# OPTIMAL SEEDING RATES FOR NEW HARD RED SPRING WHEAT CULTIVARS IN

## DIVERSE ENVIRONMENTS

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## Title

Optimal Seeding Rates for New Hard Red Spring Wheat Cultivars in

**Diverse Environments** 

By

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The Supervisory Committee certifies that this disquisition complies with North Dakota

State University's regulations and meets the accepted standards for the degree of

## DOCTOR OF PHILOSOPHY

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### ABSTRACT

Seeding rate in hard red spring wheat (HRSW) (Triticum aestivum L.) production impacts input cost and grain yield. Predicting the optimal seeding rate (OSR) for HRSW cultivars can aid growers and eliminate the need for costly seeding rate research. Research was conducted to determine the OSR of newer HRSW cultivars (released in 2013 or later) in diverse environments. Nine cultivars with diverse genetic and phenotypic characteristics were evaluated at four seeding rates in 11 environments throughout the northern Great Plains region in 2017-2018. Results from ANOVA indicated environment and cultivar were more important than seeding rate in determining grain yield. Though there was no environment x seeding rate interaction (P=0.37), OSR varied among cultivar within each environment. Cultivar x environment interactions were further explored with the objective of developing a decision support system (DSS) to aid growers in determining the OSR for the cultivar they select, and for the environment in which it is sown. Data from seeding rate trials conducted in ND and MN from 2013-2015 were also used. A novel method for characterizing cultivar for tillering capacity was developed and proposed as a source for information on tillering to be used in statistical modelling. A 10-fold repeated crossvalidation of the seeding rate data was analyzed by 10 statistical learning algorithms to determine a model for predicting OSR of newer cultivars. Models were similar in prediction accuracy (P=0.10). The decision tree model was considered the most reliable as bias was minimized by pruning methods, and model variance was acceptable for OSR predictions (RMSE=1.24). Findings from this model were used to develop the grower DSS for determining OSR dependent on cultivar straw strength, tillering capacity, and yield of the environment. Recommendations for OSR ranged from 3.1 to 4.5 million seeds ha<sup>-1</sup>. Growers can benefit from using this DSS by sowing at OSR relative to their average yields; especially when seeding new HRSW cultivars.

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### PREFACE

Seeding rate is an important factor in HRSW production as it impacts input costs and returns from grain yields. New hard red spring wheat (HRSW) (Triticum aestivum L.) cultivars are released every year, requiring frequent (and sometimes repetitive) research to determine optimal seeding rates (OSR) for these new cultivars to help growers maximize yield and economic efficiency. In addition to this, a recent study indicated environment may also need to be considered when determining OSR of HRSW cultivars. We hypothesize that cultivars sharing similar characteristics for specific genetic and phenotypic traits are likely to have similar OSR. Therefore, it may be possible to develop a predictive model for determining OSR of new cultivars based on characteristics known at the time of their release. This would help avoid the expense and resources needed to support continued seeding rate trials for new cultivars. As cultivars can be readily profiled for specific genetic traits, it may be possible to pair this genetic information with phenotypic data for advanced lines in breeding programs, to be used as inputs in a predictive model. Data specific to an environment could be incorporated into a predictive model and interfaced with a decision support system (DSS) to help growers determine the OSR for the cultivar they select to use in their fields. A DSS will benefit HRSW growers by providing them with a tool to promote seeding efficiency and maximum yield. Resolutions for the various components of this problem are detailed in the following chapters:

- 1. Seeding rate selection for optimum yield of HRSW cultivars
- 2. A standardized method for determining tillering capacity of HRSW cultivars
- Developing a decision support system to aid grower selection of optimal seeding rates for new HRSW cultivars in diverse environments
- 4. Major findings

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# LIST OF ABBREVIATIONS

CART	Classification and regression trees.
Ср	Mallow's complexity parameter.
DSS	Decision support system.
HRSW	Hard red spring wheat.
LASSO	Least absolute shrinkage and selection operator.
LSD	Least significant difference.
MAE	Mean absolute error.
MAPE	Mean absolute percent error.
NDAWN	North Dakota Agricultural Weather Network.
NDSU	North Dakota State University.
OSR	Optimal seeding rate.
QTL	Quantitative trait loci.
RMSE	Root mean squared error.
SOFATT	Seed only few, and then thin.

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# CHAPTER 1. SEEDING RATE SELECTION FOR OPTIMUM YIELD OF HRSW CULTIVARS

### Introduction

Rising production costs and relatively low market prices (Winders et al., 2016) prompts growers to seek ways to improve production efficiency and maintain profitability. Seeding rate is an important factor in HRSW production as it impacts both input costs, and returns from grain yields. Environmental limitations on yield potential will vary among cultivars dependent on biotic and abiotic stressors present during a growing season (Molero et al., 2016). It is expected that a cultivar grown under stressed conditions will establish, develop, and yield differently than the same cultivar under favorable conditions. Additionally, growth and timing of development are expected to be influenced by plant genotype.

Findings from a previous study in eastern North Dakota and western Minnesota revealed differences in OSR among HRSW cultivars (Mehring, 2016). However, seed of most of the cultivars in that study are no longer commercially available. With the continual release of new varieties (and subsequent discontinuation of older varieties), growers may benefit from knowing optimal seeding rate (OSR), specific to cultivar and environment type, will aid growers in minimizing production costs and improving wheat yield potential. Identifying factors that may aid in predicting OSR for new varieties can eliminate the need for costly experimentation, and help growers maximize productivity and economic return.

Seeding rate greatly impacts the number of established plants ha<sup>-1</sup>; one of the main determinants of yield. Grafius (1956) reported yield differences in oats (*Avena sativa* L.) that were associated with changes in panicles plant<sup>-1</sup>, representing plant response to seeding rate increases. Guitard et al. (1961) noted increases in seeding rate had a curvilinear effect on spikes

plant<sup>-1</sup> of wheat plants; whereas the effect was linear and positive for plants ha<sup>-1</sup>. Differences in yield associated with changes in seeding density and cultivar type have also been observed in barley (*Hordeum vulgare* L.) (Kirby, 1967).

Growers typically select seeding density based on past field productivity and current cost of seed. Wheat fields have commonly been planted based on a seed mass (or volume) per area unit; however, this can result in variations in the number of planted seeds ha<sup>-1</sup> by  $\geq$ 50% (Puri and Qualset, 1978). It is important for growers to seed at a rate that is based on actual seeds ha<sup>-1</sup>, and supported by regional university studies, as this can help growers establish a plant density favorable for maximum productivity and economic efficiency (Wiersma and Ransom, 2017).

An optimal plant density limits yield loss potential resulting from plant overcrowding or light-use inefficiencies (Puckridge and Donald, 1967). Reynolds et al. (1994) observed increases in early-season percent ground coverage to be positively associated with yield. These results may suggest that increasing early-season ground coverage with a high plant density may improve production efficiency compared to low plant density. Establishing an optimal plant density is especially important in resource-limited environments, to maximize nutrient capture and use efficiency throughout the plant population (Nass and Reiser, 1975).

For economical HRSW production, establishing a plant population of 3.2 to 3.5 million plants ha<sup>-1</sup> is recommended in Minnesota and North Dakota (Wiersma and Ransom, 2017). The seeding rate at which this recommended density is attained varies with crop management practices applied, seed germination percent, and conditions at planting (Khah et al., 1989; Lawrence et al., 1994; Fiez and Miller, 1995). As Wiersma and Ransom (2017) indicated stand losses typically range from 10 to 20%, seeding at a rate of 3.8 to 4.1 million seeds ha<sup>-1</sup> would provide the recommended plant population. Plant genetic characteristics can also impact OSR. Genetic selection for dwarfing genes *Rht-B1* and *Rht-D1* has contributed to reduced plant height in many modern cultivars, minimizing losses due to plant lodging. Borrell et al. (1991) observed increased yields in semidwarf *Rht-B1* and *Rht-D1* lines, and attributed gains to greater number of kernels plant<sup>-1</sup> and spikes plant<sup>-1</sup>, as a result of increased availability of assimilates normally allocated for stem development. Reductions in grain quality characteristics of protein (Law and Payne, 1983) and kernel weight (Allan and Pritchett, 1980) have also been observed at 1% and 3.5%, respectively, in cultivars possessing *Rht-B1* and/or *Rht-D1* dwarfing genes. These findings demonstrate the importance of considering dwarfing gene and seeding rate interactions when evaluating cultivar growth and yield response for determining OSR.

Though these genes impact desirable agronomic characteristics, *Rht-B1* and *Rht-D1* genes are insensitive to the growth-promoting plant hormone class of gibberellins (Hedden, 2003). Semi-dwarf cultivars possessing this gibberellin-insensitivity tend to have shortened coleoptiles and reduced seedling leaf area. These seedling characteristics increase severity of negative effects from adverse environmental conditions early in the growing season (Rebetzke et al., 2007; Amram et al., 2015). Gale and Marshall (1973) reported an increase in tillers plant<sup>-1</sup> when gibberellin-insensitive dwarf cultivars were treated with gibberellin hormone. This may suggest a plant compensatory mechanism (Stapper and Fischer, 1990) that may impact the agronomic response of cultivars possessing these genes. With the contrasting environment types in this study, environmental interactions with these genes may minimize the magnitude of this effect, potentially influencing experimental findings.

Rate of vegetative development of HRSW plants is influenced by cultivar sensitivity to day length period as wheat is a long-day plant (Syme, 1968). Cultivar possessing the sensitive

allele for photoperiod response (*Ppd-Db*) slowly progress through vegetative stages under short day conditions ( $\leq 10$  hr daylight). Understandably, this characteristic would be desirable for winter wheat cultivars seeded in late fall, but could be detrimental for spring wheat grown in the short growing seasons present in the northern Great Plains region of the U.S. Cultivars insensitive to photoperiod (*Ppd-Da*) will transition to reproductive stages even in the absence of long day periods ( $\geq 14$  hr daylight).

Breeder selection for photoperiod insensitive lines is common in modern spring wheat breeding programs as it can provide HRSW cultivars with timely maturation prior to temperatures and water deficits present in late summer season that can adversely impact yield (Beales et al., 2007). Breeding efforts are complicated by other genetic and environmental factors influencing plant development and heading timing in wheat (Keim et al., 1973; Kato and Yamashita, 1991). With a complex of factors influencing heading and anthesis timing, it is understandable how contrasting findings have been reported for yield characteristics of *Ppd-D* insensitive cultivars. Dyck et al. (2004) reported a 5% yield penalty conferred by the dominant insensitive allele; whereas others have concluded insensitivity offers a yield advantage over sensitive cultivars (Marshall et al., 1989; Worland et al., 1998). Though Beuerlein and Lafever (1989) indicated seeding rate had a minimal effect on heading timing, it would still be important to evaluate the impact of *Ppd-D1* sensitivity when evaluating cultivar yield response to seeding rate in the diverse growing environments present in ND and MN.

Plant spacing and arrangement are important agronomic factors to consider prior to planting to attain optimal yields in HRSW. In plant spacing studies in rice (*Oryza sativa* L.), Ashraf et al. (2016) noted plant densities >25 plants m<sup>-2</sup> had reduced tiller number and yields. Plant density studies in grain sorghum observed no difference in yields between clump

arrangements (2 groupings of 3 plants) and equidistant plantings (Thapa et al., 2017). However, harvest index (dry weight of grain/dry weight of aboveground biomass) of clumps was significantly higher (0.48 vs. 0.4). This difference was attributed to lower plant tiller numbers in clump arrangements compared to equidistant plantings (0.4 and 1.2 tillers plant<sup>-1</sup>, respectively). Spacing and density of established wheat plants impacts plant growth habit and ability to compete for available resources (Lemberle et al., 2001). At decreased plant densities in narrow spacing (15 cm), plant tillering increases the number of spikes m<sup>-2</sup>, providing for an average yield increase of 0.41 Mg ha<sup>-1</sup> compared to 30 cm spacing (Chen et al., 2008). Though plant leaf area and spike size significantly influence yield, number of spikes per wheat plant generally has a greater influence on plant yield (Hsu and Walton, 1971).

Fagade and de Datta (1971) reported that broadcast seeding of rice at high rates (100 kg ha<sup>-1</sup>) provided for greater number of tillers m<sup>-2</sup> compared to equidistant planting at densities ranging from 0.04 to 1.0 m<sup>-2</sup>. They also reported differences in tillering capacity of cultivars, as tiller counts were significantly different among cultivars seeded at the same density, and for individual cultivars at varying densities. When selecting an OSR for a given cultivar, it may be important to consider the tillering capacity of the cultivar. Tillering can provide plants with greater flexibility to adjust to varying densities and growing conditions (Kirby and Faris, 1972). This is an important consideration when determining OSR as a high tillering cultivar could potentially be seeded at a lower rate compared to cultivar with less tillers contributing to main stem yield. Elhani et al. (2007) experimented on this and concluded that a high tillering capacity provided cultivar with advantages in plant growth habit and yield components over low tillering cultivars, but only in non-stressed, irrigated environments. With this understanding, it also may be important to consider growth environment factors when determining cultivar OSR.

Differences in accumulation and storage of grain protein is expected with the diversity in genetic and morphological characteristics among cultivar. While environmental variables and fertility management can greatly influence protein content, adaptations in agronomic management with changes in seeding rate, have not been observed to affect grain protein content (Briggs and Ayten-Fisu, 1979; Faris and DePauw, 1981; Jenner et al., 1991).

The objectives of this research were to determine the OSR of new HRSW cultivars grown in diverse environments representing the varying wheat production regions present in ND and MN. An expectation was that grouping cultivars with similar OSR would aid in identifying factors (environmental, genetic, phenotypic) closely associated with yield and seeding rate. These factors could be used in predictive models to determine OSR prior to release of a cultivar.

### **Materials and Methods**

### **Site Description**

Experimental sites were comprised of six locations per year (2017 and 2018) and included a diverse range of growing conditions in North Dakota and Minnesota (Table 1). The 2017 site in Dickinson, ND was excluded from all analyses as it was unharvested due to excessive damage from varmint feeding. Experiments were located at North Dakota State University and the University of Minnesota research centers and experiment stations (Table 2). A research protocol was developed and distributed to university research specialists at each location. These specialists assisted by planting, maintaining, and harvesting experiments at each location (Table 2).

### **Experimental Approach**

Small plot research trials were conducted to evaluate yield response of different cultivar at incremental seeding rates. Experimental units were combined factors of cultivar (nine) and

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seeding rate (four) in a factorial arrangement, with a randomized complete block design. Agronomic management (including cultivation, fertilization, and pest management) followed current Extension recommendations relevant to each location, to ensure inputs were not a limiting variable.

Weather data were retrieved for each growing season period from the North Dakota Agricultural Weather Network (NDAWN) or from the University of Minnesota research center, for ND and MN experiments, respectively. Daily observations for temperature (°C) and accumulated rainfall (mm) were recorded by weather stations located in close proximity to experiments at each site. Observations were averaged over a 7-day period to determine a weekly mean value for each measure, and completed for 12 incremental weeks following planting.

Cultivars were selected from newer-release genotypes (2013 or later) to include specific genetic and agronomic characteristics associated with differences in plant stature and growth habit (Table 3). Four seeding rates (pure live seed) were selected to provide a range sufficient for fitting linear and nonlinear yield trends (Table 4). Germination testing by ragdoll method was performed on all seed lots. To evaluate cultivar establishment relative to seeding rate treatments, stand counts were completed in each plot around the 2-leaf stage (Zadoks 12 to 15) (Zadoks et al., 1974). Stand counts were determined by counting all emerged wheat plants within a 30 cm section of each of the three innermost rows of each plot. Counts were averaged for the three rows, and a plant density (plants m<sup>-2</sup>) was calculated for each plot.

At the Prosper location, spike population (spikes ha<sup>-1</sup>) was estimated from spike numbers obtained by placing markers 91 cm apart within two of the innermost rows, and counting all productive spikes between markers at physiological maturity (Zadoks 89).

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			Yield			Slope
Location	Latitude	Longitude	(Mg ha <sup>-1</sup> )	Soil series	Taxonomy	(%)
North Dakota						
Dickinson	46.981	-102.824	3.06‡	Arnegard	Fine-loamy, mixed, superactive, frigid Pachic Haplustolls	0-2
Hettinger	46.012	-102.647	2.71	Shambo	Fine-loamy, mixed, superactive, frigid Typic Haplustolls	0-2
Minot	48.180	-101.304	4.24	Forman-	Fine-loamy, mixed, superactive, frigid Calcic Argiudolls	3-6
				Aastad	Fine-loamy, mixed, superactive, frigid Pachic Argiudolls	
Prosper	47.003	-97.116	4.92	Kindred-	Fine-silty, mixed, superactive, frigid Typic Endoaquolls	0-2
				Bearden	Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	
Minnesota						
Crookston	47.815	-96.616	5.48	Wheatville	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	0-2
Lamberton	44.241	-95.312	4.00	Webster	Fine-loamy, mixed, superactive, mesic Typic Endoaquolls	0-2

Table 1. Location, average yield, and soil characteristics<sup>†</sup> of 2017-2018 experiment sites.

† Soil data obtained from NRCS-USDA, 2017.
‡ Average grain yield (2016-2018) reported in North Dakota and Minnesota variety trial publications.  $\infty$ 

# Table 2. Location and management details for 2017-2018 research environments in ND and MN.

		2017			2018		
Location	Managing unit	Previous crop	Seeding	Harvest	Previous crop	Seeding	Harvest
North Dakota							
Dickinson	Dickinson Research & Extension Center	HRSW†	28-Apr	-	HRSW	2-May	13-Aug
Hettinger	Hettinger Research & Extension Center	Soybean	26-Apr	3-Aug	Soybean	27-Apr	16-Aug
Minot	North Central Research & Extension Center	Soybean	21-Apr	19-Aug	Soybean	3-May	8-Aug
Prosper	Prosper Extension Cereals Program		22-Apr	21-Aug	HRSW	30-Apr	31-Jul
Minnesota							
Crookston	Northwest Research & Outreach Center	Soybean	3-May	29-Aug	Soybean	7-May	8-Aug
Lamberton	Southwest Research Outreach Center	Soybean	17-Apr	23-Aug	Soybean	7-May	10-Aug

<sup>†</sup> Soybean, Glycine max (L.) Merr.; HRSW, hard red spring wheat, Triticum aestivum, L.

Observations for agronomic measures were performed by cooperating researchers at each location. Heading date was recorded for each plot as days after planting (DAP) until >50% of the plot was headed. Plant height was measured as the average height (in cm) from the soil surface to the awns of approximately 5 spikes, with 2 areas sampled per plot. Minimal lodging was observed in all environments and excluded from results.

Cultivar	Source	Plant stature‡		Photoperiodism§	Height	Straw strength	Heading
		Rht-B1	Rht-D1	Ppd-D1	cm	(1-9)¶	DAP#
LCS Anchor	Limagrain	а	b	a	71.9	5	58
Lang-MN	UMN	а	а	a	82.6	5	61
Linkert	UMN	а	b	b	72.9	2	59
Prevail	SDSU	а	а	a	78.2	4	58
Shelly	UMN	b	а	b	77.0	5	62
Surpass	SDSU	а	а	b	79.8	7	56
SY Valda	AgriPro	а	b	b	75.9	4	60
ND VitPro	NDSU	b	а	b	80.0	4	59
TCG Wildfire	21st Century	b	a	a	86.6	4	60

Table 3. Select genetic and phenotypic characteristics<sup>†</sup> of HRSW cultivars in experiment.

<sup>†</sup> Data obtained from North Dakota HRSW variety trial selection guide (NDSU, 2017).

‡ a is wild-type allele, b is semi-dwarf allele.

§ a is insensitive allele, b is sensitive allele.

¶ Lodging score; 1 is standing erect, 9 is lying flat.

# DAP, days after planting.

Plot lengths were measured at harvest to determine area harvested by small plot combine. Plot grain yield was measured by an on-combine weigh system. Plot subsamples were collected and processed through a Clipper multi-sieve seed cleaner (Ferrell-Ross, Bluffton, IN) prior to measuring moisture content and test weight with a GAC 2100 moisture tester (DICKEY-John Corp., Minneapolis, Minnesota). Plot grain yields were corrected to a moisture content of 130 g kg<sup>-1</sup> and expressed as Mg ha<sup>-1</sup>. Grain protein content (percent) was measured using a DA 7250 NIR analyzer (Perten Instruments, Stockholm, Sweden).

Location	Factor	Treatment
Crookston, MN	Cultivar	LCS Anchor
Dickinson, ND		Lang-MN
Hettinger, ND		Linkert
Lamberton, MN		Prevail
Minot, ND		Shelly
Prosper, ND		Surpass
		SY Valda
		ND VitPro
		TCG Wildfire
	Seeding Rate	1.85 million seeds ha <sup>-1</sup>
		3.09 million seeds ha <sup>-1</sup>
		4.32 million seeds ha <sup>-1</sup>
		5.56 million seeds ha <sup>-1</sup>

Table 4. Location, factor, and treatment of RCBD of 2017-2018 experiments.

## **Statistical Analysis**

Tests for homogeneity of variance were completed prior to performing combined ANOVA in SAS 9.4 (PROC MIXED). Cultivar and seeding rate were fixed effects, and environment was random. Interactions were plotted in SAS (PROC GLIMMIX) and evaluated for crossover and non-crossover interactions (result of differences in magnitude). Mean separations were established based on protected F-test using Fisher's LSD ( $P \le 0.05$ ), with consideration for random effects from environment.

Source of variation df df equation Error terms in F-test Env [Environment] 10 env-1 Rep(Env) 22 env(r-1) A [Cultivar] 8 a-1 A MS/Env\*A MS Env x A 80 Env\*A MS/Error MS (env-1)(a-1)B [Seeding Rate] 3 (b-1) B MS/Env\*B MS Env x B 30 (env-1)(b-1)Env\*B MS/Error MS A x B A\*B MS/Env\*A\*B MS 24 (a-1)(b-1)Env x A x B 240 (env-1)(a-1)(b-1)Env\*A\*B MS/Error MS 770 (ab-1)(env)(r-1)Error \_

Table 5. Sources of variation and error terms for ANOVA of 11 environments in 2017-2018.

At the environment level, cultivar yield response to seeding rate was regressed by linear and quadratic models in SAS (PROC REG). Coefficient of determination ( $R^2$ ) and residual root mean square error (RMSE) values (from SAS output) were used to determine model best fit for data. An optimal seeding rate (OSR) was determined for each cultivar x environment combination. For cultivar with a linear response to seeding rate, the OSR was the seeding rate treatment at which maximum yield was observed. For quadratic models, the OSR was determined by evaluating the coefficients of the equation. Quadratic equations with a negative linear coefficient (second term) were assigned the lowest seeding rate treatment as the OSR. For all other quadratic models, the OSR was calculated by taking the first derivative of the equation.

### Results

Weekly mean values for maximum and minimum temperatures varied by environments and from year-to-year for locations (Figure 1). Rainfall timing and accumulation per growing season also differed among environments (Figure 2). Overall, there were no apparent trends in weather data that could be readily associated with observations for grain yield or OSR.



Figure 1. Weekly maximum and minimum temperatures after planting, 2017/2018 environments.



Figure 2. Rainfall accumulation by week after planting for 2017 and 2018 environments.

Results from the ANOVA evaluating agronomic response of HRSW cultivars to seeding rate treatments over 11 environments are included in Table 6. Environment interacted with cultivars, influencing yield, plant density, grain protein, heading date, and plant height. Environment also interacted with seeding rate, effecting plant density and grain protein. The cultivar by seeding rate interaction was not significant for any of the responses measured.

Source	df	Yield	Density†	Protein	Heading‡	Height§
A [Cultivar]	8	8.02***	2364	33.4***	7.9	169*
Env x A	80	0.68***	2740**	1.04***	6.5***	77.8***
B [Seeding Rate]	3	1.95***	2816433***	0.64	0.1	15.5
Env x B	30	0.20	4696***	0.48**	2.2	37.6*
A x B	24	0.20	1239	0.26	3.7	29.5
Env x A x B	240	0.20	1868	0.18	3.6	32.3***
Error	770	0.19	1905	0.24	3.4	22.5

Table 6. Mean square values and ANOVA results for grain yield and plant establishment of HRSW cultivars at incremental seeding rates for combined 2017-2018 environments.

\*, \*\*, and \*\*\*, indicate significance at P < 0.05, P < 0.01, and P < 0.001 respectively.

† Plant density (plants m<sup>-2</sup>) observed at 2-leaf stage (Feekes 3).

‡ Heading days after planting. No data reported for Crookston and Dickinson in 2018.

§ No data reported for 2017 Crookston environment.

## **Plant Density**

Plant density (established plants m<sup>-2</sup>) differed among environments, as cultivar interacted with environment (Table 6). This result demonstrates phenotypic plasticity of cultivars, as plant growth habit changed in response to interactive effect(s) of environment (Allard and Bradshaw, 1964; Bradshaw, 1965). Environment interacted with seeding rate effecting plant density of cultivars (Table 6). Similar results were reported in a wheat seeding rate study conducted by Geleta et al. (2002). As plant density is closely associated with yield, growers can benefit from establishing a plant density that is favorable for yield. For HRSW production in the northern Great Plains region, an established plant density of 300 to 320 plants m<sup>-2</sup> is considered optimum for early-seeded fields (Wiersma and Ransom, 2012). The number of seeds needed to attain this density will vary among growers, due to differences in equipment, agronomic management, and environmental conditions prior-to/following seeding.

With 11 environments in this study and a significant interactive effect of environment, impact of experimental and potential non-experimental variables (e.g. differences in planting equipment) on established plant density were not readily quantifiable. Therefore, instead of focusing on yield relative to established plant density, it was more efficient and practical to focus on the effect of seeding rate, as it is a fixed variable in these experiments. By taking into account their own experience with seedling emergence percentage, growers can readily adjust seeding rates relative to baseline recommendations for OSR.

## **Grain Protein**

Seeding rate did not significantly affect protein content across environments (Table 7). These results are similar to findings reported by Larter et al. (1971) and Campbell et al. (1991). Combined across environments, cultivars differed in grain protein content. These differences were attributed to the innate characteristics of the cultivar influencing protein (Table 8). Given the diversity in the genetic and phenotypic characteristics of the cultivars, relative differences in grain protein content due to the cultivar main effect were more or less in line with published levels. A significant environment by cultivar interaction was also observed for protein content (Table 6).

Table 7. The effect of seeding rate on yield and other agronomic traits for combined 2017-2018 environments.

Seeding rate	Yield	Density†	Protein	Heading‡	Height§
million seeds ha <sup>-1</sup>	Mg ha <sup>-1</sup>	plants m <sup>-2</sup>	g kg <sup>-1</sup>	DAP	cm
1.85	3.36a	165a	15.1	53	66.8
3.09	3.49b	245b	15.1	53	67.2
4.32	3.55b	322c	15.0	53	67.1
5.56	3.51b	391d	15.0	53	66.7
$LSD_{0.05}$ ¶	0.08	11	NS	NS	NS

<sup>†</sup> Plant density (plants m<sup>-2</sup>) observed at 2-leaf stage (Feekes 3).

‡ Heading days after planting (DAP). No data reported for Crookston and Dickinson in 2018.

§ Data not reported for 2017 Crookston environment.

¶ LSD values calculated based on Fisher's F-protected test.

Cultivar	Yield	Density‡	Protein	Heading§	Height
	Mg ha <sup>-1</sup>	plants m <sup>-2</sup>	g kg <sup>-1</sup>	DAP	cm
LCS Anchor	2.94a†	280	15.5ab	53	66.0ab
Lang-MN	3.52bcd	289	15.5ab	53	66.0ab
Linkert	3.36b	280	15.7a	54	68.0bc
Prevail	3.52bcd	283	14.4c	53	68.4c
Shelly	3.67de	276	14.5c	54	67.5bc
Surpass	3.57cd	278	14.8d	54	67.4bc
SY Valda	3.84e	276	14.6c	53	67.3bc
ND VitPro	3.43bc	284	15.5a	54	67.6bc
TCG Wildfire	3.45bc	284	15.3b	54	64.6a
LSD <sub>0.05</sub>	0.20	NS	0.2	NS	2.3

Table 8. Agronomic traits of HRSW cultivars for combined 2017-2018 environments.

<sup>†</sup> Values with the same letter in a column are not significantly different (P > 0.05).

<sup>‡</sup> Plant density (plants m<sup>-2</sup>) observed at 2-leaf stage (Feekes 3).

§ Heading days after planting (DAP). No data reported for Crookston and Dickinson in 2018.

¶ No data reported for 2017 Crookston environment.

Cultivar sharing similar genetic characteristics for photoperiod response (*Ppd-D1*) and/or semi-dwarf gene expression (*Rht-B1* or *Rht-D1*) did not predispose accumulation of protein at similar levels. Examples of this include protein content of ND VitPro and Shelly across environments. Though ND VitPro and Shelly are photoperiod insensitive and express the *Rht-B1b* gene, the average protein content of ND VitPro was 1.0 g kg<sup>-1</sup> greater than Shelly (calculated from Table 8). Other examples of cultivars differing in grain protein though sharing similar genetic backgrounds for photoperiod response and semi-dwarfing genes, include Lang-MN consistently having greater protein content than Prevail, and SY Valda with lower protein content than Linkert in all 11 environments. Grain protein differences between environments and cultivars are likely to be influenced by environmental factors such as differences in daily temperature fluctuation, timing of available water, and amount of accumulated rainfall, as these all can impact yield potential of cultivars and kernel development (Pan et al., 2006).

The relationship between protein content and grain yield was found to be curvilinear (Figure 3). The general trend across cultivars is with yields  $\geq 3.50$  Mg ha<sup>-1</sup>, protein content decreased as yield increased. This result coincides with previous findings by Faris and De Pauw (1981). Under lower yielding conditions (< 3.50 Mg ha<sup>-1</sup>), the positive trend between yield and grain protein content is likely representative of varying degrees of drought stress that occurred. Drought stress was likely the cause of early plant senescence, affecting the ratio of starch to protein accumulation and composition in the kernels (Rakszegi et al., 2019). Overall, these data suggest that regardless of cultivar, seeding rate had limited impact on grain protein content.



Figure 3. Protein content relative to HRSW cultivar yield, combined 2017-2018 environments.

## **Heading Date**

Heading date was not affected by changes in seeding rate, and was similar for cultivars when averaged over 11 environments (Table 6). The cultivar by environment interaction was attributed to differences in heading due to planting date and environment. Though these factors had a different magnitude of effect on growth and development of cultivars at each location, cultivars were similar in rank across locations and years for relative heading date (results not included). Based on these results, genotype and environment (not seeding rate) were the primary determinants of heading date of cultivars.

## **Plant Height**

Averaged over 11 environments, plant height was unchanged by seeding rate (Table 7). Finlay et al. (1971) had similar findings for plant heights of six barley cultivars seeded at rates of 54, 108, and 161 kg ha<sup>-1</sup>. The differences in plant height between cultivars (Table 8) was as expected given the diversity of cultivars. Cultivar response to seeding rate differed among environments (Table 6). Other studies however have reported seeding rate to have minimal effect on plant height of cultivars grown in varying environments (Pelton, 1969; Faris and De Pauw, 1981). This was likely because environments were diverse in geographic location, annual precipitation, temperature, and other environmental factors that influence phenotype and yield potential of cereal crops (Thomas et al., 1993; Frensham et al., 1998; Yan and Hunt, 2001).

## **Grain Yield**

Yield was significantly affected by seeding rate. Combined over 11 environments, grain yield increased by 0.13 Mg ha<sup>-1</sup> when the seeding rate was increased from 1.85 to 3.09 million seeds ha<sup>-1</sup> (Table 7). Seeding at rates greater than 3.09 million seeds ha<sup>-1</sup> did not increase grain yield further. Based on these results, it may seem appropriate to recommend 3.09 million seeds ha<sup>-1</sup> as optimal for yield. However, environment had a significant interactive effect with cultivar, and with seeding rate, suggesting the need to develop seeding rate recommendations that are not only variety specific, but also consider the environment in which they are grown.

When combined over environments, the cultivar x seeding rate interaction was not significant. Nevertheless, when each cultivar was characterized for its response to increasing seeding rate, they responded quite differently (Figure 4). These differences are likely due to variation among cultivar in magnitude of effect of cultivar x environment interactions, affecting grain yield (Pendleton and Dungan, 1960; Slafer and Rawson, 1994; Geleta et al., 2002). Also, as varieties were from diverse genetic backgrounds and developed by various breeding programs located in different areas of the northern Great Plains region, it was expected that cultivars would not perform the same in all environments.

When combined over environments, regression fitting of yield relative to seeding rate revealed a nonlinear relationship (Figure 5). Yield response to seeding rate for all cultivar was best fit by a quadratic model (Table 9). This indicates that growers seeding above or below the OSR for a cultivar are likely not maximizing seeding efficiency. This result is best supported by the five cultivars with a highly predictive quadratic yield response to increasing seeding rate (as indicated by  $R^2$  values  $\geq 0.89$ ). Yield was not closely associated with seeding rate for all cultivars, as yield of LCS Anchor did not respond to seeding rates ( $R^2 \leq 0.03$ ).



Figure 4. Grain yield of HRSW cultivars at seeding rates, combined 2017-2018 environments.

When averaged over cultivar and environment, the OSR was 4.39 million seeds ha<sup>-1</sup> (Table 9). Cultivar OSR ranged from 3.68 to 5.56 million seeds ha<sup>-1</sup>. Of the five cultivars with high  $R^2$  values ( $R^2 \ge 0.89$ ), Surpass had the lowest OSR of 4.02 million seeds ha<sup>-1</sup>, and Linkert had the highest OSR at 4.91 million seeds ha<sup>-1</sup>. Results for cultivar-specific OSR provide opportunities for improving efficiency of HRSW production relative to seed input, in comparison to a general recommendation (e.g. Table 7).



Figure 5. Yield response to seeding rate of HRSW cultivars, combined 2017-2018 environments.

Environment significantly interacted (P<0.001) with cultivars for grain yield (Table 6). The influence of cultivar x environment interaction was well represented by variability in yield response to seeding rate of Prevail, ND VitPro, and Lang-MN (Figure 4 and 5). When combined over environments, seeding rate was only partially predictive of grain yield of these cultivars, as  $R^2$  values were 0.43 (Prevail), 0.56 (ND VitPro), and 0.67 (Lang-MN) (Table 9).

The cultivar x environment interaction was further detailed by fitting regression functions for individual cultivar within each environment, and using regression equations to determine cultivar OSR for each environment. By pairing these cultivar-specific OSR with observed mean yield values for each environment (Table 10), differences in cultivar efficiency relative to seed input, and differences in magnitude of interactive effect of environment, became more apparent.

	Seed	ing rate (m	illion see	ds ha <sup>-1</sup> )			
Cultivar	1.85	3.09	4.32	5.56	Regression equation <sup>†</sup>	OSR‡	$R^2$
		Mg	ha-1			million seeds ha <sup>-1</sup>	
LCS Anchor	2.97	2.83	3.09	2.88	$\hat{y}=2.82+0.08x-0.01x^2$	3.68	0.03
Lang-MN	3.37	3.62	3.52	3.57	$\hat{y}=2.97+0.29x-0.03x^2$	4.32	0.67
Linkert	3.24	3.32	3.46	3.41	$\hat{y}=2.92+0.21x-0.02x^2$	4.91	0.89
Prevail	3.42	3.57	3.47	3.57	$\hat{y}=3.28+0.10x-0.01x^2$	5.13	0.43
Shelly	3.50	3.70	3.74	3.70	$\hat{y}=3.00+0.35x-0.04x^2$	4.33	0.99
Surpass	3.41	3.61	3.73	3.54	$\hat{y}=2.67+0.51x-0.06x^2$	4.02	0.95
SY Valda	3.65	3.91	3.98	3.83	$\hat{y}=2.88+0.54x-0.07x^2$	4.06	0.99
ND VitPro	3.39	3.44	3.35	3.54	$\hat{y}=3.58-0.14x+0.02x^2$	5.56	0.56
TCG Wildfire	3.31	3.45	3.55	3.51	$\hat{y}=2.90+0.27x-0.03x^2$	4.70	0.99
LSD <sub>0.05</sub>		0.0	)8§				
Combined	3.36	3.49	3.54	3.50	$\hat{y}=3.00+0.25x-0.03x^2$	4.39	0.99

Table 9. Yield response of cultivar to seeding rates, and regression equations with optimal seeding rates, based on cultivar response over 2017-2018 environments.

† Regression equation from PROC REG for quadratic best-fit model.

<sup>±</sup> OSR, optimal seeding rate; based on quadratic regression equation.

§ LSD calculated for seeding rate main effect according to Fisher's F-protected test.

Some cultivars were consistent across varying environments in producing higher or lower yields, relative to other cultivars. Of the 10 environments with significant cultivar main effect, SY Valda was the highest-yielding cultivar in eight of these environments (Table 10). LCS Anchor was the lowest yielding in all environments. These results are consistent with relative performance of these cultivars in university variety trials in North Dakota in 2016-2017 (NDSU Extension, 2016; 2017). For other cultivars, yield ranking was inconsistent across environments.

The effect of the cultivar x environment interaction was also apparent when comparing mean yield of cultivars grown in the same environment, and relative cultivar response across environments (Table 10). Cultivars grown in the Crookston 2017 environment had the highest mean yield of all environments at 5.09 Mg ha<sup>-1</sup> (range: 4.47 to 5.79 Mg ha<sup>-1</sup>). Minot 2017 was the lowest yielding environment with an average yield of 1.81 Mg ha<sup>-1</sup> (range: 1.66 to 2.13 Mg ha<sup>-1</sup>).

Cultivar OSR based on mean yield values at seeding rates combined over 11

environments ranged from 3.68 to 5.56 million seeds ha<sup>-1</sup> relative to mean yields ranging from 2.94 to 3.43 Mg ha<sup>-1</sup> (Tables 7 and 9). SY Valda and Shelly were the highest yielding cultivars at 3.84 and 3.67 Mg ha<sup>-1</sup>, respectively (Table 8). Though yield of these cultivars was similar, OSR for SY Valda was lower at 4.06 million seeds ha<sup>-1</sup> compared to 4.33 million seeds ha<sup>-1</sup> for Shelly (Table 9). As environment and cultivar interaction had variable impact on yield response of cultivars, OSR calculated from data combined over 11 environments are not likely representative of the seeding rate that will promote maximum yield in individual environments.

Yield of cultivars respective to OSR varied by environment. In the highest yielding environment (Crookston 2017), yield of these two cultivars was not significantly different, but the OSR for Shelly was lower compared to SY Valda (3.66 and 4.32 million seeds ha<sup>-1</sup>, respectively) (Table 10). Contrasting OSR of these cultivars in the lowest yielding environment (Minot 2017), while producing similar yields (P>0.05), SY Valda had a lower OSR of 4.08 million seeds ha<sup>-1</sup>, compared to Shelly of 5.56 million seeds ha<sup>-1</sup>. Other cultivars in this environment yielded similar to SY Valda and Shelly, and had varying OSR (Lang-MN, Linkert, and Prevail, at 3.69, 4.09, and 4.21 million seeds ha<sup>-1</sup>, respectively).

Specific environmental factors that may have influenced cultivar development throughout the growing season were difficult to isolate for environments. In the absence of specific parameters or qualifiers to guide an unbiased classification of environments into different groupings, the OSR for cultivars is best represented by cultivar OSR in individual environments (Table 10). However, the OSR for cultivar in each environment have a limited scope of application, as results are not robust enough to account for variability among growers and year-to-year differences in growing conditions.
					2	017				
	Cro	okston†	Pro	osper	Lam	berton	He	ttinger	M	linot
Cultivar	OSR‡	Yield	OSR	Yield	OSR	Yield	OSR	Yield	OSR	Yield
	million seeds ha <sup>-1</sup>	Mg ha <sup>-1</sup>								
LCS Anchor	2.55	4.53a§	3.96	3.98a	3.82	2.92a	3.89	1.66a	1.85	1.20a
Lang-MN	1.85	4.80b	5.20	4.68de	4.12	3.75c	3.78	2.00cde	3.69	1.88bc
Linkert	5.14	4.98b	3.26	4.39bc	4.49	3.61bc	3.09	1.83abc	4.09	1.96bc
Prevail	3.65	4.47a	5.56	4.38bc	5.56	3.81c	3.69	2.07e	4.21	1.94bc
Shelly	3.66	5.64c	3.62	5.13f	3.91	4.16d	4.26	2.06e	5.56	1.92bc
Surpass	3.09	4.99b	4.36	4.42bc	4.01	3.77c	3.74	2.07de	3.28	1.74b
SY Valda	4.32	5.79c	5.56	4.85e	4.86	4.27d	3.09	2.13e	4.08	2.10c
ND VitPro	1.85	4.89b	5.01	4.25b	5.56	3.55bc	4.16	1.91bcd	3.77	1.76b
TCG	3.58	5.71c	5.08	4.48cd	4.58	3.39b	4.67	1.76ab	3.78	1.81b
Mean	3.30	5.09	4.62	4.51	4.55	3.69	3.82	1.94	3.81	1.81

Table 10. Optimal seeding rates and mean grain yield of HRSW cultivars in 2017-2018 environments in ND and MN.

2018

		2010										
	Croc	okston	Pro	sper	Lam	berton	Het	tinger	Ν	linot	Dick	kinson
Cultivar	OSR	Yield										
	million seeds ha <sup>-1</sup>	Mg ha <sup>-1</sup>										
LCS	5.56	2.49a	1.85	3.44a	3.49	1.75a	4.32	2.81	3.42	3.97a	5.56	3.64a
Lang-MN	2.57	3.37de	5.56	4.20b	5.56	2.88fg	2.97	2.91	5.56	4.44abcd	5.56	3.80ab
Linkert	5.56	3.00b	5.56	4.16b	3.98	2.05b	1.85	3.22	3.09	4.09ab	1.85	3.64a
Prevail	5.56	3.47de	4.37	4.42bc	4.36	2.99g	3.09	3.04	1.85	4.50bcd	4.24	3.61a
Shelly	2.91	3.01bc	5.56	4.09b	5.56	2.64de	3.06	2.86	5.56	4.65cd	4.29	4.25c
Surpass	5.56	3.42de	5.56	4.63c	1.85	2.70def	3.09	3.54	3.65	4.20abc	4.10	3.82abc
SY Valda	3.88	3.66e	4.75	4.59c	4.26	2.75ef	3.69	3.14	3.90	4.80d	3.78	4.18bc
ND VitPro	3.75	3.33cd	3.85	4.37bc	5.56	2.50cd	5.56	3.40	5.56	3.96a	1.85	3.81ab
TCG	3.15	3.33d	4.32	4.13b	1.85	2.40c	1.85	3.14	5.56	4.20abc	3.50	3.64a
Mean	4.28	3.23	4.60	4.22	4.05	2.52	3.28	3.12	4.24	4.31	3.86	3.82

<sup>†</sup> Crookston and Lamberton are in Minnesota; Prosper, Hettinger, Minot, Dickinson are in North Dakota.

‡ OSR, optimal seeding rate for maximum cultivar yield; based on quadratic or linear regression equation from PROC REG best fit for data.

§ Values with the same letter within a column for individual environments are not significantly different (P>0.05) based on Fisher's LSD.

Cultivars included in this study were selected based on the presence or absence of certain genetic and phenotypic characteristics (associated with yield) to provide various groupings of cultivars for a more robust analysis. Yield response of cultivars sharing similar expression for *Rht-B*, *Rht-D*, or *Ppd-D* genes (Table 3) was variable for comparisons both within, and across environments (Table 10). Dominating interactions between environment and individual cultivar were observed in Linkert and SY Valda, which are both semi-dwarf cultivars (*Rht-Db*) and sensitive to photoperiod (*Ppd-Db*). Yield of these two cultivars differed in all environments, with the exception of Minot 2017. Shelly and ND VitPro share similar genetic traits for semi-dwarf gene *Rht-B* and photoperiod sensitivity *Ppd-D*, and these two cultivars differed in yield in 6 of the 10 environments where cultivar was a main effect.

When comparing OSR relative to yield of cultivars sharing similar key genes, Lang-MN and Prevail produced similar yields in 8 of the 10 environments that had a cultivar main effect. The OSR of these cultivars, however, varied across environments (Table 10). For Hettinger 2017, the OSR for Lang-MN was 3.78 million seeds ha<sup>-1</sup>, and 3.69 million seeds ha<sup>-1</sup> for Prevail. Contrasting results from Minot 2018, OSR for Lang-MN was 5.56 million seeds ha<sup>-1</sup>, versus 1.85 million seeds ha<sup>-1</sup> for Prevail.

One of the yield components of HRSW is the number of spikes produced per hectare. Cultivars producing similar yields, but differing in OSR, may be related to differences among cultivar in growth habit and spike production. For spike populations (spikes ha<sup>-1</sup>) combined for 2017-2018 environments at Prosper, ND, there was no interaction between cultivar and seeding rate. However, cultivar and seeding rate both had significant effects on total spikes ha<sup>-1</sup> (Table 11). Surpass, SY Valda, and ND VitPro produced similar spike populations of 7.27 million spikes ha<sup>-1</sup> (calculated mean from Table 11). LCS Anchor, Lang-MN, Linkert, Shelly, and TCG Wildfire had a lower average spike population of 6.04 million spikes ha<sup>-1</sup> (calculated from Table 11). Though spike population was similar among cultivars for each of these two groupings, yield differed among cultivar, and from year-to-year, showing that spikes are not the only component of yield that was important (Table 10).

Cultivar	1.85	3.09	4.32	5.56	Mean
			——spikes ha <sup>-1</sup>	l	
LCS Anchor	4.69	5.97	6.88	6.53	6.01
Lang-MN	5.15	6.05	5.91	6.18	5.82
Linkert	5.43	5.96	6.62	7.23	6.31
Prevail	5.91	7.28	7.46	7.62	7.06
Shelly	5.43	5.67	6.10	6.38	5.90
Surpass	5.82	6.67	8.08	8.62	7.30
SY Valda	6.08	6.89	7.41	8.58	7.24
ND VitPro	6.11	6.63	8.36	7.96	7.27
TCG Wildfire	5.23	5.93	6.63	6.82	6.15
Mean	5.54	6.34	7.05	7.32	
$LSD_{0.05}$ †		0.3	35		0.90

Table 11. Spike population of HRSW cultivars at varying seeding rates, Prosper 2017-2018.

<sup>†</sup> LSD values for mean comparisons among main effect levels from Fisher's F-protected test.

Results from spike counts within the experiments at Prosper 2017 and 2018 indicate differences in cultivar growth habit relative to seeding rate (as represented by spikes ha<sup>-1</sup>). This may represent cultivar differences in tillering capacity, as greater spacing among plants (at lower seeding rates) can allow plants to utilize compensatory mechanisms to produce additional tillers when environmental conditions are favorable and resources are available (Elhani et al., 2007). These results for variable yield response of cultivar grown in different environments further indicates the interactive effect of environment on cultivar yield and response to seeding rate.

## Conclusions

Environment and cultivar were much more important than seeding rate in determining grain yield. An optimal seeding rate for HRSW across environments and cultivars is 4.39 million seeds ha<sup>-1</sup>. Though in the combined analysis there was no environment x seeding rate interaction, OSR varied among cultivars within each environment. Some cultivars had no response to seeding rate in some environments, and growers should seed at lower rates in these scenarios. Genetic factors and spike population were not predictive of cultivar responsiveness to increasing seeding rate.

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# CHAPTER 2. A STANDARDIZED METHOD FOR DETERMINING TILLERING CAPACITY OF HRSW CULTIVARS

#### Introduction

Genotype is the primary determinant of yield potential in hard red spring wheat (HRSW) cultivars. The actual yield attained by a cultivar is greatly influenced by interactive effects from environment and agronomic management. One way that these factors can affect yield is by influencing plant growth habit and composition of yield components. For wheat grown in irrigated, intensively managed environments, production of uniculm plants is considered ideal for yield (Donald, 1968). As the majority of HRSW in the northern Great Plains region of the U.S. is seeded in dryland environments, crop management for economic production includes using cultivars with both main stem and tillers contributing to yield. Tillering can allow plants to adjust growth relative to density of neighboring plants or quality of growing conditions (Kirby and Faris, 1972). Diversity among modern HRSW cultivars includes a range of genotypes differing in plant growth habit and tillering capacity. And though an extensive record of publications have documented efforts employed to identify quantitative trait loci (QTL) associated with tillering in wheat, no findings have been published to date identifying a specific gene associated with tillering traits (Richards, 1988; Li et al., 2002).

Agronomic management for sustainable HRSW production includes selecting a seeding rate that will maximize efficiency of production for every plant. For example, a high tillering cultivar could be seeded at a lower rate compared to cultivar with a lower number of tillers contributing to total grain yield. When determining the seeding rate optimal for cultivar yield, it is important to consider the tillering capacity of a cultivar to avoid economic losses due to unnecessary seed costs (overseeding) or uncaptured yield (underseeding). Underseeding to promote plant tillering is likely to impact yield, though differences in reportings for percent of total grain yield contributed by tillers (range 6.7% to 46.9%) to make it difficult to determine a definitive percent (Destro et al., 2001; Otteson et al., 2008). Additionally, underseeding a field with a low tillering cultivar can limit leaf area index, reducing the amount of radiative light intercepted ha<sup>-1</sup>, and thereby lowering production efficiency and subsequent yield at harvest (Fischer and Kohn, 1966). Richards and Townley-Smith (1987) noted that greater leaf area index proved to be a disadvantage for high tillering cultivars subjected to early drought conditions occurring prior to anthesis. When drought conditions were present only after anthesis, pre-anthesis vegetative growth was estimated to contribute 60% of total grain yield.

Though it is apparent that differences among cultivars in tillering capacity and plant leaf area influence yield, Hsu and Walton (1971) noted spikes plant<sup>-1</sup> generally has a greater influence on plant yield. Applying this understanding on a field scale, Holliday (1960) noted yield gains observed in high tillering stands could be attributed to contributions from spikes, based on the understanding of spike photosynthetic efficiency reported by Archbold and Mukerjee, 1942). Elhani et al. (2007) conducted tillering experiments and concluded that a high tillering capacity provided cultivars with advantages in plant growth habit and yield components over low tillering cultivars, but only in non-stressed, irrigated environments. Hucl and Baker (1988) noted that though tillers m<sup>-2</sup> is closely associated with spikes m<sup>-2</sup> (r = 0.84), spikes m<sup>-2</sup> is a poor determinant of yield ( $R^2 = 0.005$ ). Considering diversity in tillering capacity among cultivars and production potential across environments, these findings reinforce the importance of considering cultivar tillering capacity when determining optimal seeding rates to maximize yield throughout the northern Great Plains region.

Though environment can influence tillers plant<sup>-1</sup> produced by a cultivar, it can be expected to observe similar relative responses in other cultivars across environments. Klepper et al. (1982) indicated that the process of wheat plant development is unchanged across environment types, but noted that environment affects the rate at which development occurs. This can be used to explain the reporting from Friend (1965) that reduced tiller numbers in mature wheat plants was not attributable to the plant's inability to form tiller buds, but rather the lack of tillers emerging from axillary buds. Though environment and agronomic factors can impact tillering, it can be expected that genotypes will not to be differentially affected by these factors. Work completed by Richards (1988) provides support for this statement as varying planting timing of spring-seeded wheat produced similar changes in spikes plant<sup>-1</sup> among tillering cultivars. Evaluations of tiller numbers of cultivar seeded in various arrangements (adaptations in spacing and rectangularity) indicated arrangement had no interactive effect on cultivar tillering (Auld et al., 1983). Carr et al. (2003) found that no rank changes were observed among HRSW cultivars when evaluating tillage and seeding rate effects on tiller production. In general, these studies all document cultivar responses to agronomic practices that are similar in scale across cultivars.

Though differences in wheat plant tiller number in response to varying treatments for row spacing, seeding rate, and planting method have been extensively published, documentation is minimal for current methods used by breeders and researchers to assess tillering characteristics of individual cultivar. Hucl and Baker (1988) utilized various approaches for evaluating tillering characteristics of wheat genotypes based on spikes plant<sup>-1</sup>. One approach included single row planting and subsequent thinning to 10 plants m<sup>-2</sup> for sampling of 5 plants to represent each genotype. Additional approaches included solid-seeded (2.40 million seed ha<sup>-1</sup>) and space-

planted (est. 0.12 million seeds ha<sup>-1</sup>) conditions to determine spikes plant<sup>-1</sup> for relative comparisons among genotypes. The objective of the space-planted method was to provide growing conditions with minimal competition among plants to promote plant tillering for full evaluation of genotype tillering capacity. Of the 373 genotypes originally evaluated, the genotypes with the 10 lowest, and 10 highest values for spikes plant<sup>-1</sup> were reported as low tillering and high tillering genotypes, respectively. This study demonstrated various experimental approaches used to identify high and low tillering genotypes, based on relative rank among genotypes for spikes plant<sup>-1</sup>. However, not all HRSW cultivars available to growers are designated as having characteristics of high tillering or low tillering. This reinforces the importance of developing a standardize system for assessing cultivar tillering capacity.

Without a standardized system for assessing cultivars for tillering characteristics, there is greater uncertainty in optimal seeding rates for HRSW cultivars due to additional error associated with subjective evaluation of cultivar tillering habits. Developing a standardized method for assessing tillering capacity of genotypes provides breeding programs with a tool to readily determine this important characteristic to include in the description of new cultivars upon release.

The objectives of this research were to determine a method for assessing tillering of HRSW cultivars and develop a standardized approach for characterizing cultivar tillering capacity. Various seeding techniques were applied in differing plant spacing arrangements to evaluate tillering habit and spikes plant<sup>-1</sup> of diverse HRSW cultivars.

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## **Materials and Methods**

## **Site Description**

Three different experiments were used in this study to profile various assessment methods. All experiments were established at the agricultural research site near Prosper, ND (47.003° -97.116°), with a soil type that is characterized as somewhat poorly drained consisting of a complex of Kindred (fine-silty, mixed, superactive, frigid Typic Endoaquolls) and Bearden (fine-silty, mixed, superactive, frigid Aeric Calciaquolls) soils with a minimal slope (0-2%). Experiment 1 was established in 2017 and 2018 (two environments). Experiment 2 and Experiment 3 were each established in 2018. Sites were cropped to HRSW in the year prior. Sites received disc tillage in the fall prior, and a field cultivator in the spring, prior to planting. Agronomic management (including cultivation, fertilization, and pest management) followed NDSU extension recommendations to ensure inputs were not an additional source of variance.

## **Experimental Approach**

Experiment 1 was conducted concurrently within the small plot seeding rate experiment described in Chapter 1. Experimental units were 5.5 m<sup>2</sup> plots in a randomized complete block design with a factorial arrangement of cultivar and seeding rate. Treatments included combinations of four seeding rates and nine HRSW cultivars (Table 4) seeded in 7 rows at 18 cm spacing with a Great Plains no-till drill (Kincaid Research, Haven, KS). Cultivars were selected to include a diversity of genetic backgrounds and phenotypes (Table 9). Plant counts were completed within each plot around the 2-leaf stage (Zadoks 12 to 15) (Zadoks et al., 1974) by placing markers 91 cm apart within two of the innermost rows and counting all wheat plants between markers. At physiological maturity (Zadoks 89), all productive spikes between markers were counted and averaged over early-season plant counts in each sampling area. Values from

both areas sampled were averaged to determine average spikes  $plant^{-1}$  for each plot. Spikes  $plant^{-1}$  was selected (versus spikes m<sup>-2</sup> or stems m<sup>-2</sup>) to evaluate various plant spacing arrangements to determine the method most appropriate for evaluating cultivar tillering abilities. As Hucl (1986) reported spacing arrangement comparisons based on spikes plant<sup>-1</sup> were only slightly correlated (r = 0.33), the focus will be on relative cultivar response across the various methods.

Space-planted methods were used in Experiment 2 and Experiment 3 to promote cultivar expression of tillering phenotype by minimizing competition among plants (Hucl and Baker; 1988). Experiment 2 was a randomized complete block design with four replicates. Nine HRSW cultivars were assigned as treatments. Experimental units were 5.5 m<sup>2</sup> plots seeded with a Hege 1000 no-till planter (Hege Company, Waldenburg, Germany) in 4-rows with 30 cm spacing. A SOFATT (seed only few, and then thin) method was applied by seeding at a fixed rate of 215 000 seeds ha<sup>-1</sup> with the objective to establish wheat plants within each plot at an equidistant of 30 cm for both intra-row and inter-row spacing. Plots were thinned in early June to remove excess wheat plants, including any plants spaced <18 cm from a neighboring plant (Figure 6). Spikes plant<sup>-1</sup> was measured at physiological maturity (Zadoks 89) to allow plants to reach full tillering potential (Hucl, 1986). Intra-row spacing of plants (in meters) and mean spikes plant<sup>-1</sup> were recorded for each plot by averaging spike counts of 8 plants sampled from each plot (Figure 7).

Experiment 3 was a randomized complete block design with 24 replicates. Nine HRSW cultivars were assigned as treatments. Experimental units were 0.09 m<sup>2</sup> single-seed hills planted with a 4-row hill plot planter at equidistant spacing (30 cm x 30 cm). Spikes plant<sup>-1</sup> were determined at physiological maturity for each cultivar replicate.



Figure 6. Experiment 2 plot of HRSW plants (a) seeded at rate of 215 000 seeds ha<sup>-1</sup> and (b) same plot after thinning established plants to equidistant spacings of 30 cm.



Figure 7. Intrarow spacing and spikes plant<sup>-1</sup> were determined for eight plants selected from experimental plots at physiological maturity (Zadoks 89).

## **Statistical Analysis**

Analysis of variance of Experiment 1 data was performed for 2017 and 2018 environments using PROC MIXED in SAS 9.4 (SAS Institute Inc., Cary, NC). Variance of environments was considered homogenous and data was combined for ANOVA using PROC MIXED in SAS 9.4. Following the approach outlined by Carmer et al. (1989), environment was assigned a random effect, and fixed effects were cultivar and seeding rate. A one-way ANOVA was completed for Experiment 2 in PROC MIXED and for Experiment 3 in PROC GLIMMIX evaluating spikes plant<sup>-1</sup> of cultivar treatments. Mean separations were completed according to F-protected LSD values ( $P \le 0.05$ ) determined in PROC MIXED for Experiment 1 and Experiment 2, and PROC GLIMMIX for Experiment 3 as an unequal number of cultivar replicates were present.

## **Parameterization Methods**

Three parameterization methods were evaluated as potential approaches for determining parameters for classifications of the tillering capacity rating system. These methods included the 'Means Comparisons approach', the 'Z-score approach', and the 'Standardized Distribution approach'.

For the 'Mean Comparisons approach', SAS output for LSD mean separations of spikes plant<sup>-1</sup> were used to assign parameters as qualifiers associated with each classification of the tillering capacity rating system (low, moderate, high). As an objective was to develop a standardized system that could be applied in various environmental settings, this required a robust system with the capacity to assign tillering capacity ratings to cultivar from diverse genetic backgrounds that were assessed in differing growing conditions. To account for the effects of these experiment-specific variables in regards to cultivar tillering expression, it was determined that cultivar tillering capacity should be evaluated only after accounting for potential differences in spike densities across experiments. This was done by using Z-score transformations for spikes plant<sup>-1</sup> observations in each experiment. This approach has been utilized by numerous agricultural and ecological studies, to account for variability across environments (Laundre and Reynolds, 1993; Ellsworth et al., 1998; Rahman et al., 2009). The parameterization method used was described as the 'Z-score approach', which proposed that standardized z-scores calculated from spikes plant<sup>-1</sup> observations, could be used to determine cultivar tillering capacity based on relative tillering performance of the cultivar. The 'Z-score approach' required a data transformation step in SAS (PROC STANDARD) to calculate standardized z-scores from observations for spikes plant<sup>-1</sup> as:

$$z-score = \frac{x-\bar{x}}{s}$$

where x is observed spikes plant<sup>-1</sup>,  $\bar{x}$  is experimental mean, and *s* is experimental standard deviation (Clark-Carter, 2014). This adjusted the scale of plant response for spikes plant<sup>-1</sup> to have a mean of 0, and standard deviation of 1.

The 'Standardized Distribution approach' was the third parameterization method evaluated. This method was somewhat of a continuation of the 'Z-score approach', as standardized z-scores were used to calculate spikes plant<sup>-1</sup> estimates to represent data relative to the distribution of the population as:

$$\hat{\mathbf{x}} = (z \text{-score}^* \sigma) + \mu$$

where,  $\hat{x}$  is estimated spikes plant<sup>-1</sup>, z-score is z-score of spikes plant<sup>-1</sup> observation,  $\sigma$  is standard deviation of population, and  $\mu$  is population mean.

## **Results and Discussion**

## **Assessing Cultivar Tillering**

# **Experiment** 1

Results from the ANOVA of Experiment 1 revealed spikes plant<sup>-1</sup> was consistent for

cultivar from year-to-year (Table 12). This is in line with findings of Klepper et al. (1982), and

offers support that seasonal differences in environmental conditions are not likely to influence

results when assessing tillering of HRSW cultivars to determine tillering capacity.

Table 12. Mean square values and ANOVA results for spikes plant<sup>-1</sup> of HRSW cultivars at seeding rate treatments, combined 2017-2018 experiments in Prosper, ND.

Source	df	Mean square
A [Cultivar]	8	1.14*
Env x A	8	0.32
B [Seeding Rate]	3	24.83**
Env x B	3	0.27
A x B	24	0.13
Env x A x B	24	0.17
Error	140	0.18

\*, \*\*, and \*\*\*, indicate significance at  $P \le 0.05$ ,  $P \le 0.01$ , and  $P \le 0.001$  respectively.

As cultivars were of diverse genetic backgrounds and phenotypes, it was not surprising that spikes plant<sup>-1</sup> differed among cultivars (Table 13). Cultivars with higher tiller number ( $\geq$ 2.54 spikes plant<sup>-1</sup>) included SY Valda, Prevail, and ND VitPro. Cultivars producing a moderate number of spikes were Surpass, Linkert, and Shelly, at spikes plant<sup>-1</sup> of 2.50, 2.33, and 2.21 spikes plant<sup>-1</sup>, respectively. TCG Wildfire, LCS Anchor, and Lang-MN were low tillering cultivars (<2.18 spikes plant<sup>-1</sup>) in Experiment 1.

Incremental increases in seeding rate had an inverse effect on spikes plant<sup>-1</sup> (Table 14). An increase in seeding rate from 1.85 to 3.09 million seeds ha<sup>-1</sup>, reduced the number of spikes plant<sup>-1</sup> from 3.3 to 2.5 spikes plant<sup>-1</sup>, respectively. At the highest seeding rates of 4.32 and 5.56 million seeds ha<sup>-1</sup>, plant spikes plant<sup>-1</sup> was the lowest at 1.9 and 1.8 spikes plant<sup>-1</sup>, respectively. Similar responses to seeding rate were reported by Joseph et al. (1985), where 3.7, 2.3, and 1.8 spikes plant<sup>-1</sup> were observed at a seeding rate of 1.86, 3.72, and 5.58 million seeds ha<sup>-1</sup>, respectively.

Cultivar	Spikes plant <sup>-1</sup>
LCS Anchor	2.15ab†
Lang-MN	2.06a
Linkert	2.33abcd
Prevail	2.60d
Shelly	2.21abc
Surpass	2.50bcd
SY Valda	2.64d
ND VitPro	2.54cd
TCG Wildfire	2.17abc
mean	2.36
LSD <sub>0.05</sub>	0.38

Table 13. Spikes plant<sup>-1</sup> of HRSW cultivars observed at 2017-2018 environments, Prosper, ND.

<sup>†</sup> Values with the same letter in a column are not significantly different.

Table 14. Spikes plant<sup>-1</sup> of HRSW at seeding rates, 2017-2018 Prosper environments.

Seeding rate	
(million seeds ha <sup>-1</sup> )	Spikes plant <sup>-1</sup>
1.85	3.3
3.09	2.5
4.32	1.9
5.56	1.8
mean	2.4
$LSD_{0.05}$	0.3
CV	18.0

Other studies have indicated similar negative trends in number of spikes plant<sup>-1</sup> as plant density increased, including observations of 29.4, 18.6, 7.2, 2.1, and 0.7 spikes plant<sup>-1</sup> at 1.4, 7, 35, 184, and 1078 plants m<sup>-2</sup>, respectively, and reduction in spikes from 5.6 to 3.1 spikes plant<sup>-1</sup>, as plant density was increased from 75 to 200 plants m<sup>-2</sup> (Puckridge and Donald, 1967; Medd et

al., 1985). As a consistent decrease in spikes plant<sup>-1</sup> was observed across cultivars seeded at increasing rates, agronomic management appeared to be limiting cultivar tillering potential.

Though spikes plant<sup>-1</sup> was influenced by seeding rate, changes in seeding rate did not differentially affect cultivar spikes plant<sup>-1</sup> (Table 12). Simmons et al. (1982) reported similar findings for barley (*Hordeum vulgare* L.) genotypes that varied in tillering capacity. Cultivar differences in tillering capacity and growth habit may explain why greater differences were not see among cultivars seeded at different rates in Experiment 1. Plants established in densely-seeded conditions are expected to have greater intraspecies competition compared to plants in space-planted conditions. As neighboring plants compete for available resources, growth habit can vary depending on the intensity of competition among rivaling intraspecies and interspecies plants (Goldberg, 1990; Schenk et al., 1999).

To further evaluate cultivar spikes plant<sup>-1</sup> relative to seeding rate, cultivar spike counts from the 2018 environment were used for one-way ANOVA for each seeding rate factor level. The purpose of this was to evaluate each seeding rate level as a potential approach for assessing cultivar tillering capacity. Results in Table 15 indicate assessments to determine cultivar tillering capacity should not be completed in HRSW production fields, as spikes plant<sup>-1</sup> was similar among cultivar at seeding rates greater than 1.85 million seeds ha<sup>-1</sup>. Though differences in spikes plant<sup>-1</sup> were observed among cultivar seeded at 1.85 million seeds ha<sup>-1</sup>, the differences were minimal and not likely to be readily detected in field evaluations. However, it is still important to note that spikes plant<sup>-1</sup> of cultivars became more dissimilar as seeding rate decreased. Increased cultivar expression of tillering habit with incremental decreases in seeding rate was represented by a continual decrease in correlation coefficient (*r*) values when comparing treatments (Table 15). In summary, Experiment 1 confirmed the effects of plant density on HRSW tillering, primarily in relation to the level of competition among neighboring plants (Goldberg, 1990; Schenk et al., 1999). As a consistent decrease in spikes plant<sup>-1</sup> was observed across cultivars as seeding rate increased, tillering assessments based on plants seeded at production-level densities (1.85 to 5.56 million seeds ha<sup>-1</sup>), will not represent the full tillering capacity of a cultivar.

	Method						
		Seeding rate (mil	lion seeds ha <sup>-1</sup> )				
Cultivar	1.85	3.09	4.32	5.56			
		spikes	plant <sup>-1</sup>				
LCS Anchor	2.50a†	2.23	1.61	1.59			
Lang-MN	2.67ab	2.13	1.54	1.50			
Linkert	3.55cd	2.11	2.15	1.57			
Prevail	3.02abcd	2.63	1.87	1.66			
Shelly	3.09abcd	2.13	1.58	1.35			
Surpass	3.22bcd	2.49	1.98	1.89			
SY Valda	3.63d	2.74	2.44	1.97			
ND VitPro	3.23bcd	2.80	2.19	1.96			
TCG Wildfire	2.99abc	1.95	2.05	1.58			
Mean	3.10	2.36	1.93	1.68			
$LSD_{0.05}$	0.64	NS	NS	NS			
CV	11.8	14.9	17.4	15.4			

Table 15. Spikes plant <sup>-1</sup> of HRSW cultivars at various planting methods in 2018 at Prosper	:, NI	D.
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	Pearson's correlation coefficient $(r)$
1.85 vs 3.09	
3.09 vs 4.32	$0.52^{NS}$
4.32 vs 5.56	0.76 <sup>*</sup>

 $\dagger$  Values with the same letter in a column are not significantly different (*P*>0.05).

\* Significant at  $P \leq 0.05$ ; NS, not significant.

# **Experiment** 2

Spaced-plantings with the SOFATT method in Experiment 2 promoted cultivar

expression of tillering phenotype as mean spikes plant<sup>-1</sup> was 22.1 spikes plant<sup>-1</sup> (Table 16).

Cultivar diversity was apparent as responses in spikes plant<sup>-1</sup> were normally distributed, and

spikes plant<sup>-1</sup> differed among cultivars. Cultivars with the greatest tillering were ND VitPro and Shelly at 25.9 spikes plant<sup>-1</sup> each. Cultivars with moderate tillering were Lang-MN and Prevail (each with 23.4 spikes plant<sup>-1</sup>), and Surpass and LCS Anchor producing 21.3 and 21.0 spikes plant<sup>-1</sup>, respectively. Linkert, SY Valda, and TCG Wildfire were the lowest tillering cultivars, as all averaged <20 spikes plant<sup>-1</sup>.

Cultivar	Experiment 2	Experiment 3
	spikes	plant <sup>-1</sup>
LCS Anchor	21.0ab	21.8ab
Lang-MN	23.4bc	23.5bc
Linkert	19.5a	22.2ab
Prevail	23.4bc	26.4cd
Shelly	25.9c	23.1bc
Surpass	21.3ab	22.0ab
SY Valda	19.5a	20.5ab
ND VitPro	25.9c	26.6d
TCG Wildfire	18.9a	19.7a
Mean	22.1	22.9
CV	11.4	21.5

Table 16. Spikes plant<sup>-1</sup> of HRSW cultivars in space-planted experiments, Prosper 2018.

<sup>†</sup> Values with the same letter in a column are not different based on Fisher's LSD (P>0.05).

An unexpected result in this experiment was that Shelly was among the highest tillering cultivars, as this cultivar was one of the lower tillering cultivars in Experiment 1 (Table 14). This may represent adaptive abilities of this cultivar, allowing for adjustments in growth habit relative to the amount of intraspecies competition. This ability may not be realized/manifest until Shelly is grown at wider intra-row spacings. Increased tillering expression with greater intra-row spacing may be represented by other cultivars, as spikes plant<sup>-1</sup> were not correlated (r = -0.23; P=0.547) when comparing cultivars at 1.85 million seeds ha<sup>-1</sup> in Experiment 1 and at spaced-plantings in Experiment 2. However this may not be the case, as there was no interaction for cultivar by seeding rate in Experiment 1, and Shelly did not have an apparent advantage over

other cultivars when different seeding density methods were used to assess tillering capacity of cultivars. In general, the spaced-planting approach in Experiment 2 provided an effective method for assessing tillering of HRSW cultivars as the greater growing area of the experimental units helped minimize experimental error and maximize tillering potential of cultivars, as competition from neighboring plants was limited.

## **Experiment 3**

Experiment 3 had poor emergence and uneven establishment across replicates, which was attributed to dry soil conditions at planting. Spikes plant<sup>-1</sup> differed among cultivar at spaced-plantings used in Experiment 3, and the mean spikes plant<sup>-1</sup> was 22.9 spikes plant<sup>-1</sup> (Table 16). The highest tillering cultivar was ND VitPro (26.6 spikes plant<sup>-1</sup>). The cultivar with the lowest amount of tillers was TCG Wildfire with 19.7 spikes plant<sup>-1</sup>. The six other cultivars had moderate tillering as spikes plant<sup>-1</sup> ranged from 20.5 to 26.4 spikes plant<sup>-1</sup> among cultivars. The lack of differences among cultivars was a result of high standard error values due to the broad range of spikes plant<sup>-1</sup> observed for each cultivar (Figure 8).



Figure 8. Spikes plant<sup>-1</sup> of HRSW cultivars evaluated at spaced-plantings in Experiment 3.

This error was likely exacerbated by unfavorable environmental conditions at varying intensity throughout the site, and additional error within the experiment (CV=21.5) that was unaccounted for (Table 16). Based on these results, the space-planted method used in Experiment 3 is not likely to provide an accurate assessment of tillering of HRSW cultivars.

#### Summary

These results indicate the spaced-planting method used in Experiment 2 provided for the most accurate assessment of cultivar tillering. However, as results for cultivar spikes plant<sup>-1</sup> in Experiment 3 were correlated with Experiment 2 results (r=0.79; P=0.011), this indicated that both space-planted methods provided for similar tillering expression of cultivars. In summary, spaced-plantings are needed to properly assess tillering of HRSW cultivars. Overall, it is apparent that ND VitPro is a high tillering cultivar, and SY Valda and TCG Wildfire are low tillering.

## **Determining Cultivar Tillering Capacity**

#### **Parameterization Methods**

Three parameterization methods were identified as potential approaches for differentiating the classes of low, moderate, and high, of the tillering capacity rating system. Parameters determined by the 'Mean Comparisons approach' [using letter(s) from mean separations based on LSD values] were selected based on SAS output for mean separations  $(P \le 0.05)$  for cultivar spikes plant<sup>-1</sup> in Experiment 2 (Table 16). As four groupings of cultivars were indicated by mean comparisons based on Fisher's LSD, parameters for each rating class were relatively easy to distinguish (Table 17). However, in experiments where the number of treatments or level of precision results in greater than four letter groupings, defining parameters for each tillering capacity rating class would be a highly subjective process. Also, in experiments with unequal replication where alternative mean separations tests (such as Tukey procedure, or Tukey-Kramer test) are most appropriate, results would not be readily comparable across experiments. As these factors are additional sources of error and uncertainty that are not easy to account for, this is likely the reason why as of now, there is no standardized method that is widely used when assigning tillering capacity ratings to HRSW cultivars.

Table 17. Parameterization used for grouping HRSW cultivars based on tillering capacity in Experiment 2.

		Tillering capacity	
Parameterization method	Low	Moderate	High
Mean separations	a†	ab / bc	с
Z-score	< -0.6745	-0.6745 to 0.6745	> 0.6745
Spikes plant <sup>-1</sup> (est.)	< 19.7‡	19.7 to 24.4	> 24.4

† Based on F-protected LSD values (P < 0.05).

 $\ddagger$  Estimated spikes plant<sup>-1</sup> based on standardized distribution; calculated as: population mean  $\pm$  0.6745\*standard deviation of population.

Parameterization by the 'Z-score approach' (data transformation to standardized z-score values) provided a quantitative method for determining cultivar tillering capacity ratings. As spikes plant<sup>-1</sup> responses in Experiment 2 followed a normal distribution, z-score values at the first quartile (Q<sub>1</sub>) and third quartile (Q<sub>3</sub>) were used as parameters for assigning tillering capacity ratings (Table 17). Cultivar with an average z-score of <-0.6745 were considered to have a low tillering capacity. Cultivar with a z-score  $\geq$ -0.6745 and  $\leq$ 0.6745 were considered to have a moderate tillering capacity. Cultivar with a high tillering capacity had a z-score  $\geq$ 0.6745. This parameterization method was very easy to complete, and interpretation of results is not limited to the dataset evaluated, as data were adjusted to a standardized scale, with a mean of zero and standard deviation of one.

Z-scores for cultivar spikes plant<sup>-1</sup> can be readily compared across experiments to determine relative tillering of cultivars in differing environments. This was best demonstrated by

comparing results from Experiment 1 and Experiment 2. Though expression of tillering phenotypes was limited in Experiment 1, differences in spikes plant<sup>-1</sup> were observed among cultivar. These results were used to demonstrate cultivar tillering response at densities present in grower production fields (Experiment 1) compared to growth in spaced-plantings (Experiment 2). To account for the large difference in mean spikes plant<sup>-1</sup> between Experiment 1 and Experiment 2, standardized z-score values were calculated for spikes plant<sup>-1</sup> observations in each experiment. This adjusted the response scale of each experiment to be on the same relative scale (Figure 9). Though data from Experiment 1 data were not used to determine cultivar tillering capacity, Figure 9 demonstrates how z-scores can be used to standardize observations to a scale that is relative to the experimental mean. Therefore, relative response of a cultivar can be compared when more than one experimental dataset is used to determine cultivar tillering capacity.



Figure 9. Observations for spikes plant<sup>-1</sup> (a) and standardized Z-scores based on transformed values for spikes plant<sup>-1</sup> (b) in Experiment 1 and Experiment 2 at Prosper, ND in 2018.

The 'Standardized Distribution approach' (standardized z-scores transformed to spikes plant<sup>-1</sup> estimates based on population distribution) was the third parameterization method used. For this method, parameters for tillering capacity ratings were set at Q<sub>1</sub> and Q<sub>3</sub>, and represented as estimated values for spikes plant<sup>-1</sup>; where  $\mu = 22.1$  and  $\sigma = 3.5$ . Spikes plant<sup>-1</sup> was estimated as 19.7 and 24.4 spikes plant<sup>-1</sup> at Q<sub>1</sub> and Q<sub>3</sub>, respectively (Table 17). This method is most relevant for application in future tillering studies.

With the diversity of cultivars and n=36 spikes plant<sup>-1</sup> observations in Experiment 2, it was surmised that results from Experiment 2 were representative of most HRSW cultivars currently available to growers for production. Therefore, the spikes plant<sup>-1</sup> parameters outlined in Table 17 can be readily used by researchers to determine tillering capacity of cultivar when using the tillering assessment method from Experiment 2. If the mean and standard deviation of any subsequent experiments were to differ from the population ( $\mu = 22.1$ ;  $\sigma = 3.5$ ), values from the subsequent experiment could be considered samples of the population, and thereby readily adjusted by solving for *X* (estimated spikes plant<sup>-1</sup>) in the z-score equation, using the z-score for the sampled value, and the standardized distribution for the population ( $\mu$ ,  $\sigma$ ).

# **Cultivar Tillering Profiles**

The ranking for tillering capacity was similar across parameterization methods for each cultivar (Table 18). Shelly and ND VitPro have a relatively high tillering capacity, whereas Linkert, SY Valda, and TCG Wildfire have relatively low tillering capacity. Cultivars with moderate capacity for tillering include LCS Anchor, Surpass, Prevail, and Lang-MN. Genetic influences on plant tillering were represented in Experiment 2, as cultivars with similar expression for a particular trait, have the same tillering capacity. This was demonstrated by the

high tillering capacity cultivars (Shelly and ND VitPro), which are both photoperiod insensitive (*Ppd-D1b*) and express the *Rht-B1* gene for semi-dwarf phenotype.

Plant tillering also appears to be affected by genes other than *Ppd-D1b*, as other photoperiod insensitive cultivars (Linkert and SY Valda) have a low tillering capacity. These low tillering cultivars also have a semi-dwarf phenotype; however, this phenotype is imparted by *Rht-D1* semi-dwarf gene expression in these low tillering cultivars. These contrasting responses likely represent the effects of *Ppd-D1* interactions with other genes (including semi-dwarf genes *Rht-B1* and *Rht-D1* as potential interactors) discussed by Gonzalez et al. (2005). This is further supported by observations for Prevail and Lang-MN, as they both possess wild-type allele for semi-dwarf gene expression, are similar for sensitivity to photoperiod, and both identified as having moderate tillering capacity (Table 18).

Cultivar	Photoperiod response	Dwarfing gene	Me Compa	ean arisons	Z-sco	ore	Spik plan	tes t <sup>-1</sup>
TCG Wildfire†	Sensitive	Rht-B1	а	L‡	-0.92	L	18.9	L
SY Valda	Insensitive	Rht-D1	а	L	-0.75	L	19.5	L
Linkert	Insensitive	Rht-D1	а	L	-0.73	L	19.5	L
LCS Anchor	Sensitive	Rht-D1	ab	М	-0.31	М	21.0	Μ
Surpass	Insensitive	Wild-type	ab	М	-0.23	М	21.3	Μ
Prevail	Sensitive	Wild-type	bc	Μ	0.37	М	23.4	Μ
Lang-MN	Sensitive	Wild-type	bc	М	0.38	М	23.4	Μ
Shelly	Insensitive	Rht-B1	с	Н	1.10	Н	25.9	Η
ND VitPro	Insensitive	Rht-B1	c	Н	1.10	Н	25.9	Η

Table 18. Tillering capacity of HRSW cultivars as determined by parameterization methods.

<sup>†</sup> Cultivar ranked by tillering capacity; low to high.

‡ Tillering capacity; where L is low, M is moderate, and H is high tillering cultivar.

Prior studies in barley have indicated the importance of evaluating tiller density (stems plant<sup>-1</sup>) relative to the number of spikes plant<sup>-1</sup>, as tiller mortality differs with genotype and can negatively impact grain yield (Kirby, 1967; Kirby and Jones, 1977). As tiller mortality differed

among cultivars in Experiment 2, these results indicate tiller production and survival are greatly influenced by HRSW genotype (Figure 10). Tiller density, spikes plant<sup>-1</sup>, and tillering capacity did not appear to influence tiller mortality of cultivars. These results offer additional support to the understanding that genotype greatly influences tiller mortality.



Figure 10. Stem and spikes plant<sup>-1</sup> (difference represents tiller mortality) of HRSW cultivars in spaced-plantings in Experiment 2 at Prosper, ND 2018. Mean separations for tiller mortality were determined by LSD test. Cultivar with same letter are not significantly different (P>0.05). † TC, tillering capacity.

Though tiller mortality differed among cultivars, tiller production and survival was consistent for all cultivars, as indicated by high correlation coefficients ( $r \ge 0.93$ ) between stem density and spikes plant<sup>-1</sup> (Table 19). This reveals that HRSW plants are able to self-regulate tiller formation to consistently produce a certain number of viable spikes relative to the number of tillers formed. Lang-MN and Surpass appear to have produced a more variable number of yield-contributing spikes relative to the number of tillers formed.

Cultivar	Tillers plant <sup>-1</sup>	Spikes plant <sup>-1</sup>	Tiller mortality†	r‡	$\Pr >  r $
	stems plant <sup>-1</sup>	spikes plant-1	n plant <sup>-1</sup>		
TCG Wildfire§	19.4	18.9	0.5	0.99	0.006
SY Valda	20.3	19.5	0.8	0.98	0.021
Linkert	20.5	19.5	1.0	0.99	0.003
LCS Anchor	22.3	21.0	1.3	0.97	0.026
Surpass	22.2	21.3	0.9	0.93	0.066
Prevail	25.0	23.4	1.6	0.98	0.020
Lang-MN	24.1	23.4	0.7	0.94	0.057
Shelly	26.2	25.9	0.3	0.99	0.001
ND VitPro	27.0	25.9	1.1	0.99	0.003
Experimentwise	23.0	22.1	0.9	0.99	< 0.0001
$LSD_{0.05}$ ¶	3.7	3.0	0.7		

Table 19. Spikes plant<sup>-1</sup> of HRSW cultivars in spaced-plantings in Experiment 2, Prosper 2018.

 $\dagger$  Tiller mortality = (stems plant<sup>-1</sup> – spikes plant<sup>-1</sup>).

 $\ddagger r$ , Pearson's correlation coefficient.

§ Ranked by tillering capacity; low to high.

¶ LSD value based on Fisher's F-protected test ( $P \le 0.05$ ).

These observations revealed the importance of considering the plant structure used to assess and determine tillering capacity of HRSW cultivars, as determining tillering capacity based on stems plant<sup>-1</sup> could potentially lead to a different tillering capacity when based on spikes plant<sup>-1</sup>. However, this was not the case in Experiment 2 as cultivar tillering capacity based on tiller density or spikes plant<sup>-1</sup> arrived at the same tillering capacity rating (results not included). As spikes plant<sup>-1</sup> is a primary component of wheat yield, cultivar tillering capacity based on spikes plant<sup>-1</sup> is more relevant to growers making seeding and crop management decisions, in comparison to cultivar characteristics for forming tillers; especially as not all tillers will produce spikes contributing to final yield.

## Method Validation

To validate the application of these approaches for determining cultivar tillering capacity, experimental means from eight similar space-planted tillering studies conducted at Prosper, ND and Crookston, MN in 2014 and 2015, were used to determine tillering capacity of 12 HRSW cultivars (Table 20). Observations for tiller density (stems plant<sup>-1</sup>) were used for this validation, as spikes plant<sup>-1</sup> was not reported. Z-scores and standardized values for cultivar tiller density guided the selection of tillering capacity rating for each cultivar (Table 20).

Results for the validation set were similar to Experiment 2 results as interactions between photoperiod gene *Ppd-D1* and semi-dwarfing genes (*Rht-B1* and *Rht-D1*) appear to influence plant tillering response. The most revealing finding is that the two cultivar that are photoperiod insensitive (*Ppd-D1a*) and express the *Rht-B1b* allele, both have a high tillering capacity. This is a favorable result as similar findings were revealed in Experiment 2, as Shelly and ND VitPro are both cultivars with high tillering capacity (Table 18). This response could be influenced by QTL reported by Borras-Gelonch (2012), who indicated QTLs at a similar locus were associated with tillering and phenology characteristics. Eagles et al. (2014) suggested *Ppd-D1* interactions with alternate gene(s) (that also have effect on tillering) as a possible explanation for contrasting yield responses observed in genotypes with similar genetic background for *Ppd-D1*. In comparison to these high tillering capacity. As it has been noted by Addisu et al. (2010) that *Rht-D1* plants can have reduced biomass and greater harvest index in comparison to *Rht-B1* plants, these differences in tillering capacity observed in photoperiod insensitive cultivars are understandable.

c. 1:	Photoperiod	Dwarfing	Observed	Standardized	7	Tillering
Cultivar	response	gene	stems plant <sup>-1</sup>	stems plant <sup>-1</sup>	Z-score	capacity
Samson†	Sensitive	Rht-B1	16.8	17.7‡	-1.54	L§
Kelby	Sensitive	Rht-D1	19.3	19.7	-0.96	L
Briggs	Insensitive	Wild-type	19.6	19.9	-0.89	L
Oklee	Sensitive	Wild-type	20.4	20.6	-0.70	L
Rollag	Insensitive	Rht-D1	20.6	20.8	-0.65	Μ
Kuntz	Sensitive	Rht-D1	22.0	21.9	-0.33	Μ
Vantage	Insensitive	Wild-type	23.2	22.8	-0.04	Μ
Knudson	Sensitive	Rht-B1	25.8	24.9	0.56	Μ
Marshall	Insensitive	Rht-D1	26.1	25.2	0.63	Μ
Albany	Insensitive	Rht-B1	28.3	27.0	1.15	Н
Sabin	Sensitive	Wild-type	28.9	27.4	1.29	Н
Faller	Insensitive	Rht-B1	29.7	28.1	1.48	Н
Mean			23.4	23.0	0.00	
Std. Dev.			4.3	3.4	1.00	

Table 20. Tillering of HRSW cultivars in space-planted experiments in ND and MN, 2014-2015.

<sup>†</sup> Cultivar ranked by tillering capacity; low to high.

 $\ddagger$  Based on standardized distribution of z-scores with  $\mu = 23.0$  and  $\sigma = 3.4$  stems plant<sup>-1</sup>.

§ Tillering capacity rating based on z-score parameterization. L, Low; M, Moderate; H, High.

Cultivars with photoperiod sensitivity have variable tillering capacity profiles as no apparent groupings were observed for sensitive cultivars with similar genetic background for traits affecting plant height. As data was compiled over multiple years and locations, it is likely that these results represent a robust assessment of tillering habit of these cultivar, sufficient to determine the tillering capacity that accurately characterizes each cultivar.

## Conclusion

Assessing cultivar tillering to determine tillering capacity was best represented by the SOFATT method used in Experiment 2. Cultivar tillering habit is best represented by average spikes plant<sup>-1</sup> of multiple plants sampled from a cultivar grown at spaced-plantings (inter-row and intra-row spacing at  $30 \pm 12$  cm). Plants grown at high population densities in grower production fields are not likely to represent full tillering potential of cultivar. Researchers can

use results for average spikes plant<sup>-1</sup> to determine tillering capacity rating for each cultivar, based on raw or transformed z-score values for spikes plant<sup>-1</sup>. The Z-score approach is most relevant for researchers evaluating a diverse selection of HRSW genotypes (e.g. advanced breeding lines, variety trials). The Standardized Distribution approach is also useful, as researchers can establish values for spikes plant<sup>-1</sup> that can be used as parameters in future studies to readily determine cultivar tillering capacity rating.

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# CHAPTER 3. DEVELOPING A DECISION SUPPORT SYSTEM TO AID GROWER SELECTION OF OPTIMAL SEEDING RATES FOR NEW HRSW CULTIVARS IN DIVERSE ENVIRONMENTS

#### Introduction

Genetic improvement through continued breeding efforts leads to the development of new hard red spring wheat (HRSW) cultivars that typically provide a yield advantage over cultivars released in prior years (Austin et al., 1980). Adaptations in plant growth habit, phenotypic traits, or physiological processes related to stress, are a few examples of ways that newer cultivars may provide increased yield potential over older cultivars (Austin et al., 1989; Christopher et al., 2008; Reynolds et al., 2012). Growers have shown preference for newer cultivars, primarily driven by the opportunity for increased grain yield potential and protein content (Dahl et al., 2004). This prompts public and private seed organizations to continuously release new HRSW cultivars, resulting in the subsequent 'retirement' of older cultivars. When these new cultivars are first released, they are not accompanied by a seeding rate recommendation. Growers rely on accurate recommendations for optimal seeding rates (OSR), to avoid economic losses due uncaptured yield (underseeding) or excess seed and fertilizer waste (overseeding).

University extension specialists commonly provide seeding rate recommendations for new cultivars based on prior seeding rate studies of cultivars released in the preceding years. After these "new" cultivars are subsequently tested in multi-year seeding rate studies, the actual OSR can greatly differ from the original extension recommendation. These differences can reveal 2+ years of reduced yields and economic losses (Mehring, 2016). Though this reinforces the importance of proper seeding rate selection, with the continued release of new cultivars (and subsequent discontinuation of older cultivars), determining OSR for each cultivar is expensive, time-consuming, and repetitive research. Furthermore, environment factors (e.g. yield potential, annual precipitation, seasonal temperature) impact cultivar yield, and have an interactive effect on seeding rate (Fisher, 1985; Geleta et al., 2002; Lloveras et al., 2004). Briggs and Ayten-Fisu (1979) noted the importance of including diverse environments in seeding rate studies of new cultivars; especially as some environment and cultivar combinations favor lower seeding rates. This demonstrates the importance of evaluating cultivar yield and agronomic response at different seeding rates, and in diverse growing conditions, to ensure robustness in the OSR recommendation for a cultivar.

Decision support systems have been developed to address agricultural production problems related to soil, nutrient, and precipitation, with the objective to reduce economic losses for growers and promote sustainability by minimizing environmental impact (Bonfil et al., 2004; Wang et al., 2010). These type of systems can provide environment-specific management recommendations based on location and field-specific information provided as inputs in a computer-based algorithmic model. Currently, most of these decision support systems are focused on nutrient or disease management. Small et al. (2015) developed a decision support system to aid growers in managing late blight disease in potatoes (*Solanum tuberosum* L.). Weather data, crop information, and grower management practices were all variables incorporated into this system that would alert growers when conditions were favorable for late blight, so growers could ensure timely management for disease prevention.

Other decision support systems have been developed that are specific to crop management, but they are commonly modeled in high productivity regions (i.e. southern U.S.), and thereby likely to be highly-sensitive to even slight changes in input variables. Developing a predictive model for determining OSR for new cultivars could eliminate the time lag, expense, and repetition of the current method with field trials. This type of model could be coupled with environment-specific data and incorporated into a decision support system to allow for the varying effects of environmental interactions to be accounted for when determining an OSR for a new cultivar.

Regression functions (linear and nonlinear) are commonly used to model agronomic responses in seeding rate studies (Geleta et al., 2002; Lemerle, 2004). Regression equations from these models are useful when considering yield tradeoffs relative to seeding rate changes and can also be used to determine an estimate for OSR (Wiersma, 2002). However, when these models are fit to only one set of data, predictions produced by the model can be greatly biased and parameters have large standard error (Jones and Carberry, 1994). Various methods of splitting of datasets can be used to minimize these errors when conducting statistical analyses (Crowley, 1992). A prior HRSW seeding rate study conducted in ND and MN produced regression models predictive for grain yield by dividing the original dataset into two subsets (Mehring, 2016). This method represents the validation set approach.

When using the validation set approach, only a portion of the dataset (training set) is used to fit a predictive model. The other portion of the dataset (validation set) is then used to test the fit of the training model. Results for this test include the root mean squared error (RMSE) value, which provides an estimate for model accuracy as it represents the test error associated with differences in predicted and observed values. Akin to using several regression functions to identify a regression model best-fit for data, comparisons among models produced by various statistical learning methods can be readily accomplished by evaluating RMSE values (James et al., 2014). This process of evaluating the accuracy (fit) of these predictive models is called model assessment. Model assessment is critical for identifying and selecting the machine learning method that will best represent the data, while minimizing bias and error.

The validation approach is an efficient way to develop and test a predictive model. However, decreasing the number of observations used to train the model will inherently decrease the power of the test, increasing the likelihood of committing a Type-II error (fail to reject the null hypothesis, when the null hypothesis is false). As it is unlikely that training set data will be exactly representative of the validation set data, validation-trained models are likely to have higher RMSE values compared to models fit to only one dataset. To address these issues, crossvalidation approaches are used in place of the traditional validation approach. Cross-validation is a resampling method that is used to perform multiple 'model-training' iterations prior to producing a final model that is based on the average fit of these iterations. Wu et al. (2012) demonstrated the benefits of cross-validation in regression-based modeling as they noted reduced bias in predicted values and a lower RMSE value compared to one-time regression analysis. An improvement on this method can be made by dividing the original dataset, and performing multiple cross-validation iterations on each subset, then averaging these results to determine a final model. This k-fold cross-validation method is a considerable improvement on the validation approach, as it can provide for a stable, reliable predictive model. The application of the k-fold cross-validation method has been demonstrated previously in various ecological and agricultural studies (Wiens et al., 2008; Yost et al., 2018.).

Numerous algorithms have been developed to guide classification of data to produce decision trees that are user friendly as they do not require extensive knowledge to interpret. In experiments with multiple levels for each independent variable, the classification and regression trees (CART) algorithm can be used to readily produce decision trees. The use of this approach was demonstrated by Waheed et al. (2006), as they applied the CART decision tree algorithm to classify experimental plots based on irrigation use, weed management, and fertilization.

The objective of this research was to develop a decision support system (DSS) to improve grower selection of optimal seeding rates for newer HRSW cultivars sown in the varying growing environments throughout North Dakota and Minnesota. This DSS will benefit HRSW growers by providing them with a tool to promote optimal seeding efficiency and maximum yield for sustainable production.

#### **Materials and Methods**

## **Site and Experiment Description**

Data from seeding rate trials conducted in ND and MN from 2013-2015 and 2017-2018 (32 total environments) were compiled for this research. Four locations from 2013-2015 experiments at Prosper, ND and Crookston, Hallock, and Perley, MN. Two locations were from 2014 and 2015 experiments at Kimball, and Lamberton, MN. Experiment locations in 2017 and 2018 included Dickinson (2017 only), Hettinger, Minot, and Prosper, in ND, and Crookston, and Lamberton, in MN. Location and site descriptions for combined dataset are in Table 21.

The optimal seeding rate was determined for each cultivar x environment combination based on regression equation output from SAS 9.4 (PROC REG). The model considered best fit for data (linear or quadratic) was determined by maximizing  $R^2$  and minimizing RMSE values. For linear fits, OSR was the seeding rate treatment at which maximum yield was observed. For quadratic fits, OSR was determined by evaluating the coefficients of the equation. Quadratic equations with a negative linear coefficient (second term) were assigned the lowest seeding rate treatment as the OSR. For all other quadratic models, the OSR was calculated by solving the first derivative of the quadratic equation.

Location <sup>‡</sup>	Soil series	Taxonomy	Slope
			%
North Dakota			
Dickinson	Arnegard	Fine-loamy, mixed, superactive, frigid Pachic Haplustolls	0-2
Hettinger	Shambo	Fine-loamy, mixed, superactive, frigid Typic Haplustolls	0-2
Minot	Forman	Fine-loamy, mixed, superactive, frigid Calcic Argiudolls	3-6
	Aastad	Fine-loamy, mixed, superactive, frigid Pachic Argiudolls	3-6
Prosper	Kindred	Fine-silty, mixed, superactive, frigid Typic Endoaquolls	0-2
	Bearden	Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	0-2
Minnesota			
Hallock	Northcote	Very-fine, smectitic, frigid Typic Epiaquerts	0-1
Perley	Fargo	Fine, smectitic, frigid Typic Epiaquerts	0-1
Crookston	Wheatville	Coarse-silty over clayey, mixed over smectitic, superactive, frigid Aeric Calciaquolls	0-2
Lamberton	Webster	Fine-loamy, mixed, superactive, mesic Typic Endoaquolls	0-2
	Normania	Fine-loamy, mixed, superactive, mesic Aquic Hapludolls	0-2
Kimball (2014)	Fairhaven	Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls	0-2
Kimball (2015)	Dakota	Fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Argiudolls	2-6
	Ridgeport	Coarse-loamy, mixed, superactive, mesic Typic Hapludolls	2-6

Table 21. Location and soil characteristics<sup>†</sup> of environments, 2013-2015 and 2017-2018.

<sup>†</sup> Soil data obtained from NRCS-USDA, 2018.

‡ Ordered by longitude, west to east.

# **Data Structure**

Environments and cultivars were characterized prior to modelling. Environments were characterized based on latitude and longitude (decimal degrees), planting date (d of the year), and average HRSW yield (Mg ha<sup>-1</sup>) observed in environment for the respective year (Table 22). These factors were selected as they can be readily determined by growers (or estimated based on field records from prior years) to be used as inputs in a DSS. The use of continuous variables to represent environments was used to minimize bias when grouping similar data across environments, and reduce model overfitting, that could increase error in OSR prediction. This also ensured models were robust, and thereby relevant to a greater number of growers.

Location <sup>†</sup>				Planting	Harvest	Yield
Year	Latitude	Longitude	Previous crop	date	date	(Mg ha <sup>-1</sup> )
Dickinson, ND	46.981	-102.824				
2018			HRSW§	2-May	13-Aug	3.82
Hettinger, ND	46.012	-102.647				
2017			Soybean	26-Apr	3-Aug	1.94
2018			Soybean	27-Apr	16-Aug	3.09
Minot, ND	48.180	-101.304				
2017			Soybean	21-Apr	19-Aug	1.81
2018			Soybean	3-May	8-Aug	4.31
Prosper, ND	47.003	-97.116				
2013			Soybean	16-May	22-Aug	4.69
2014			Soybean	27-May	3-Sep	4.43
2015			Soybean	9-Apr	21-Aug	4.67
2015			Soybean	22-May	25-Aug	3.62
2017			HRSW	22-Apr	21-Aug	4.51
2018			HRSW	30-Apr	31-Jul	4.22
Hallock, MN	48.802	-96.982				
2013			Soybean	16-May	3-Sep	7.27
2014			Soybean	23-May	6-Sep	5.45
2015			Soybean	16-Apr	13-Aug	5.62
Perley, MN	47.151	-96.752				
2013			Soybean	8-May	16-Aug	5.80
2014			Soybean	22-May	2-Sep	6.00
2015			Soybean	13-Apr	11-Aug	7.03
Crookston, MN	47.815	-96.616				
2013			Soybean	10-May	8-Aug	6.14
2013			Soybean	29-May	26-Aug	6.38
2014			Soybean	17-May	27-Aug	4.95
2014			Soybean	4-Jun	27-Aug	4.55
2015			Soybean	23-Apr	21-Aug	6.35
2015			Soybean	22-May	25-Aug	5.38
2017			Soybean	3-May	29-Aug	5.09
2018			Soybean	7-May	8-Aug	3.23
Lamberton, MN	44.241	-95.312				
2014			Soybean	21-Apr	20-Aug	5.14
2015			Soybean	4-Apr	12-Aug	5.62
2015			Soybean	27-Apr	12-Aug	4.55
2017			Soybean	17-Apr	23-Aug	3.69
2018			Soybean	7-May	10-Aug	2.52
Kimball, MN	45.417	-94.324				
2014			Soybean	26-Apr	14-Aug	5.54
2015			Soybean	8-Apr	31-Jul	5.97

Table 22. Location and year details for ND and MN environments in combined dataset.

<sup>†</sup> Ordered by longitude, west to east.

‡ Environment not included in analysis due to >40% stand loss.
§ HRSW, hard red spring wheat, *Triticum aestivum*, L.; Soybean, *Glycine max* (L.) Merr.

Specific phenotypic and genetic traits were used to characterize cultivars (Table 23).

Data specific to each cultivar included gene expression for *Ppd-D* (photoperiod response), *Rht-B* and *Rht-D* (semi-dwarfing genes), and phenotypic characteristics for plant height, tillering capacity, straw strength, and heading date (days to maturity).

	Photoperiod					
	response	Semi-dwarf	Tillering	Plant	Straw	
Cultivar	(Ppd-D1)	gene	capacity	height†	strength	Heading
			z-score	cm	1 to 9‡	DAP§
Albany	Insensitive	Rht-B1	1.33¶	77.0	5	63
LCS Anchor	Sensitive	Rht-D1	-0.23	71.9	4	58
Briggs	Insensitive	wild-type	-1.03	83.3	7	57
Faller	Insensitive	Rht-B1	1.70	83.3	5	61
Kelby	Sensitive	Rht-D1	-1.10	72.6	4	58
Knudson	Sensitive	Rht-B1	0.63	78.0	5	60
Kuntz	Sensitive	Rht-D1	-0.37	75.4	4	60
Lang-MN	Sensitive	wild-type	0.37	82.6	5	61
Linkert	Insensitive	Rht-D1	-0.83	72.9	2	59
Marshall	Insensitive	Rht-D1	0.73	78.2	4	63
Oklee	Sensitive	wild-type	-0.80	80.5	6	58
Prevail	Sensitive	wild-type	0.67	78.2	4	58
Rollag	Insensitive	Rht-D1	-0.73	75.9	3	59
Sabin	Sensitive	wild-type	1.47	78.0	6	61
Samson	Sensitive	Rht-B1	-1.77	73.9	3	60
Shelly	Insensitive	Rht-B1	1.07	77.0	5	62
Surpass	Insensitive	wild-type	-0.27	79.8	6	57
SY Valda	Insensitive	Rht-D1	-0.90	75.9	4	60
Vantage	Insensitive	wild-type	-0.07	77.5	2	64
ND VitPro	Insensitive	Rht-B1	1.33	80.0	4	59
TCG Wildfire	Sensitive	Rht-B1	-1.20	86.6	4	60

Table 23. Genetic and phenotypic characteristics of HRSW cultivars.

<sup>†</sup> Agronomic measures for phenotypic traits averaged from HRSW variety trial results (NDSU, 2014-2018; Univ. of MN, 2008-2018).

‡ 1-9; 1 is erect, 9 is lying flat.

§ DAP, days after planting.

¶ Rating based on 'Z-score approach'; High,  $\geq 0.67$ ; Moderate, 0.66 to -0.67; Low, <0.67.

Agronomic measures compiled from HRSW variety trial data from ND (NDSU, 2014-2018) and MN (Univ. of MN, 2008-2018) were used to characterize cultivar for phenotypic traits. Tillering capacity was determined according to the 'Z-score approach' described in Ch. 2 (Table 17).

# **Statistical Analysis and Model Development**

Analysis and modelling were completed in R 3.5.3 statistical software (R Development Core Team, 2019) with the *caret* package (Kuhn et al., 2016). Variable independence was verified by Pearson's correlation test prior to modelling. Highly correlated variables ( $r \ge |0.8|$ ) were excluded to minimize multicollinearity and overfitting of models. Models were fit by various statistical learning algorithms that have been demonstrated in prior agronomic and crop improvement studies (Williams et al., 1979; Piaskowski et al., 2016; Sharif et al., 2016). These modelling approaches included ridge regression, elastic net, least absolute shrinkage and selection operator (LASSO) regression, stepwise regression, decision tree, and random forest.

A *k*-fold repeated cross-validation was performed with two different settings for k (k=5 and k=10) to produce resampling measures for assessing models and determining tuning parameters for each model based on estimates for test error associated with each learning algorithm (James et al., 2014). Data were split into k random subsets, with k-1 subsets used as a training set, and the remaining subset withheld from the training step and used as the validation set; repeated for k iterations. The model with the lowest root mean squared error (RMSE) value was selected as the optimal model (Breiman et al., 1984).

For comparing performance of statistical learning algorithms, mean absolute error (MAE) was used. Evaluations of error within each model were based on RMSE. Mallow's complexity parameter (Cp) statistic was used to guide variable selection at each split in the decision tree. The variable producing the lowest Cp value at a split was selected as the primary variable at that

branching point. Variable importance measures were used to identify variables primarily impacting OSR prediction (Ruβ and Brenning, 2010).

#### Results

Cultivar and environment variables were considered independent as values for Pearson's correlation coefficient were all acceptable ( $r \le |0.8|$ ). Initial models were prone to overfitting to specific latitude and longitude, so these variables were excluded from analyses. This coincides with the objective of this study, to develop a predictive model that is relevant to a broad audience of growers. Models overfit to individual locations or environments are not robust, and likely to be poor predictors of OSR for the same location in future years.

The 10-fold repeated cross-validation was most representative of the dataset as models fit for each learning algorithm were more accurate than models fit by the 5-fold repeated crossvalidation (Figure 11). This is because the additional subsets in the 10-fold provided for a more robust model, as the ratio of data comprising the training and validation sets were 316:35 samples for the 10-fold, and 281:70 samples for the 5-fold. With greater representation of cultivar and environment data in each 10-fold train set, and fewer samples in each validation set, the final model for each algorithm was fit after 'viewing' the dataset from multiple angles.

Model accuracy was compared among the different learning algorithms to determine if one method was superior to another in predicting OSR for HRSW cultivars in different environments. Mean absolute error (MAE) was similar across models (Table 24). This revealed no superiority among statistical learning algorithms in producing a model with a lower amount of predictive error (Figure 12).



Figure 11. Root mean squared error (RMSE) of models fit by statistical algorithms for determining optimal seeding rate by *k*-fold repeated cross-validation of HRSW cultivars and environments in seeding rate dataset. SW, stepwise regression; LS, lasso regression; RG, ridge regression; EN, elastic net regression; TR, decision tree; RF, random forest. Models labeled with a '2' included two-way interactions as potential model parameters.

	Mean	Pearson's
	Absolute	Correlation
Method	Error†	Coefficient
		r
Stepwise	1.003	0.30§
Lasso	1.011	0.31
Lasso2‡	1.008	0.40
Ridge	1.010	0.31
Ridge2	0.993	0.39
Elastic Net	1.001	0.31
Elastic Net2	0.997	0.34
Decision Tree	1.019	0.39
Random Forest	0.994	0.51
Random Forest2	1.020	0.60
Mean	1.006	
$LSD_{0.05}$	NS	

Table 24. Mean absolute error and correlation with OSR for statistical learning algorithms fit by 10-fold repeated cross-validation of HRSW cultivars in different environments in ND and MN.

<sup>†</sup> Mean absolute error between predicted and observed values for OSR.

‡ 2 indicates two-way interactions were included as parameters in model.

§ Correlation between resampled values of model-predicted OSR and observed OSR.

As models were similar when considering overall accuracy of predicting OSR, the accuracy of predictions by individual models was further evaluated. Models fit by stepwise and penalization regressions (lasso, ridge, elastic net) were not predictive of OSR, as all models had  $R^2 \le 0.12$  (Table 25). The addition of two-way interactions as parameters in penalization regressions had minimal effect on fit of models, as RMSE values were reduced by only 0.03, 0.09, and 0.13, for lasso, elastic net, and ridge regressions, respectively (Table 25). Considering the diversity of environments, and prior knowledge of environment interactions with both seeding rate and cultivars (documented in Ch. 1), these results are understandable. Tree-based algorithms were included in this study to further evaluate OSR within various environment settings.



Figure 12. Results from comparisons of model accuracy (based on MAE, mean absolute error) among OSR predictive models produced by 10-fold repeated cross-validation of seeding rate dataset and fit by various statistical learning algorithms. EN, elastic net regression; LS, lasso regression; RF, random forest; RG, ridge regression; SW, stepwise; TR, decision tree. Models labeled with a 2 indicate two-way interactions were included as model parameters.

Table 25. Fit and error of regression models fit by 10-fold repeated cross-validation of seeding rate dataset.

Method	$R^2$	RMSE†
Stepwise	0.11	1.221
Lasso	0.10	1.231
Lasso2‡	0.11	1.228
Ridge	0.10	1.224
Ridge2	0.12	1.211
Elastic Net	0.11	1.221
Elastic Net2	0.12	1.212

† RMSE, root mean squared error.

‡ 2 indicates two-way interactions were included as parameters in model.

For the decision tree algorithm, the 10-fold repeated cross-validation provided a selection of 10 decision tree models. The model selected for the final decision tree had a RMSE of 1.2386 (Table 26). As RMSE values are reported in the same units as OSR (million seeds ha<sup>-1</sup>), and OSR observations were recorded to three decimals in the seeding rate dataset, one may postulate that any of the models from iterations 6, 8, or 9 could have been selected for the final decision tree. To avoid bias in this decision, the final model for the decision tree was automatically selected in R, by including a data step for making the selection based on the iteration with the lowest RMSE value. To prevent overfitting of the decision tree model, Mallow's complexity parameter (Cp) used to guide variable selection at each potential branching point was 0.0151 (Table 26). Branching ceased when all variables at a potential branch point produced a Cp value > 0.0151. The OSR at each terminal node (leaf) is the mean OSR of the data comprising that node (Figure 13).

Iteration	RMSE†	Ср
1	1.2650	0.0057
2	1.2629	0.0060
3	1.2633	0.0063
4	1.2537	0.0077
5	1.2487	0.0083
6	1.2395	0.0097
7	1.2386	0.0151
8	1.2390	0.0187
9	1.2411	0.0433
10	1.2669	0.0734

Table 26. Modelling summary from the 10 iterations of the decision tree algorithm.

† RMSE, root mean squared error; Cp, Mallow's complexity parameter.

The global model from the decision tree algorithm was predictive of OSR with 67% accuracy (based on 100 – MAPE (mean absolute percent error). The R model output for the decision tree algorithm revealed variables impacting OSR (Figure 13). Nodes (branching points)

included both phenotypic characteristics (straw strength, tillering capacity) and environment (yield of the environment). Based on variable importance measures (Pratt, 1987) reported in R (scaled relative to 1), the primary variable influencing OSR in the decision tree model was straw strength, with a relative variable importance of 25.7% (Figure 14). Other variables affecting OSR included yield of the environment (21.0%), tillering capacity (17.6%), and plant height (17.3%). *Rht-D* and *Rht-B* partially influenced OSR determined by the decision tree at 13.4% and 5.0%, respectively. According to the decision tree model, cultivar differences in gene expression for *Ppd-D* (photoperiod response) did not influence OSR.

The root node in the decision tree differentiated OSR based on cultivar straw strength rating (Figure 13). This follows previous reportings of differences in OSR for cultivars varying in straw characteristics that affected lodging potential (Faris and DePauw, 1980). The model also indicated environment interacted with cultivars, causing differential effects on OSR depending on straw strength and average yield of the environment (Figure 13). This is similar to what Otteson et al. (2007) documented for genotype x environment interactions, where different seeding rates were considered optimal for yield. For HRSW cultivars with favorable straw strength (rating <5), tillering capacity was a determinant of OSR, but only in environments with average yield  $\geq$  3.2 Mg ha<sup>-1</sup> (Figure 13). This likely demonstrates cultivar phenotype expression as determined by growing conditions. This is explained by the understanding that in resourcelimited environments (e.g. water or nutrient deficiencies), expression of plant phenotype(s) associated with yield can be severely restricted (Richards et al., 2010; Wasson et al., 2012). This is further demonstrated by findings of Hucl and Baker (1990) for HRSW cultivars grown in semi-arid environments in Canada (average yield of 3.55 Mg ha<sup>-1</sup>).



Figure 13. Decision tree model for selecting optimal seeding rate for HRSW cultivars in differing environments in ND and MN. Straw strength rating (1-9; 1 is erect, 9 is lying flat) for varieties in HRSW variety trial publications from NDSU (2014-2018) and Univ. of MN (2008-2018). Tillering capacity determined from tillering evaluations of HRSW cultivars at spaced plantings. Number of samples and percent of whole dataset are reported for root, nodes and leaves. RMSE, root mean squared error.

Though cultivars differed in tillering capacity, OSR for maximum yield was similar among cultivars under these growing conditions. Variables absent from the final decision tree were plant height and all of the genetic traits (*Rht-B*, *Rht-D*, *Ppd-D*). However, as previously indicated, all of these variables (except *Ppd-D*) were of importance to the decision tree model, thereby of influence on OSR (Figure 14).



Figure 14. Results for Variable Importance for decision tree model. Importance is relative to 1.

Based on the decision tree model, growers seeding in high yielding (average yield  $\geq 5.5$  Mg ha<sup>-1</sup>) or moderate yielding (average yield 5.4 to 3.2 Mg ha<sup>-1</sup>) environments, should seed at a rate of 4.5 million seeds ha<sup>-1</sup>, unless they are seeding a cultivar with known phenotypic characteristics requiring a lower seeding rate (i.e. poor straw strength [rating  $\geq 5$ ] or high

tillering capacity) (Figure 13). Growers in low yielding environments (average yield <3.2 Mg ha<sup>-1</sup>) can maximize yield by seeding HRSW at a rate of 3.7 million seeds ha<sup>-1</sup>, except when seeding a cultivar with poor straw strength (rating  $\geq$  5), where an OSR of 3.9 million seeds ha<sup>-1</sup> is more favorable for yield (Figure 13). In general, OSR for these environment types differentiated by average yield are similar to recommendations made by Holliday (1960) and Donald (1963), where environments with greater resource availability are expected to have higher OSR. Figure 15 was produced to provide growers with a DSS to readily determine OSR based on their selection for HRSW cultivar and the environment in which it is sown.



Figure 15. Decision support system (DSS) for growers to determine optimal seeding rates for HRSW cultivars sown in diverse yielding environments in ND and MN.

Though the level of variance was slightly higher for the decision tree model compared to linear regression models, the trade-off was for reduced bias in OSR predictions produced by the decision tree model. Similar to the other algorithms included in this study, the accuracy of the OSR produced by the decision tree model are greatly dependent on the data used to develop the model. This is why it was important to utilize the same resources when characterizing cultivars. Additionally, with year-to-year variability in temperature, rainfall accumulation, and other environmental factors influencing wheat growth in each environment (e.g. Figures 1 and 2), average grain yield was used to characterize environments. This is primarily because yield as a model parameter allows growers to readily determine OSR based on yields on their individual operations.

The recommendations outlined in the DSS improve the accuracy of predictions for OSR (Model RMSE = 1.17 million seeds ha<sup>-1</sup>; Cross-validation RMSE = 1.24 million seeds ha<sup>-1</sup>) in comparison to the current generalized recommendation of Wiersma and Ransom (2017) for 3.8 to 4.1 million seeds ha<sup>-1</sup> (RMSE = 1.27 million seeds ha<sup>-1</sup>). However, as RMSE values for the terminal nodes (leaves) in the decision tree model ranged from 1.0 to 1.5 million seeds ha<sup>-1</sup>, there are apparent limitations in these findings due to the amount of error in predicted versus observed OSR values. Variability in the OSR recommendations at each terminal node could be reduced by allowing additional branching points, however this would lead to overfitting of the decision tree model and reduce the scope of these findings. This indicates that growers should not simply default to the OSR indicated by the DSS, but rather utilize information from this tool to guide seeding rates of newer HRSW cultivars. Growers can adapt seeding rates as needed, to account for operational differences in agronomic and environmental factors influencing OSR relative to yield (Figure 14).

#### Conclusions

Environment and phenotypic characteristics for straw strength and tillering capacity, influence the seeding rate that is optimal for yield in HRSW production. For environments where average yield is  $\geq$  3.2 Mg ha<sup>-1</sup>, the OSR is generally higher in comparison to OSR for lower yielding environments (4.5 versus 3.7 million seeds ha<sup>-1</sup>) and when seeding cultivars with high tillering capacity. Adjustments to OSR can also be expected when seeding cultivars with poor straw strength (rating  $\geq$  5). Breeders and agronomists should utilize this information to focus efforts on characterizing advanced breeding lines and new cultivars for specific genetic and phenotypic traits influencing OSR. Growers can benefit from these findings by adapting seeding rates relative to their average yields; especially when seeding new HRSW cultivars.

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#### **CHAPTER 4. MAJOR FINDINGS**

Evaluating the seeding response of new hard red spring wheat (HRSW) (*Triticum aestivum* L.) cultivars in diverse growing environments revealed that environment x genotype interactions were more important than seeding rate per se, for determining grain yield. As seeding rate studies are commonly conducted to provide growers with information relevant to their production region, it is likely that most HRSW seeding rate studies conducted in the northern Great Plains region are not likely to include the same scale of diversity in environments comprised in this study (based on composition/extent of geospatial location, growing season length, environment yield potential). With this in consideration, it is likely that environment x genotype interactions are not a variable of influence (especially as a dominating variable) in most seeding rate studies. However, this study revealed that environment can greatly impact the optimal seeding rate (OSR) for maximum yield of HRSW cultivars, with the OSR ranging from 3.1 to 4.5 million seeds ha<sup>-1</sup> depending on the environment where tested.

As phenotype is a manifestation of environment influences on genotype, it was important to also include phenotypic characteristics as potential variables when performing statistical analysis and modelling to develop a model for predicting the OSR of new HRSW cultivars. Phenotypic traits used as potential variables in modelling were selected based on prior findings of traits associated with grain yield (e.g. straw strength, height, days to heading). Tillering capacity was also selected as a trait to characterize cultivars, as spikes ha<sup>-1</sup> is a yield component. However, as there is currently no widely-accepted method for determining the tillering capacity of HRSW cultivars, the SOFATT (seed only few, and then thin) method was developed and demonstrated as a standardized method for assessing cultivar tillering and determining tillering capacity. Results from the SOFATT method were used as measures representing the tillering capacity of the cultivars included in the statistical analysis and modelling portion of this study.

Including various statistical learning algorithms in the modelling step revealed that the regression-only models were not predictive of cultivar OSR in diverse environments, as these models had  $R^2 \leq 0.12$ . Additionally, Random Forest models were prone to overfitting, and thereby lacked in the robustness needed to produce accurate OSR predictions. The statistical learning algorithm that provided for a predictive model that could readily support a decision support system (DSS) was the decision tree algorithm. The decision tree model identified straw strength and tillering capacity as primary variables influencing OSR, representing the environment x genotype interactions identified in the original analysis of variance (Figure 13). Depending on the yield of the environment in which a cultivar is grown, environment may not have an interactive influence on cultivar that affects OSR. For environments where average yield is  $\geq$  3.2 Mg ha<sup>-1</sup>, the OSR is generally higher in comparison to OSR for lower yielding environments (4.5 versus 3.7 million seeds ha<sup>-1</sup>). However, when growing a cultivar with poor straw strength (rating  $\geq$  5) in high yielding environments (5.5 Mg ha<sup>-1</sup>), it is recommended that growers seed at a reduced rate of 3.1 million seeds ha<sup>-1</sup>, to reduce potential losses due to lodging. Overall, the DSS developed in this study reveals important information that can be applied by growers, breeders, and agronomists to improve seeding efficiency and promote maximum yield of new HRSW cultivars.

### **APPENDIX**

Table A1. Mean squares and significance from ANOVA of yield and other agronomic traits, Crookston 2017.

Source	df	Yield	Density	Loss	Protein
Rep	2	4.68***	3024	146	0.06
A [Cultivar]	8	3.03***	$2864^{*}$	$214^{*}$	5.19***
B [Seeding Rate]	3	0.07	300084***	1907***	0.12
A*B	24	0.12	1883	128	0.10
Error	70	0.09	1335	99	0.07

\*, \*\*, and \*\*\*, indicate significance at P < 0.05, P < 0.01, and P < 0.001 respectively.

Table A2. Mean squares and significance from ANOVA of yield and other agronomic traits, Hettinger 2017.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	$0.54^{***}$	139	123	0.23	2.3	22.3
A [Cultivar]	8	0.32***	1290	77	2.62***	1.6	53.9***
B [Seeding Rate]	3	0.36***	295633***	539	0.69**	1.0	4.5
A*B	24	0.04	2731	195	0.14	3.2	$20.4^{**}$
Error	70	0.05	2990	240	0.15	2.2	8.8

\*, \*\*, and \*\*\*, indicate significance at P<0.05, P<0.01, and P<0.001 respectively.

Table A3. Mean squares and significance from ANOVA of yield and other agronomic traits, Lamberton 2017.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	0.83*	3310	141	0.43	1.8	40.1
A [Cultivar]	8	1.93***	2883*	344*	5.25***	19.0**	28.3
B [Seeding Rate]	3	1.21***	217829***	2634***	$0.70^{*}$	4.4	12.7
A*B	24	0.18	845	103	0.17	3.6	32.2
Error	70	0.19	1345	126	0.24	5.4	22.2

\*, \*\*, and \*\*\*, indicate significance at *P*<0.05, *P*<0.01, and *P*<0.001 respectively.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	0.15	1358	107	3.41***	0.04	19.5
A [Cultivar]	8	$0.79^{***}$	5066	303	3.36***	$14.7^{**}$	112.8**
B [Seeding Rate]	3	0.28	272966***	1923***	1.42***	1.0	75.6
A*B	24	0.18	2573	131	$0.22^{*}$	4.9	46.3
Error	70	0.13	2553	191	0.11	4.1	35.4

Table A4. Mean squares and significance from ANOVA of yield and other agronomic traits, Minot 2017.

\*, \*\*, and \*\*\*, indicate significance at P<0.05, P<0.01, and P<0.001 respectively.

Table A5. Mean squares and significance from ANOVA of yield and other agronomic traits, Prosper 2017.

Source	df	Yield	Density	Protein	DTH	Height
Rep	2	0.12	1755	2.15***	1.3	46.9
A [Cultivar]	8	1.37***	870	$6.07^{***}$	4.4	$58.8^{**}$
B [Seeding Rate]	3	$0.58^{***}$	263850***	0.45	2.5	11.1
A*B	24	$0.14^{*}$	2600	0.19	5.9	36.8*
Error	70	0.08	2000	0.24	3.6	20.0

\*, \*\*, and \*\*\*, indicate significance at *P*<0.05, *P*<0.01, and *P*<0.001 respectively.

Table A6. Mean squares and significance from ANOVA of yield and other agronomic traits, Crookston 2018.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	0.85**	116	7.7	2.60***	5.2	9.1
A [Cultivar]	8	$1.46^{***}$	5942*	483**	2.37***	2.3	$10.1^{*}$
B [Seeding Rate]	3	0.04	364820***	1825***	0.30	0.6	5.5
A*B	24	0.18	2054	140	0.22	2.9	$7.0^{*}$
Error	70	0.16	2190	161	0.15	2.7	4.1

\*, \*\*, and \*\*\*, indicate significance at P<0.05, P<0.01, and P<0.001 respectively.

Source	df	Yield	Density	Loss	Protein	Height
Rep	2	$1.06^{*}$	7188	682 <sup>*</sup>	2.82**	42.5
A [Cultivar]	8	$0.67^{*}$	1715	173	3.06***	63.4*
B [Seeding Rate]	3	0.42	184244***	3497***	0.32	13.6
A*B	24	0.30	1897	157	0.28	26.0
Error	70	0.28	2538	198	0.37	27.0

Table A7. Mean squares and significance from ANOVA of yield and other agronomic traits, Dickinson 2018.

\*, \*\*, and \*\*\*, indicate significance at P<0.05, P<0.01, and P<0.001 respectively.

Table A8. Mean squares and significance from ANOVA of yield and other agronomic traits, Hettinger 2018.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	2.15*	1471	186	0.81	0.3	0.8
A [Cultivar]	8	0.72	3656	199	$2.29^{***}$	2.1*	368***
B [Seeding Rate]	3	0.29	239193***	2960***	0.25	0.3	135*
A*B	24	0.32	1557	118	0.20	0.9	84**
Error	70	0.46	1795	130	0.28	0.8	38

\*, \*\*, and \*\*\*, indicate significance at *P*<0.05, *P*<0.01, and *P*<0.001 respectively.

Table A9. Mean squares and significance from ANOVA of yield and other agronomic traits, Lamberton 2018.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	2.40***	898	6.8	12.74***	11.7	4.7
A [Cultivar]	8	1.92***	1843	190*	4.57***	7.2	64.1**
B [Seeding Rate]	3	0.02	160248***	1944***	0.40	2.3	11.9
A*B	24	0.05	1130	116	0.31	6.1	21.7
Error	70	0.07	1086	90	0.52	4.2	21.0

\*, \*\*, and \*\*\*, indicate significance at *P*<0.05, *P*<0.01, and *P*<0.001 respectively.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	0.13	4477	380	0.14	0.1	8.6
A [Cultivar]	8	1.09**	1772	125	4.03***	6.1	93.9**
B [Seeding Rate]	3	0.35	252645***	739**	0.08	4.5	58.2
A*B	24	0.51	1466	108	0.04	2.5	26.2
Error	70	0.40	1746	134	0.06	3.9	31.2

Table A10. Mean squares and significance from ANOVA of yield and other agronomic traits, Minot 2018.

\*, \*\*, and \*\*\*, indicate significance at P<0.05, P<0.01, and P<0.001 respectively.

Table A11. Mean squares and significance from ANOVA of yield and other agronomic traits, Prosper 2018.

Source	df	Yield	Density	Loss	Protein	DTH	Height
Rep	2	0.20	612	14	2.67**	15.8*	1.7
A [Cultivar]	8	$1.50^{***}$	1866	167	4.96***	2.3	16.6
B [Seeding Rate]	3	0.34	311877***	1032***	0.68	1.3	26.3
A*B	24	0.18	1178	82	0.21	2.4	19.6
Error	70	0.19	1383	98	0.45	3.7	17.1

\*, \*\*, and \*\*\*, indicate significance at P < 0.05, P < 0.01, and P < 0.001 respectively.

Table A12. Summary of regression analysis results for HRSW cultivar response to seeding rates, Crookston 2017.

Cultivar	RMSE†	$R^2$	Regression equation‡	Yield (ŷ)§	$OSR^{\dagger}(x)$
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.401	0.20	$\hat{y}=4.43+0.14x-0.03x^2$	4.53	2.55₽
Lang-MN	0.060	0.94	$\hat{y}=5.35-0.24x+0.02x^2$	4.80	1.85
Linkert	0.148	0.93	$\hat{y}=3.52+0.67x-0.07x^2$	4.98	5.14
Prevail	0.013	0.99	$\hat{y}=3.89+0.37x-0.05x^2$	4.47	3.65
Shelly	0.085	0.64	$\hat{y}=5.21+0.27x-0.04x^2$	5.64	3.66
Surpass	0.165	0.22	ŷ=4.82+0.04x	4.99	3.09
SY Valda	0.116	0.52	ŷ=5.56+0.06x	5.79	4.32
ND VitPro	0.075	0.97	$\hat{y}=5.97-0.47x+0.04x^2$	4.89	1.85
TCG Wildfire	0.024	0.99	$\hat{y}=5.01+0.46x-0.06x^2$	5.71	3.58

† RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Cultivar	RMSE†	$R^2$	Regression equation <sup>‡</sup>	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.189	0.58	$\hat{y}=0.75+0.54x-0.07x^2$	1.66	3.89P
Lang-MN	0.036	0.99	ŷ=0.67+0.81x-0.11x <sup>2</sup>	2.00	3.78
Linkert	0.068	0.09	ŷ=1.87-0.01x	1.83	3.09
Prevail	0.011	0.99	$\hat{y}=1.07+0.64x-0.09x^2$	2.07	3.69
Shelly	0.077	0.97	$\hat{y}=0.55+0.82x-0.10x^2$	2.06	4.26
Surpass	0.046	0.96	$\hat{y}=1.17+0.56x-0.08x^2$	2.07	3.74
SY Valda	0.100	0.13	ŷ=2.20-0.02x	2.13	3.09
ND VitPro	0.148	0.56	$\hat{y}=1.25+0.35x-0.04x^2$	1.91	4.16
TCG Wildfire	0.112	0.86	$\hat{y}=0.92+0.41x-0.04x^2$	1.76	4.67

Table A13. Summary of regression analysis results for HRSW cultivar response to seeding rates, Hettinger 2017.

<sup>†</sup> RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{s}$   $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Predictions for OSR outside range of treatments adjusted to low (1.85) or high (5.56) rate.

Table A14.	Summary	of regression	analysis res	ults for HRSW	' cultivar response	to seeding rates,
Lamberton	2017.					

Cultivar	RMSE†	$R^2$	Regression equation <sup>‡</sup>	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.335	0.14	$\hat{y}=2.39+0.33x-0.04x^2$	2.92	3.82₽
Lang-MN	0.418	0.41	$\hat{y}=2.38+0.76x-0.09x^2$	3.75	4.12
Linkert	0.121	0.96	ŷ=1.63+1.01x-0.11x <sup>2</sup>	3.61	4.49
Prevail	0.137	0.94	$\hat{y}=4.07-0.39x+0.08x^2$	3.81	5.56
Shelly	0.135	0.76	$\hat{y}=3.17+0.58x-0.07x^2$	4.16	3.91
Surpass	0.375	0.60	$\hat{y}=1.89+1.07x-0.13x^2$	3.77	4.01
SY Valda	0.166	0.79	$\hat{y}=3.34+0.45x-0.05x^2$	4.27	4.86
ND VitPro	0.018	0.99	ŷ=3.79-0.21x+0.03x <sup>2</sup>	3.55	5.56
TCG Wildfire	0.027	0.99	$\hat{y}=0.27+1.56x-0.17x^2$	3.39	4.58

† RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Cultivar	RMSE†	$R^2$	Regression equation <sup>‡</sup>	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.195	0.76	$\hat{y}=2.15-0.45x+0.04x^2$	1.20	1.85P
Lang-MN	0.104	0.84	$\hat{y}=0.97+0.57x-0.08x^2$	1.88	3.69
Linkert	0.062	0.99	$\hat{y}$ =-0.06+1.13x-0.14x <sup>2</sup>	1.96	4.09
Prevail	0.159	0.59	$\hat{y}=1.22+0.39x-0.05x^2$	1.94	4.21
Shelly	0.041	0.99	ŷ=1.09+0.22x	1.92	5.56
Surpass	0.185	0.59	$\hat{y}=1.24+0.38x-0.06x^2$	1.74	3.28
SY Valda	0.434	0.06	$\hat{y}=1.67+0.24x-0.03x^2$	2.10	4.08
ND VitPro	0.102	0.85	$\hat{y}=0.79+0.59x-0.08x^2$	1.76	3.77
TCG Wildfire	0.565	0.24	$\hat{y}=0.55+0.77x-0.10x^2$	1.81	3.78

Table A15. Summary of regression analysis results for HRSW cultivar response to seeding rates, Minot 2017.

† RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{s}$   $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Predictions for OSR outside range of treatments adjusted to low (1.85) or high (5.56) rate.

Table A16.	Summary	of regression	analysis res	ults for HRSW	<sup>7</sup> cultivar response	to seeding rates,
Prosper 201	7.					

Cultivar	RMSE†	$R^2$	Regression equation‡	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.050	0.99	$\hat{y}=2.25+1.00x-0.13x^2$	3.98	<b>3.96</b> ₽
Lang-MN	0.036	0.98	$\hat{y}=3.97+0.32x-0.03x^2$	4.68	5.20
Linkert	0.014	1.00	$\hat{y}=3.83+0.43x-0.07x^2$	4.39	3.26
Prevail	0.226	0.42	$\hat{y}$ =4.28-0.03x+0.01x <sup>2</sup>	4.38	5.56
Shelly	0.160	0.79	$\hat{y}=3.99+0.73x-0.10x^2$	5.13	3.62
Surpass	0.232	0.90	$\hat{y}=1.98+1.28x-0.15x^2$	4.42	4.36
SY Valda	0.325	0.06	$\hat{y}=5.10-0.18x+0.03x^2$	4.85	5.56
ND VitPro	0.028	0.79	$\hat{y}=4.10+0.07x-0.01x^2$	4.25	5.01
TCG Wildfire	0.316	0.81	$\hat{y}=2.71+0.82x-0.08x^2$	4.48	5.08

<sup>†</sup> RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Cultivar	RMSE†	$R^2$	Regression equation <sup>‡</sup>	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.162	0.86	$\hat{y}=3.91-0.92x+0.13x^2$	2.49	5.56 <b>P</b>
Lang-MN	0.133	0.94	$\hat{y}=3.10+0.40x-0.08x^2$	3.37	2.57
Linkert	0.035	0.96	ŷ=2.66+0.09x	3.00	5.56
Prevail	0.422	0.44	$\hat{y}=3.63-0.26x+0.05x^2$	3.47	5.56
Shelly	0.250	0.32	$\hat{y}=2.82+0.19x-0.03x^2$	3.01	2.91
Surpass	0.331	0.60	$\hat{y}=3.25-0.08x+0.03x^2$	3.42	5.56
SY Valda	0.032	0.99	$\hat{y}=1.98+0.99x-0.13x^2$	3.66	3.88
ND VitPro	0.125	0.08	$\hat{y}=3.18+0.09x-0.01x^2$	3.33	3.75
TCG Wildfire	0.163	0.07	$\hat{y}=3.25+0.07x-0.01x^2$	3.33	3.15

Table A17. Summary of regression analysis results for HRSW cultivar response to seeding rates, Crookston 2018.

† RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Predictions for OSR outside range of treatments adjusted to low (1.85) or high (5.56) rate.

Table A18. Su	mmary of regression	analysis results	for HRSW	cultivar response	to seeding rates,
Dickinson 201	8.	-		_	-

Cultivar	RMSE†	$R^2$	Regression equation‡	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.592	0.54	$\hat{y}=3.59-0.26x+0.07x^2$	3.64	5.56 <b>P</b>
Lang-MN	0.140	0.74	ŷ=3.35+0.12x	3.80	5.56
Linkert	0.185	0.04	ŷ=3.80-0.10x+0.01x <sup>2</sup>	3.64	1.85
Prevail	0.050	0.99	$\hat{y}=1.68+1.04x-0.12x^2$	3.61	4.24
Shelly	0.220	0.63	$\hat{y}=3.19+0.56x-0.07x^2$	4.25	4.29
Surpass	0.187	0.88	$\hat{y}=1.79+1.13x-0.14x^2$	3.82	4.10
SY Valda	0.094	0.98	$\hat{y}=1.75+1.48x-0.20x^2$	4.18	3.78
ND VitPro	0.759	0.25	ŷ=5.51-0.94x+0.11x <sup>2</sup>	3.81	1.85
TCG Wildfire	0.016	0.99	$\hat{y}=3.22+0.29x-0.04x^2$	3.64	3.50

† RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Cultivar	RMSE†	$R^2$	Regression equation <sup>‡</sup>	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.643	0.00	ŷ=2.75+0.02x	2.81	4.32₽
Lang-MN	0.405	0.16	$\hat{y}=2.68+0.21x-0.04x^2$	2.91	2.97
Linkert	0.752	0.32	ŷ=5.31-1.25x+0.16x <sup>2</sup>	3.22	1.85
Prevail	0.393	0.16	ŷ=3.24-0.09x	3.04	3.09
Shelly	0.032	1.00	$\hat{y}=1.68+0.90x-0.15x^2$	2.86	3.06
Surpass	0.024	0.75	ŷ=3.62-0.02x	3.54	3.09
SY Valda	0.609	0.16	$\hat{y}=2.10+0.65x-0.09x^2$	3.14	3.69
ND VitPro	0.205	0.83	$\hat{y}$ =4.43-0.77x+0.12x <sup>2</sup>	3.40	5.56
TCG Wildfire	0.297	0.22	$\hat{y}=3.75-0.33x+0.04x^2$	3.14	1.85

Table A19. Summary of regression analysis results for HRSW cultivar response to seeding rates, Hettinger 2018.

<sup>†</sup> RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Predictions for OSR outside range of treatments adjusted to low (1.85) or high (5.56) rate.

Table A20.	Summary of	regression a	analysis res	ults for HI	RSW cult	ivar response t	to seeding ra	ates,
Lamberton	2018.	-	-			-	_	

Cultivar	RMSE†	$R^2$	Regression equation <sup>‡</sup>	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.065	0.92	$\hat{y}=1.06+0.47x-0.07x^2$	1.75	3.49 <b>₽</b>
Lang-MN	0.065	0.92	$\hat{y}=3.13-0.24x+0.04x^2$	2.88	5.56
Linkert	0.139	0.62	$\hat{y}=1.33+0.41x-0.05x^2$	2.05	3.98
Prevail	0.051	0.27	$\hat{y}=2.88+0.06x-0.01x^2$	2.99	4.36
Shelly	0.197	0.50	$\hat{y}=3.15-0.37x+0.06x^2$	2.64	5.56
Surpass	0.030	0.95	ŷ=2.96-0.07x	2.70	1.85
SY Valda	0.027	0.99	$\hat{y}=1.47+0.69x-0.08x^2$	2.75	4.26
ND VitPro	0.120	0.62	$\hat{y}=2.68-0.17x+0.03x^2$	2.50	5.56
TCG Wildfire	0.012	0.97	ŷ=2.53-0.03x	2.40	1.85

† RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.
Cultivar	RMSE†	$R^2$	Regression equation <sup>‡</sup>	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.359	0.59	$\hat{y}=2.75+0.85x-0.12x^2$	3.97	3.42₽
Lang-MN	0.024	1.00	ŷ=3.38+0.29x	4.44	5.56
Linkert	0.271	0.00	ŷ=4.12-0.01x	4.09	3.09
Prevail	0.325	0.82	ŷ=6.56-1.00x+0.10x <sup>2</sup>	4.50	1.85
Shelly	0.026	0.99	ŷ=4.04+0.17x	4.65	5.56
Surpass	0.198	0.91	$\hat{y}=1.79+1.54x-0.21x^2$	4.20	3.65
SY Valda	0.613	0.31	$\hat{y}=3.12+0.99x-0.13x^2$	4.80	3.90
ND VitPro	0.200	0.96	$\hat{y}=5.13-1.10x+0.18x^2$	3.96	5.56
TCG Wildfire	0.519	0.57	$\hat{y}$ =5.55-1.02x+0.16x <sup>2</sup>	4.20	5.56

Table A21. Summary of regression analysis results for HRSW cultivar response to seeding rates, Minot 2018.

<sup>†</sup> RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Predictions for OSR outside range of treatments adjusted to low (1.85) or high (5.56) rate.

Table A22.	Summary	of regression	analysis re	sults for H	RSW cultiva	r response to	seeding 1	rates,
Prosper 201	18.							

Cultivar	RMSE†	$R^2$	Regression equation‡	Yield (ŷ)§	OSR† (x)
				Mg ha <sup>-1</sup>	million seeds ha <sup>-1</sup>
LCS Anchor	0.057	0.98	ŷ=5.10-0.93x+0.11x <sup>2</sup>	3.44	1.85₽
Lang-MN	0.195	0.72	$\hat{y}=3.63+0.21x-0.01x^2$	4.20	5.56
Linkert	0.024	0.99	ŷ=3.77+0.10x	4.16	5.56
Prevail	0.533	0.26	$\hat{y}=3.33+0.57x-0.07x^2$	4.42	4.37
Shelly	0.362	0.63	$\hat{y}=3.88-0.09x+0.03x^2$	4.09	5.56
Surpass	0.083	0.77	$\hat{y}=4.97-0.26x+0.04x^2$	4.63	5.56
SY Valda	0.048	0.99	$\hat{y}=3.40+0.58x-0.06x^2$	4.59	4.75
ND VitPro	0.171	0.87	$\hat{y}=2.53+1.10x-0.14x^2$	4.37	3.85
TCG Wildfire	0.315	0.00	ŷ=4.14+0.0005x	4.13	4.32

† RMSE, root mean squared error; OSR, optimal seeding rate.

‡ Regression equation from PROC REG.

 $\hat{y}$ , maximum yield; observed for linear model, calculated for quadratic model.

Predictions for OSR outside range of treatments adjusted to low (1.85) or high (5.56) rate.

	Seeding	g rate (mi	illion see	ds ha <sup>-1</sup> )			
Cultivar	1.85	3.09	4.32	5.56	Regression equation <sup>†</sup>	OSR; (x)	$R^2$
		——Mg	ha <sup>-1</sup> ——			million seeds ha <sup>-1</sup>	
LCS Anchor	3.66	4.17	4.18	3.92	$\hat{y}=2.25+1.00x-0.13x^2$	3.96§	0.99
Lang-MN	4.47	4.65	4.81	4.80	$\hat{y}=3.97+0.32x-0.03x^2$	5.20	0.98
Linkert	4.40	4.52	4.46	4.18	$\hat{y}=3.83+0.43x-0.07x^2$	3.26	1.00
Prevail	4.32	3.09	4.32	5.56	$\hat{y}$ =4.28-0.03x+0.01x <sup>2</sup>	5.56	0.42
Shelly	5.04	5.19	5.37	4.90	$\hat{y}=3.99+0.73x-0.10x^2$	3.62	0.79
Surpass	3.79	4.68	4.60	4.60	$\hat{y}=1.98+1.28x-0.15x^2$	4.36	0.90
SY Valda	4.94	4.59	5.04	4.84	$\hat{y}=5.10-0.18x+0.03x^2$	5.56	0.06
ND VitPro	4.21	4.23	4.29	4.27	$\hat{y}=4.10+0.07x-0.01x^2$	5.01	0.79
TCG Wildfire	3.87	4.67	4.52	4.84	$\hat{y}$ =2.71+0.82x-0.08x <sup>2</sup>	5.08	0.81

Table A23. Summary of grain yield (Mg ha<sup>-1</sup>) at incremental seeding rates and results of best fit regression model for select HRSW cultivars, Prosper 2017.

† Regression equation from PROC REG.

‡ OSR, optimal seeding rate.

§ Predictions for OSR outside range of treatments adjusted to low (1.85) or high (5.56) rate.

Table A24. Least squares mean values for grain yield response of HRSW cultivars to seeding rates, 2017-2018 experiments in ND and MN.

Environment	Seeding rate (million seeds ha <sup>-1</sup> )							
(Location+Year)	1.85	3.09	4.32	5.56	OSR†			
		]	Mg ha <sup>-1</sup>		million seeds ha <sup>-1</sup>			
Crookston17‡	5.074	5.082	5.156	5.033	3.61			
Prosper17	4.299a§	4.540b	4.647b	4.535b	3.94			
Minot18	4.172	4.331	4.296	4.449	4.59			
Prosper18	4.073	4.247	4.230	4.345	3.98			
Dickinson18	3.639	3.853	3.916	3.878	4.28			
Lamberton17	3.407a	3.675b	3.902b	3.787b	5.56			
Crookston18	3.178	3.249	3.224	3.272	4.47			
Hettinger18	3.196	2.999	3.154	2.996	1.85			
Lamberton18	2.474	2.526	2.538	2.535	4.58			
Hettinger17	1.792a	2.057c	2.005bc	1.920bc	5.56			
Minot17	1.678	1.874	1.907	1.786	5.56			

<sup>†</sup> OSR, optimal seeding rate; based on quadratic best fit regression equation from PROC REG.

‡ Environments ordered by mean observed yield, high to low.

§ Values sharing a letter within a row are not significantly different (P>0.05) by Fisher's LSD.

			2017					201	8		
Cultivar	Crookston†	Prosper	Lamberton	Hettinger	Minot	Crookston	Prosper	Lamberton	Hettinger	Minot	Dickinson
	g kg <sup>-1</sup>										
LCS Anchor	15.3d‡	14.8cd	15.5c	14.4bc	15.6c	17.0c	14.6de	15.9e	16.6c	15.6c	15.0cde
Lang-MN	15.2d	15.1d	16.1d	14.1b	15.7cd	17.0c	14.3bcd	15.7de	16.3bc	15.6c	15.1de
Linkert	15.8e	14.6c	15.7cd	14.6c	16.2e	17.0c	14.9e	15.2cd	17.1d	15.8cd	15.4e
Prevail	14.7c	13.0a	14.0a	13.5a	14.8a	15.9a	12.9a	14.1a	16.0ab	14.5a	14.5bc
Shelly	14.0a	13.6b	14.7b	13.6a	15.1b	16.5b	13.7b	14.6ab	15.6a	14.5a	13.7a
Surpass	15.3d	13.8b	14.7b	13.6a	15.3b	16.1a	14.0bc	14.2a	16.4c	15.1b	14.8bcd
SY Valda	14.2b	13.5b	14.7b	13.6a	14.6a	15.9a	13.8b	14.8bc	16.0ab	14.6a	14.5b
ND VitPro	15.6e	14.8cd	15.6c	14.6c	15.9d	16.8bc	14.8de	15.3cde	16.3bc	15.9d	15.2de
TCG Wildfire	14.2ab	14.5c	15.6c	14.5c	15.8cd	16.8bc	14.5cde	15.2bcd	16.4bc	15.6c	15.0de
Mean	14.9	14.2	15.2	14.0	15.4	16.6	14.2	15.0	16.3	15.2	14.8
LSD <sub>0.05</sub>	0.2	0.4	0.4	0.3	0.3	0.3	0.5	0.6	0.4	0.2	0.5

Table A25. Mean separations for grain protein content of HRSW cultivars in 2017 and 2018 environments.

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<sup>+</sup> Crookston and Lamberton, Minnesota; Prosper, Hettinger, Minot, and Dickinson, North Dakota.

‡ Values with the same letter within a column are not significantly different (P>0.05) based on Fisher's LSD.

Table A26. Mean separations for plant density response of HRSW cultivars to seeding rates, 2017 and 2018 environments.

Seeding rate			2017					201	8		
(million seeds ha <sup>-1</sup> )	Crookston†	Prosper	Lamberton	Hettinger	Minot	Crookston	Prosper	Lamberton	Hettinger	Minot	Dickinson
					—— plants 1	m <sup>-2</sup> ———					
1.85	175a‡	175a	166a	151a	167a	188a	165a	144a	175a	146a	165a
3.09	250b	244b	251b	237b	235b	287b	252b	216b	246b	226b	252b
4.32	345c	346c	306c	318c	314c	381c	344c	274c	324c	297c	293c
5.56	415d	395d	379d	394d	400d	457d	411d	323d	392d	372d	363d

† Crookston and Lamberton, Minnesota; Prosper, Hettinger, Minot, and Dickinson, North Dakota.

‡ Values with the same letter within a column are not significantly different (P>0.05) based on Fisher's LSD.

	2017					2018					
Cultivar	Crookston†	Prosper	Lamberton	Hettinger	Minot	Crookston	Prosper	Lamberton	Hettinger	Minot	Dickinson
	DAP (d after planting)										
LCS Anchor	-	58.3‡	57.7	55.8	55.1	47.4	51.1	50.3	53.9	47.6	-
Lang-MN	-	58.3	59.0	55.6	56.1	48.3	51.0	49.8	52.9	46.9	-
Linkert	-	58.3	60.3	55.0	56.8	47.8	50.8	50.8	53.9	47.8	-
Prevail	-	57.7	58.0	56.1	56.8	48.1	51.0	49.2	54.0	47.3	-
Shelly	-	59.3	60.5	55.6	56.3	48.6	50.3	49.7	53.6	48.0	-
Surpass	-	58.2	60.8	55.8	57.8	47.7	52.0	49.3	53.4	46.5	-
SY Valda	-	59.5	58.8	55.1	58.3	48.8	51.0	48.7	53.6	46.6	-
ND VitPro	-	58.8	60.9	55.4	58.1	47.9	50.9	49.8	54.0	48.7	-
TCG Wildfire	-	57.9	60.5	55.8	58.2	47.7	50.9	51.1	53.0	46.9	-
Mean		58.5	59.6	55.6	57.0	48.0	51.0	49.9	53.6	47.4	
Planting date	3 May	22 April	17 April	26 April	21 April	7 May	30 April	7 May	2 May	3 May	2 May

Table A27. Mean separations for heading date of HRSW cultivars in 2017 and 2018 environments.

97 <sup>†</sup> Crookston and Lamberton, Minnesota; Prosper, Hettinger, Minot, and Dickinson, North Dakota.

<sup>‡</sup> Data unreported for Crookston 2017 and Dickinson 2018.

Environment	Yield	Density	Protein	DTH	Height
Crookston17	6.0	12.3	1.8	-	-
Crookston18	12.2	14.3	2.4	3.4	2.6
Dickinson18	13.8	18.8	4.1	-	6.7
Hettinger17	11.2	19.9	2.8	2.7	5.5
Hettinger18	21.8	14.9	3.2	1.7	9.1
Lamberton17	11.7	13.3	3.3	3.9	7.4
Lamberton18	10.6	13.8	4.8	4.1	6.6
Minot17	20.2	18.1	2.1	3.6	11.7
Minot18	14.7	16.1	1.6	4.2	7.8
Prosper17	6.2	15.4	3.5	3.3	6.3
Prosper18	10.4	12.7	4.8	3.8	6.3

Table A28. Summary of coefficient of variation (CV) values for agronomic traits from experiments in 2017 and 2018 environments.

Table A29. Mean squares and significance from ANOVA of spike population (spikes ha<sup>-1</sup>), 2017 and 2018 experiments at Prosper, ND.

Source	df	2017	2018
Rep	2	-	-
A [Cultivar]	8	6.6***	5.0***
B [Seeding Rate]	3	18.5***	16.2***
A*B	24	1.26	0.50
Error	70	0.85	0.57

Table A30. Mean squares and significance from ANOVA of spike population, 2017-2018 experiments in Prosper, ND.

Source	df	Spike population
A [Cultivar]	8	9.80*
Env x A	8	1.83*
B [Seeding Rate]	3	34.4**
Env x B	3	0.32
A x B	24	0.91
Env x A x B	24	0.86
Error	140	0.71

\*, \*\*, and \*\*\*, indicate significance at P < 0.05, P < 0.01, and P < 0.001 respectively.

2017	2017 2018	
	-million spike	s ha <sup>-1</sup>
6.15a	4.93a	5.54a
6.94b	5.74b	6.34b
7.80c	6.29c	7.05c
7.92c	6.73d	7.32c
7.20	5.92	6.56
	2017 6.15a 6.94b 7.80c 7.92c 7.20	2017 2018   ——million spike   6.15a 4.93a   6.94b 5.74b   7.80c 6.29c   7.92c 6.73d   7.20 5.92

Table A31. Least squares mean values for spike population of HRSW at seeding rates, 2017-2018 experiments at Prosper, ND.

<sup>†</sup> Values followed by same letter in a column are not different (P>0.05) based on Fisher's LSD.

Table A32. Least squares mean values for spike population of HRSW cultivars at Prosper, ND, 2017-2018 environments.

Cultivar	2017	2018	Combined	
	————million spikes ha <sup>-1</sup> ————			
LCS Anchor	6.84a†	5.19ab	6.01a	
Lang-MN	6.19a	5.45ab	5.82a	
Linkert	6.55a	6.07cd	6.31ab	
Prevail	8.03b	6.10cde	7.06bc	
Shelly	6.87a	4.92a	5.90a	
Surpass	8.17b	6.42de	7.30c	
SY Valda	7.77b	6.71e	7.24c	
ND VitPro	7.82b	6.71e	7.27c	
TCG Wildfire	6.56a	5.74bc	6.15a	
LSD <sub>0.05</sub>	0.75	0.62	0.90	

<sup> $\dagger$ </sup> Values followed by same letter in a column are not different (*P*>0.05) based on Fisher's LSD.

	Variable associated with indicated rank of importance				
Model	1st	2nd	3rd	4th	
Stepwise	Straw	Tiller†	Rht-D	Height	
Lasso	Straw	Tiller	Rht-D	Yenv	
Lasso2	Yenv:Straw‡	Rht-D	Rht-D:Yenv	Yenv:Height	
Ridge	Straw	Tiller	Rht-D	Yenv	
Ridge2	Yenv:Straw	Rht-D:Yenv	Rht-B:Tiller	Yenv:Tiller	
Elastic net	Straw	Tiller	Rht-D	Yenv	
Elastic net2	Yenv:Straw	Yenv:Tiller	Rht-D:Yenv	Straw	
Decision Tree	Straw	Yenv	Tiller	Height	
Random Forest	Tiller	Straw	Height	Yenv	
Random Forest2	Yenv:Tiller	Yenv:Straw	Tiller:Straw	Ppd-D:Yenv	

Table A33. Top ranked variables indicated as important parameters in various models.

<sup>†</sup> Tiller, tillering capacity; Yenv, yield of the environment.

‡ Interacting variables indicated by ':' in models including two-way interactions.

Table A34. Mean squared values and ANOVA results from comparisons of model accuracy for 10 statistical learning algorithms fit by 10-fold repeated cross-validation of seeding rate dataset.

Source	df	MAE†
Fold	9	-
A [Algorithm]	9	$0.0009^{NS}$
Error	360	0.0005

† MAE, mean absolute error.

NS, nonsignificant.

Table A35. Modelling summary for decision tree algorithm.

Level	C <sub>p</sub>	splits	Rel. error	Root node error x. Rel. error
		n		
1	0.0734	0	1.000	1.609
2	0.0433	1	0.927	1.491
3	0.0187	2	0.883	1.421
4	0.0151	4	0.846	1.361



Figure A1. Unpruned decision tree from 10-fold repeated cross-validation of seeding rate dataset.



Figure A2. Resampling error (RMSE, root mean squared error) relative to level of Mallow's complexity parameter value.



Figure A3. Density plot of resampling error (RMSE, root mean squared error) from OSR predictions of the final decision tree model.



Figure A4. Resampling error (RMSE, root mean squared error) associated with increasing number of variables in decision tree model.



Figure A5. Random forest model with minimal branching from 10-fold repeated cross-validation of seeding rate dataset.



Figure A6. Random forest model with maximum branching from 10-fold repeated cross-validation of seeding rate dataset.



Figure A7. Root mean squared error relative to number of trees fit by random forest algorithm.



trees

Figure A8. Root mean square error relative to number of trees fit by random forest algorithm with two-way interactions.



Figure A9. Modelling summary for random forest algorithm.