas affected by gas and soil sampling foot traffic, and do not collect subsamples from rows immediately next to each other or near adjacent plots. Separate weeds from crop and combine the subsamples.

Dry biomass samples at 60°C and determine the whole-sample dry weight. Thresh samples and weigh grain. This method will give total biomass yield, grain yield, and residue (leaves + stems) yield by difference. Retain all grain and a subsample of residue (either 50 plants or a 2–3-inch diameter bundle).

Grind grain and residue subsample using a #3 Wiley Mill, ensuring the ground material can pass through a 1mm diameter screen. Analyze N content of grain and residue by combustion analysis.

Total N uptake will be determined from grain and residue yield and N contents and be used to calculate nitrogen use efficiency (NUE) as the apparent crop recovery of fertilizer and N supply:

$$\text{Fertilizer N Recovery} = \frac{\text{Treatment Aboveground N Uptake} - \text{Control Plot Aboveground N Uptake}}{\text{Added Fertilizer N}}$$

$$\text{N Supply Recovery} = \frac{\text{Treatment Aboveground N Uptake} - \text{Control Plot Aboveground N Uptake}}{\text{Preplant Soil N} + \text{Added Fertilizer N}}$$

Combine Harvest – Grain Quality

Determine harvest grain yields using an on-board system and correct to 14% moisture. Adjust for the hand harvested biomass samples.

Retain a 2 kg sub-sample to measure Thousand Kernel Weight (i.e., seed mass; g per 1000 kernels) and Kernel Test Weight (i.e., grain bulk density; kg per hL). Determine whole grain protein content of the subsample using near infrared reflectance spectroscopy (NIRS) technology.

ECONOMIC ANALYSIS

A simple economic analysis will be conducted for the period of the study based on the following:

- Incremental cost of production will be determined based on typical fuel and labour costs for field traffic to split apply nitrogen
- Incremental cost of inhibited urea will be determined
- Total cost of fertilizer for each treatment will be determined
- Gross return from grain yield and protein will also be determined

The change in profitability (i.e., whether the 4R practice is at no additional cost to the farmer or not) will be concluded from:

$$\frac{\text{Change in return from grain yield and protein} + \text{Reduction in the cost of untreated urea}}{\text{Incremental Cost of Production}}$$

APPENDIX – UPDATES TO THE 2024 FIELD PROTOCOL

2024 Field Protocol		2025 Field Protocol
Field Selection – Field Layout	–	You may reuse your randomized plot layout from one year to the next provided you have moved your site to a different field.
General Agronomy & Field Measures – At the Time of Seeding	–	Including diatomaceous earth as a drying agent when you treat the urea can prevent caking and improve flow through the drill.
Soil Sampling	–	You do not need to collect routine 0-15 cm soil samples each time you gas sample. Do not continue this sampling after harvest.
	<i>Growing Season N Transformation (Routine 0-15 cm)</i> Collect composite soil samples from each of the 52 plots.	Collect composite soil samples from only the Control and 100% rate treatments (i.e., Treatments 3, 6, 9, 12, and 13) in each block, for a total of 20 plots. Because these samples do not exceed 15 cm, a manual auger or push probe is recommended over a hydraulic auger.
	<i>Post Harvest Residual N (0-15 cm and 15-60 cm)</i>	While gas sampling continues to freeze-up, the post-harvest 0-15 cm and 15-60 cm cores are the last soil samples collected for the growing season.
Gas Sampling	Direct Emissions (N₂O) Frequency of sampling is based on expected emissions events: • 3 times a week for the 3-4 weeks following seeding, then reduce to • 2 times a week up until flowering, then reduce to • 1 time a week until freeze-up	Frequency of sampling is based on expected emissions events: • 2 to 3 times each week from seeding up to and for three weeks following in-season application of UAN, then reduce to • 1 time a week until freeze-up
	Indirect Emissions (NH₃)	Move the dositube after a rain event. If the soil is moist, move it weekly; if the soil is dry, move it every two weeks. Replace dositubes with fresh ones when the current tube is nearly spent (i.e. almost all the reagent has changed colour) or if it has fallen off the stake.