Optimal planting date and populations in dual-use Kernza intermediate wheatgrass (*Thinopyrum intermedium*)-legume intercropping systems in Wisconsin

by

Oluwakorede J. Olugbenle

Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Science

(Agroecology)

At the

University of Wisconsin - Madison

2021

ACKNOWLEDGMENTS

I would like to thank my advisor, Valentin Picasso, for his continual guidance when I am lost, encouragement when I feel down, and support whenever I need it over the past two years.

Thank you to the members of my committee, Julie Dawson and Ken Albrecht, for their time in answering my questions, providing feedback and discussions.

Thank you to the Picasso lab, previous and current members for their support, help, teachings, and laughs either at the field, lab or office.

Thank you to Andy Vanloocke and Gretchen Zdorkowski for their advice, encouragement, and help during my graduate school applications.

Thank you to the farm crew at Arlington and Lancaster Agricultural Research Stations.

Thank you to my family who have supported me in my endeavors, even if they do not understand it fully! Thank you to my friends who always put a smile on my face whenever I am down.

Thanks to God for making this all possible.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

Chapter 3

Supplemental Tables

LIST OF FIGURES

Chapter 2

Chapter 3

Chapter 1 - Introduction

Thesis overview

This introductory chapter provides a review of literature on Kernza intermediate wheatgrass agronomic management and intercropping for dual use (forage and grain) cropping systems. The second chapter is a manuscript to be submitted for publication with the results and discussion of the optimal planting date research study. The third chapter presents the results and observations over the course of three years in an ongoing study evaluating performance of breeding populations of Kernza in Wisconsin, USA, intercropped with legumes.

Kernza intermediate wheatgrass

Global cropping systems are based on monocultures of annual crop species (e.g., corn, soybeans; Pimentel et al., 2012). They are important for the farming industry as they provide grains for food, livestock feed, biomass for fuel and farm income (Smil, 1994). However, they annually require tillage, replanting, and external inputs (e.g., fertilizers, pesticides, fossil fuel), which contribute to soil erosion, nutrient and pesticide losses, and in turn water pollution (Jungers et al., 2018; Pimentel et al., 2012; Ryan et al., 2018). There have been suggested actions to improve agricultural systems incrementally with the planting of cover crops (Martinez-Feria et al., 2016, Gabriel & Quemada, 2011), however the problems persist on a large scale across the US Midwest, and throughout the world (Borrelli et al., 2020).

Many landscapes in the Upper Midwest include topography where annual crop production places soils at high risk for erosion (Pimentel et al., 2012). Perennial crops improve sustainability compared to annual crops because their extensive root systems reduce soil erosion, nutrient and pesticide losses, and pesticide use (Crews et al., 2018; Glover et al., 2010), which lowers farmer expenses due to decreased annual inputs and costs. Recently there has been increased research efforts to domesticate and breed perennial cereals for seed yield, including the cool-season grass, intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) (Bajgain et al., 2020; Cattani, 2016; DeHaan & Van Tassel, 2014; Hybner et al., 2012).

Intermediate wheatgrass is a cool-season perennial grass that was initially introduced from Asia and Europe to the U.S. in the 1930s and was mainly used as a forage crop (Ogle et al., 2011). The Rodale Institute (Kurztown, PA) conducted the initial grain breeding work, and later on germplasm was transferred to The Land Institute (Salina, KS) where domestication and breeding efforts have continued (DeHaan et al., 2014), with field evaluations up to the tenth cycle focused on grain yield in the year 2021 (Crain et al., 2021). Although there has been a lot of progress in domestication and breeding of intermediate wheatgrass, farmers still lack agronomic knowledge on how to manage the crop for establishment and grain harvest (Lanker et al., 2019).

Intermediate wheatgrass requires vernalization to flower and produce seed in the next growing season (Ivancic et al., 2021). Thus, intermediate wheatgrass is typically planted early in the fall ensuring enough time for germination, shoot and root establishment (Jungers et al., 2018; Zimbric et al., 2020). The plant is exposed to a cold period in its vegetative stage during winter and resumes growth and development the following spring (Ivancic et al., 2021). Midwest farmers need more information on the establishment methods of Kernza intermediate wheatgrass such as planting date, row spacing, etc. (Lanker et al., 2019) which motivated our first experiment. Due to pioneer farmers receiving seeds late and there being no previous research on optimal planting date of Kernza some farmers were curious if planting after September will affect yield (Lanker et al., 2019).

This thesis is framed under the concept of perennial dual-use crops to improve the sustainability of current systems. This project aims to develop best agronomic management practices for producing perennial grain and forage crops in order to integrate them into the Wisconsin cropping systems. This thesis focuses on two management practices: planting date and breeding population of intermediate wheatgrass for intercropping systems.

Intercropping and legume species

The long-term vision of introducing more perennials in agriculture, involves increasing diversity by the use of intercropping as a tool (Ryan et al., 2018). Mixed intercropping is growing more than one crop at the same time (Brooker et al., 2015). Intercropping is advantageous because it can aid with improving yields, strengthening soils, and minimizing weed competition (Vandermeer, 1989). Intercropping grasses with legumes provides several advantages for forage mixtures and crops (Picasso et al., 2008). Two legumes well adapted to Wisconsin are alfalfa (*Medicago sativa* L.*)* and red clover (*Trifolium pratense* L.*).* Both are able to fix nitrogen, improve soil quality, and intercropping them with grasses also means an increase in biodiversity (Jensen et al., 2020).

Alfalfa, the United States most important forage legume, has a taproot that can penetrate up to 9 meters. It grows best in soils that have high fertility, neutral in pH, and are well drained. It is poorly adapted to saline or wet soils and is intolerant to flooding, however it has good drought tolerance. Water use exceeds that of many annual row crops that have a shorter period of vegetative growth, but water use is increased by high air temperatures and low humidity (Sheaffer & Evers, 2007). Intermediate wheatgrass intercropped with alfalfa showed a reduced grain yield relative to a fertilized monoculture of Intermediate wheatgrass over four years (Tautges et al., 2018).

Red clover is a perennial legume that has a taproot with secondary branches, but its roots do not penetrate the soil to the extent of alfalfa. It has a good tolerance to acidic soils, and poor tolerance to basic soils. It is fairly tolerant to cold, drought, and wet soils. It is best adapted where moisture is sufficient and summer temperatures are moderately cool to warm. Although it has relatively less drought and heat tolerance than alfalfa it is adapted to a wide range of soil types, except those in areas prone to drought or excess moisture. It has lower yields and shorter stand life than alfalfa. Its seedlings are more competitive than other small seeded legumes (Sheaffer & Evers, 2007). Intermediate wheatgrass intercropped with red clover showed a higher crude protein concentration, suggesting nitrogen transfer from legumes to the grass (Favre et al., 2019).

Research goal and objectives

The goal of this thesis is to increase our understanding of dual-use intermediate wheatgrass cropping systems in Wisconsin. Specifically, research was conducted to 1) determine the effects of planting date on Kernza intermediate wheatgrass grain yield, and intermediate wheatgrass biomass yield, 2) determine the effects of red clover planting season on Kernza intermediate wheatgrass grain yield, and intermediate wheatgrass biomass yield, 3) identify breeding population(s) that maximized Kernza intermediate wheatgrass grain yield, and intermediate wheatgrass biomass yield.

References

Bajgain, P., Zhang, X., Jungers, J. M., DeHaan, L. R., Heim, B., Sheaffer, C. C., Wyse, D. L., & Anderson, J. A. (2020). 'MN- Clearwater', the first food-grade intermediate wheatgrass (Kernza perennial grain) cultivar. *Journal of Plant Registrations*, *14*(3), 288–297. https://doi.org/10.1002/plr2.20042.

Borrelli, P., Robinson, D. A., Panagos, P., Lugato, E., Yang, J. E., Alewell, C., Wuepper, D., Montanarella, L., & Ballabio, C. (2020). Land use and climate change impacts on global soil erosion by water (2015-2070). *Proceedings of the National Academy of Sciences of the United States of America*, *117*(36), 21994–22001. https://doi.org/10.1073/pnas.2001403117

Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., Hawes, C., Iannetta, P. P. M., Jones, H. G., Karley, A. J., Li, L., Mckenzie, B. M., Pakeman, R. J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C. A., Zhang, C., … White, P. J. (2015). Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, *206*(1), 107–117. https://doi.org/10.1111/nph.13132

Cattani, D.J. (2016). Selection of a perennial grain for seed productivity across years: intermediate wheatgrass as a test species. *Canadian Journal of Plant Science*, *97*(3), 516-524. https://doi.org/10.1139/cjps-2016-0280

Crain, J., DeHaan, L., & Poland, J. (2021). Genomic prediction enables rapid selection of high-performing genets in an intermediate wheatgrass breeding program. *The Plant Genome*, *14*(2), 1–17. https://doi.org/10.1002/tpg2.20080

Crews, T. E., Carton, W., & Olsson, L. (2018). Is the future of agriculture perennial? Imperatives and opportunities to reinvent agriculture by shifting from annual monocultures to perennial polycultures. *Global Sustainability*, *1*, e11. https://doi.org/10.1017/sus.2018.11

Dehaan, L. R., & Van Tassel, D. L. (2014). Useful insights from evolutionary biology for developing perennial grain crops. *American Journal of Botany*, *101*(10), 1801–1819. https://doi.org/10.3732/ajb.1400084

DeHaan, L. R., Wang, S., Larson, S. R., Cattani, D. J., Zhang, X., & Kantarski, T. (2014). Current efforts to develop perennial wheat and domesticate *Thinopyrum intermedium* as a perennial grain. *Perennial Crops for Food Security Proceedings of the FAO Expert Workshop*, 72–89.

Favre, J. R., Castiblanco, T. M., Combs, D. K., Wattiaux, M. A., & Picasso, V. D. (2019). Forage nutritive value and predicted fiber digestibility of Kernza intermediate wheatgrass in monoculture and in mixture with red clover during the first production year. *Animal Feed Science and Technology*, *258*. https://doi.org/10.1016/j.anifeedsci.2019.114298

Gabriel, J. L., & Quemada, M. (2011). Replacing bare fallow with cover crops in a maize cropping system: Yield, N uptake and fertiliser fate. *European Journal of Agronomy*, *34*(3), 133–143. https://doi.org/10.1016/J.EJA.2010.11.006

Glover, J. D., Reganold, J. P., Bell, L. W., Borevitz, J., Brummer, E. C., Buckler, E. S., Cox, C. M., Cox, T. S., Crews, T. E., Culman, S. W., DeHaan, L. R., Eriksson, D., Gill, B. S., Holland, J., Hu, F., Hulke, B. S., Ibrahim, A. M. H., Jackson, W., Jones, S. S., … Xu, Y. (2010). Increased food and ecosystem security via perennial grains. *Science*, *328*(5986), 1638–1639. https://doi.org/10.1126/science.1188761

Hybner, R. M. (2012). Intermediate wheatgrass (*Thinopyrum intermedium* L.): An introduced conservation grass for use in Montana and Wyoming. *United States Department of Agriculture Natural Resources Conservation Service Plant Materials Technical Note No. MT-80.* https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/mtpmctn11288.pdf

Ivancic, K. A., Locatelli, A., Tracy, W, F., & Picasso. V, D. (2021). Kernza intermediate wheatgrass (*Thinopyrum intermedium*) response to a range of vernalization conditions. *Canadian Journal of Plant Science*. https://doi.org/10.1139/CJPS-2020-0251

Jensen, E. S., Carlsson, G., & Hauggaard-Nielsen, H. (2020). Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: A global-scale analysis. *Agronomy for Sustainable Development*, *40*(1). https://doi.org/10.1007/s13593-020-0607-x

Jungers, J. M., Frahm, C. S., Tautges, N. E., Ehlke, N. J., Wells, M. S., Wyse, D. L., & Sheaffer, C. C. (2018). Growth, development, and biomass partitioning of the perennial grain crop *Thinopyrum intermedium*. *Annals of Applied Biology*, *172*(3), 346–354. https://doi.org/10.1111/aab.12425

Lanker, M., Bell, M., & Picasso, V. D. (2019). Farmer perspectives and experiences introducing the novel perennial grain Kernza intermediate wheatgrass in the US Midwest. *Renewable Agriculture and Food Systems*, *35*(6), 653–662. https://doi.org/10.1017/S1742170519000310

Martinez-Feria, R. A., Dietzel, R., Liebman, M., Helmers, M. J., & Archontoulis, S. V. (2016). Rye cover crop effects on maize: A system-level analysis. *Field Crops Research*, *196*, 145–159. https://doi.org/10.1016/J.FCR.2016.06.016

Ogle, D., St. John, L., & Tober, D. (2011). Plant guide for intermediate wheatgrass (*Thinopyrum intermedium*). *USDA-Natural Resources Conservation Service*, Idaho and North Dakota Plant Materials Centers. https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/idpmspg10369.pdf

Picasso, V.D., Brummer, E.C., Liebman, M., Dixon, P.M. and Wilsey, B.J. (2008). Crop species diversity affects productivity and weed suppression in perennial polycultures under two management strategies. *Crop Science*, *48*(1), 331-342. https://doi.org/10.2135/cropsci2007.04.0225

Pimentel, D., Cerasale, D., Stanley, R. C., Perlman, R., Newman, E. M., Brent, L. C., Mullan, A., & Chang, D. T. I. (2012). Annual vs. perennial grain production. *Agriculture, Ecosystems and Environment*, *161*, 1–9. https://doi.org/10.1016/j.agee.2012.05.025

Ryan, M. R., Crews, T. E., Culman, S. W., Dehaan, L. R., Hayes, R. C., Jungers, J. M., & Bakker, M. G. (2018). Managing for multifunctionality in perennial grain crops. *BioScience*, *68*(4), 294–304. https://doi.org/10.1093/biosci/biy014

Sheaffer, C.C. & Evers G.W. (2007). Cool-season Legumes for Humid Areas. In R.F. Barnes, C.J. Nelson, K.J. Moore, M. Collins. (Ed). Forages, Volume 2: The science of grassland agriculture. (Sixth edition, 808). Ames, Iowa: Blackwell.

Smil, V. (1994). *Energy in world history*. Winnipeg: University of Manitoba.

Tautges, N. E., Jungers, J. M., Dehaan, L. R., Wyse, D. L., & Sheaffer, C. C. (2018). Maintaining grain yields of the perennial cereal intermediate wheatgrass in monoculture v. bi-culture with alfalfa in the upper Midwestern USA. *The Journal of Agricultural Science*, *156*(6), 758-773. https://doi.org/10.1017/S0021859618000680

Vandermeer, J. H., (1989). The ecology of intercropping. *Cambridge: Cambridge University Press*.

Chapter 2 - Optimal planting date of Kernza intermediate wheatgrass intercropped with red clover

Abstract

Intermediate wheatgrass [IWG; *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is a cool-season perennial grass that is being developed as a perennial grain for commercial use under the trade name Kernza®. Farmers in the North-central United States are curious about the appropriate time to plant intermediate wheatgrass. This research aimed to identify the optimal planting date of Kernza intermediate wheatgrass in North-Central United States in dual-use systems intercropped with red clover (*Trifolium pratense* L.). Treatments were a combination of two factors: Kernza planting dates (August to October, and April) and red clover planting season (in the fall with Kernza or frost seeded in the spring). The experiment was established in two locations (Arlington and Lancaster, WI) and two years (2017 and 2018), and grain and forage yield measured for two years after planting. Optimal planting date was estimated based on a 30 year average of remaining growing degree days (GDD) to end of GDD accumulation. Maximum Kernza grain yield was a mean of 489 kg ha^{-1} in the first year at Arlington between 721 and 972 GDD, and at Lancaster the mean yield was 351 kg ha^{-1} in the first year between 630 and 664 GDD. At 972 and 865 GDD, the spring seeded red clover treatments maximized IWG summer biomass at 6811 and 5158 kg ha^{-1} in the first year at Arlington and Lancaster respectively. At Arlington and Lancaster, overall planting date did not have an effect in the second year. Planting as early as possible in the fall minimized the amount of weed biomass accumulated in the first year and the weed biomass overall was reduced by the

second year. Planting during the spring did not produce any grain yield in the first year at Arlington and produced similar second year yields at both locations compared to the optimal fall plantings, with a mean of 135 kg ha^{-1}. Red clover biomass in the first year was maximized at 103 GDD with a value of 705 kg ha^{-1} at Lancaster but did not have an effect in the second year. In Wisconsin, planting by 15 September maximized yields 50% of the time, planting by 8 September maximized yields 90% of the time and planting red clover in spring maximized Kernza yields in the first year.

Introduction

Kernza, a new perennial grain, has been developed through conventional breeding for increased seed production of intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) at The Land Institute; Salina, KS, USA, the second selection cycle increased seed yield around 77% (DeHaan et al., 2014). As a dual-use crop, grain and forage of intermediate wheatgrass can present multiple income streams to farmers (Jungers et al., 2017). Although there has been much progress in breeding, such as the production of the first food grade intermediate wheatgrass cultivar (Bajgain et al., 2020), there is little agronomic knowledge for the management of Kernza as a dual use crop (Duchene et al., 2021). Kernza intermediate wheatgrass, a cool-season grass, has a vernalization requirement, where the plant needs exposure to cold in its vegetative stage during winter in order to flower (Ivancic et al., 2021) similar to winter wheat.

Farmers are requesting more information on the establishment methods of Kernza intermediate wheatgrass such as optimal planting date and row spacing (Lanker et al., 2019). There has been no previous research on optimal planting date for Kernza grain and forage in the North Central US, thus farmers are curious about the effect of planting date on yield. Research on another cereal dual use crop, winter wheat (*Triticum aestivum* L.) has shown that planting date affects yield, Blue et al. (1990) showed that winter wheat established in Nebraska maximized grain yields with planting dates that accumulated about 400 growing degree days (with a base temperature of 4.44°C) from planting until December 31. In Wisconsin, a study showed that a September 3 planting date with an approximated 790 GDD produced the highest grain yield for the winter wheat and there was no advantage of planting at an earlier date. When the seeding rate was increased from 42 to 56 seeds/sq ft, a September 23 planting date obtained comparable yields (Dahlke et al., 1993). According to Conley et al. (2015) planting late reduces yield because the crop does not germinate or emerge effectively in the fall due to the lower air and soil temperature. Conley et al. (2015) also suggest that winter wheat should be planted between September 20 and October 5, avoiding planting too late with low temperature and too early with potential active pests (i.e, Aphids) that transmit viruses such as barley yellow dwarf virus.

Intercropping, a tool for introducing more perennials in agriculture and increasing biodiversity, is advantageous because it can aid with minimizing weed competition, improving yields, fixing nitrogen in the soil which helps decrease synthetic fertilizer use and runoff (Picasso et al., 2008; Jensen et al., 2020). Although, intercropping legumes with Kernza may affect the optimal planting date because of competition or facilitation effects (Ashworth et al., 2015).

The first objective of this study was to determine the effects of planting date on Kernza intermediate wheatgrass grain yield, intermediate wheatgrass summer biomass, intermediate wheatgrass fall biomass, total intermediate wheatgrass production (summer and fall), red clover biomass, overall forage production (intermediate wheatgrass and red clover), and weed biomass. We expected that planting in early fall (mid to late September) would achieve higher forage and grain production in the following growing season, as well as better weed control. The second objective was to determine the effects of red clover planting season on Kernza grain yield, intermediate wheatgrass biomass, and red clover biomass. We expect that the fall seeded red clover will compete with the Kernza intermediate wheatgrass lowering the yield compared to the spring seeded red clover.

Material and Methods

Experimental Design and Management

The experiment was established at two locations at University of Wisconsin Agricultural Research Stations: Arlington (43°18’9.47″N, 89°20’43.32″W) and Lancaster (42°49′52.56″N, 90°48′1.78″W) in the fall of 2017 and 2018 and yield data collected for two subsequent years. None of the experiments were irrigated. The treatment design was factorial with two factors: intermediate wheatgrass planting date and red clover planting season. Kernza intermediate wheatgrass were planted at 5 different planting dates: late August, mid-September, late September, mid-October, late October and in April (spring) next year (Table 1). In 2017 and 2018 the planting dates at both locations were not always the same, due to weather conditions it was difficult to get into the field at times and had some plantings delayed or missed entirely. All plots were intercropped with red clover; either the red clover planted in the fall at the same time as the intermediate wheatgrass or frost seeded in the spring. Arlington and Lancaster were managed differently because with differing plot sizes the planting density also changed as well, and with differing soil fertility each location had its own fertilization requirements to maintain productivity (Table 2). The field plot design was complete randomized blocks with 4 replications for each location and planting year. At Arlington treatments were allocated as a full factorial, and at Lancaster treatments were allocated as a split-plot with red clover planting season as the whole plot and intermediate wheatgrass planting date as split-plot. We chose a split-plot for Lancaster because it was a location further from University of Wisconsin-Madison's campus, so the Lancaster research station staff implemented the plantings, and a split-plot design was simpler than a full factorial design. The Kernza seed for both locations were from The Land Institute (Salina, KS) selection cycle 4, harvested in August 2017 at Arlington, WI, except for the Arlington 2018 planting, which used seed originating from The Land Institute selection cycle 5, harvested in August 2018 at Arlington, WI. Red clover was variety "FF 9615" from LaCrosse Seeds.

Soils were Plano silt loam with 2 to 6% slope (PaB – Web Soil Survey, 2021) at Arlington and Fayette silt loam with 6 to 12% slope (FaC2 – ibid.) at Lancaster. At Arlington, which was previously tall fescue (*Festuca arundinacea* L.), in both years, individual plots were rototilled with a "Land Pride RTA2570" rototiller to prepare a fresh seedbed before each planting. At Lancaster, which was previously corn (*Zea mays* L.), in both years the land was disked with a "Ford 230 disk" and cultipacked with a "Kewanee 85 cultimulcher" due to the field being dry and to firm up the seedbed prior to the first scheduled planting. At Arlington a "Carter small plot forage drill" was used for planting and at Lancaster a "Great Plains 1006 no-till grain drill" was used for planting.

At Arlington, the 2017 planting had the spring planted IWG plots mowed to 10cm to control weeds in early July 2018. At Lancaster, the 2017 planting had the spring planted IWG plots mowed to 15cm to control weeds in early July 2018. No weed control implemented for 2018 fall and spring planting in any location.

Forage and grain sampling

Grain and forage were collected by hand-harvesting one 50-by-50 cm quadrat at the soil level with a sickle, to include two Kernza intermediate wheatgrass rows. The seed heads from one 50-by-50 cm quadrat per plot were cut and dried at 29 °C for at least five days then threshed manually to estimate grain yield. The summer forage samples were collected from the same quadrat area as the grain. All species in the quadrat (IWG, red clover, and weeds) were harvested, placed in paper bags and separated manually in the lab. Forage samples were then placed in a forced-air dryer at 52 °C for at least five days. Fall forage samples were collected in the first year only at each site, according to the same protocol as the summer forage harvest. Dry matter yields per hectare were then extrapolated from the quadrat data on an area basis.

Table 1. Kernza planting dates and accumulated fall growing degree days (base 0 °C) until frost for the 2017 and 2018 seeding years at Arlington and Lancaster, WI, USA.

	Arlington				**Lancaster**			
	2017		2018		2017		2018	
Planting date treatment	Planting date	GDD until frost	Planting date	GDD until frost	Planting date	GDD until frost	Planting date	GDD until frost
Late August	30 Aug	972	-	-	-	-	30 Aug	865
Mid-September	15 Sep	728	-	-	13 Sep	798	13 Sep	597
Late September	27 Sep	468	21 Sep	420	28 Sep	473	27 Sep	335
Mid-October	20 Oct	162	14 Oct	138	10 Oct	291	12 Oct	156
Late October	-	-	30 Oct	54	26 Oct	103	-	-
Spring	30 Apr 2018	-	8 Apr 2018	-	15 Apr 218	-	3 Apr 2018	-

Table 2. Management practices, seeding and harvest dates for the Kernza planting date experiment seeded in 2017 and 2018 at Arlington and Lancaster, WI, USA.

	Arlington		Lancaster	
Seeding year	2017	2018	2017	2018
Plot size	2.6 by 3.4 m^2	1.7 by 3 m^2	3 by 4.6 m^2	
Kernza planting density	13.5 kg ha^{-1}		10.6 kg ha^{-1}	
Row spacing	30.5 cm		38.1 cm	
Red clover fall planting density	7.8 kg ha^{-1}		14.1 kg ha^{-1}	
Red clover frost seeding date	2-Mar-18	8-Apr-19	12-Mar-18	2-Apr-19
Red clover frost seeding density	11.2 kg ha^{-1}	10.1 kg ha^{-1}	10.1 kg ha^{-1}	
Fertilization rate year 1	44.8 kg/ha^{-1} of N	0	56 kg/ha^{-1} of N 44.8 kg/ha^{-1} of P_2O_5 84 kg/ha^{-1} of K_2O	102 kg/ha^{-1} of N 33.6 kg/ha^{-1} of P_2O_5 257.6 kg/ha^{-1} of K_2O
Fertilization date year 1	Mid May & mid June 2018		Late Mar 2018	May-19
Fertilization rate year 2	44.8 kg/ha^{-1} of N	42 kg/ha^{-1} of N	102 kg/ha^{-1} of N 33.6 kg/ha^{-1} of P_2O_5 257.6 kg/ha^{-1} of K_2O	0
Fertilization date year 2	Mid June 2019	Mid June 2019	May-19	
Summer harvest date 1st year	7-Aug-18	8-Aug-19	3-Aug-18	30-Jul-19
Fall harvest date 1st year	26-Oct-18	22-Oct-19	15-Oct-18	25-Oct-19
Summer harvest date 2nd year	6-Aug-19	30-Jul-20	30-Jul-19	4-Aug-20

Temperature and Precipitation

Temperature and precipitation records were obtained from the online database of the National Weather Service (NWS, 2021). Daily average temperatures and the equation from McMaster and Wilhelm (1997), was used to calculate growing degree days in Celsius with a base temperature of 0 °C. The equation used was:

$$GDD = [(T_{MAX}+T_{MIN})/2]-T_{BASE}.$$

T_{MAX} and T_{MIN} are daily maximum and minimum air temperature, respectively, while T_{BASE} is the base temperature (0 °C). GDD accumulation initiated at planting and ended when average daily temperatures remained below the base temperature for 5 consecutive days (Jungers et al., 2018; Favre et al., 2019). In 2018 the average temperature warmed up earlier after winter than 2019 or 2020 at both locations. Also, in 2018 average temperature after planting started to cool earlier than the previous year. Average temperature did not vary a lot between locations with the majority of the months sharing the same temperature (Figure 1). In all four years, Lancaster received more precipitation than Arlington, 2018 and 2019 being the wettest year for Lancaster and Arlington respectively. September 2019 in Lancaster was recorded as the wettest month in all four years between locations with just over 470 mm of precipitation (Figure 2).

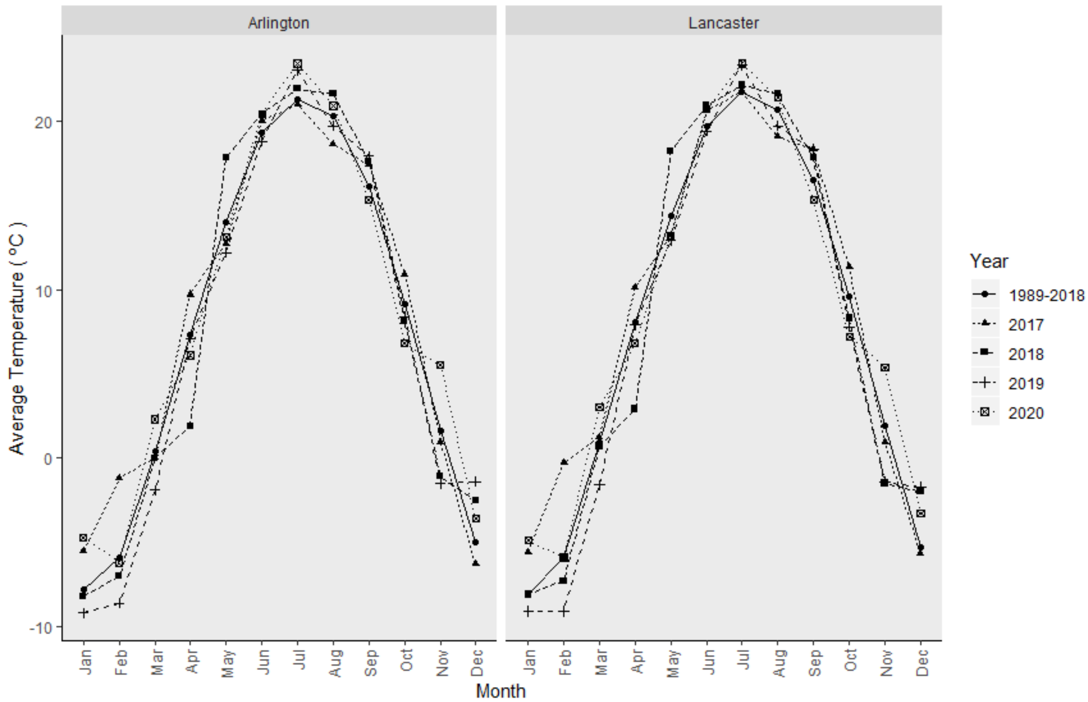


Figure 1 Average monthly temperature (°C) at Arlington and Lancaster for 2017-2020 and 30 year average 1989-2018 (Data from NWS)

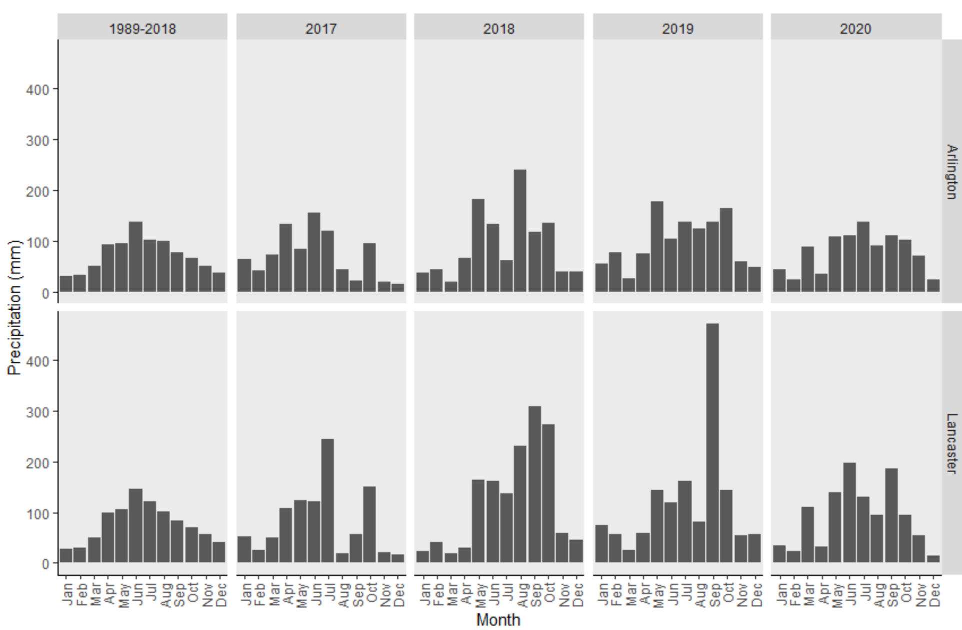


Figure 2 Monthly precipitation (mm) at Arlington and Lancaster for 2017-2020 and 30 year average 1989-2018 (Data from NWS)

Statistical Analyses

Statistical analyses were conducted using PROC MIXED procedure in SAS 9.4 (SAS Institute, 2021) software. Analysis of variance were conducted by location and age of stand, using a model for a randomized complete block design:

$$Y_{mnop} = \boldsymbol{\mu} + S_m + C_n + F_{o(m)} + B_{p(m)} + S{*}C_{mn} + C{*}F_{no(m)} + E_{mnop}$$

where Y_{mnop} = Kernza grain yield, intermediate wheatgrass summer biomass yield, fall biomass, total intermediate wheatgrass biomass (summer + fall), red clover summer biomass, overall biomass (IWG + red clover), or weed biomass; μ = the overall mean; S_m = effect of seeding year; C_n = effect of red clover planting season; $F_{o(m)}$ = effect of fall growing degree days nested within seeding year (treated as a factor); $B_{p(m)}$ = effect of blocks nested within seeding year; $S{*}C_{mn}$, $C{*}F_{no(m)}$ = effect of the two way interactions and E_{mnop} = random residual. Least square means were calculated. When significant, differences among treatments were further investigated with a Tukey test and considered significant at $p < 0.05$. For Arlington we had a full factorial, so all effects were tested against the residual. For Lancaster we had a split-plot design with red clover planting season as whole plot and fall growing degree days as split-plot, so the random interaction $C{*}B_{np(m)}$ was used as an error to test the differences between clover levels (main plot) and the residual was used as an error for testing differences between fall Kernza planting date.

Using the least square means obtained previously, linear, quadratic, and cubic regression equations and p-values were obtained in PROC REG procedure in SAS 9.4 (SAS Institute, 2021) software for intermediate wheatgrass grain, biomass yield, and weed biomass against accumulated fall growing degree days until end of GDD accumulation for each combination of location, age of stand, and clover planting season. Best fit equations for each variable were selected based on the AIC values (see Supplemental tables). With the best fit model, optimal planting date in GDD were determined for each situation.

In order to convert GDD into calendar dates for the optimal planting dates, a 30 year mean was calculated for the end of GDD accumulation. This was accomplished by obtaining daily weather records from the past 30 years (NWS, 2021). For each year, the date for the end of GDD accumulation, when average daily temperatures remained below 0 °C for 5 consecutive days) was identified first, and from that date, accumulation of GDD was calculated backwards until the required GDD was reached. Later, each date that reached the required GDD was converted to a numerical day of the year (i.e., 1 Jan = 1 to 31 Dec = 365) and the mean for the 30 years was found. The mean value was then converted back to its corresponding date. The 90% probability of accumulating the required GDD was obtained by first ordering the numerical day of year values in ascending order for all 30 years. Then a cumulative relative frequency was calculated for each day of year value.

Results

At Arlington, there was a significant interaction between red clover planting season and fall growing degree days in the first year on intermediate wheatgrass fall biomass, red clover biomass and weed biomass. No other variables had interactions that were significant in the first year. In the second year all variables showed an interaction between red clover planting season and fall growing degree days (Table 3). In the first year at Arlington only there was a significant interaction between seeding year and red clover planting season. No other variables had interactions that were significant in both years. At

Lancaster, there was a significant interaction between red clover planting season and fall growing degree days in the first year on intermediate wheatgrass fall biomass. No other variables had interactions that were significant in the first year. In the second year only the only intermediate wheatgrass summer biomass and red clover biomass showed an interaction between red clover planting season and fall growing degree days. No other variables had interactions that were significant in the second year (Table 3). No variable had a seeding year and red clover planting season interaction in both years.

Table 3. P-values from the analysis of variance by location and age of stand for the effect of block, seeding year (2017 and 2018), red clover seeding season (fall and spring), accumulation of growing degree days from planting to end of GDD accumulation with a base temperature of 0 °C from planting to when average daily temperatures remained below the base temperature for 5 consecutive days in the fall) and their respective interaction for Kernza grain yield, intermediate wheatgrass summer biomass, fall biomass, total intermediate wheatgrass biomass (summer + fall), red clover summer biomass, total biomass (IWG + red clover) and weed summer biomass. P-values with * significant at < 0.05 and ** significant at < 0.01.

	Kernza grain yield		IWG summer biomass		IWG fall biomass	Total IWG biomass	Red clover biomass		Overall biomass (IWG + Red clover)		Weed biomass	
	Age 1	Age 2	Age 1	Age 2	Age 1	Age 1	Age 1	Age 2	Age 1	Age 2	Age 1	Age 2
Arlington												
Block	0.38	0.07	0.41	0.32	0.35	0.49	0.26	0.6	0.26	< 0.01**	0.17	0.02*
Seeding year (S)	< 0.01**	< 0.01**	< 0.01**	< 0.01**	< 0.01**	< 0.01**	< 0.01**	< 0.01**	0.01*	< 0.01**	0.53	0.38
Clover (C)	0.04*	0.12	0.09	0.29	0.08	0.05	0.14	0.02*	0.15	0.18	0.03*	0.59
Fall GDD (F)	< 0.01**	0.12	< 0.01**	< 0.01**	< 0.01**	< 0.01**	< 0.01**	0.21	< 0.01**	0.46	< 0.01**	< 0.01**
S*C	0.28	0.63	0.44	0.63	0.72	0.39	0.02	0.57	0.95	0.76	0.47	0.13
C*F	0.08	< 0.01**	0.14	< 0.01**	< 0.01**	0.21	< 0.01**	< 0.01**	0.42	< 0.01**	< 0.01**	< 0.01**
Lancaster												
Block	0.52	0.36	0.66	0.03*	0.59	0.6	0.5	0.36	0.37	0.02*	0.67	0.1
Seeding year (S)	0.78	0.03*	0.03*	0.25	< 0.01**	0.01	0.82	0.04*	0.07	0.35	0.06	0.01*
Clover (C)	0.73	0.18	0.47	0.36	0.28	0.91	0.04*	0.08	0.21	0.53	0.84	0.72
Fall GDD (F)	< 0.01**	0.12	< 0.01**	< 0.01**	< 0.01**	< 0.01**	0.17	0.35	< 0.01**	0.06	< 0.01**	0.01*
S*C	0.81	0.38	0.31	0.41	0.66	0.46	0.79	0.21	0.33	0.91	0.66	0.35
C*F	0.61	0.22	0.23	0.04*	0.05*	0.38	0.1	< 0.01**	0.49	0.19	0.53	0.08

Fall growing degree days from planting until end of GDD accumulation had an effect on grain yield, intermediate wheatgrass fall biomass, total intermediate wheatgrass biomass, red clover biomass, and total biomass at both locations in the first year with the exception of red clover biomass, which just had an effect at Arlington (Table 3). Fall growing degree days also had an effect on both years of intermediate wheatgrass summer biomass and weed biomass at both locations.

At Arlington, red clover planting season had an effect on grain yield and weed biomass in the first year and red clover biomass in the second year. No other variable showed an effect of red clover planting season in both years (Table 3). Seeding year had an effect on all variables except weed biomass in both years. At Lancaster, red clover planting season had an effect on red clover biomass in the first year. No other variable showed an effect of red clover planting season in both years (Table 3). Seeding year had an effect on intermediate wheatgrass summer biomass, intermediate wheatgrass fall biomass, and total intermediate wheatgrass biomass in the first year. In the second year, seeding year had an effect on grain yield, red clover biomass, and weed biomass. No other variable showed an effect of seeding year in both years.

Grain yield

As planting dates get later in the fall, the accumulation of GDD from planting to end of GDD accumulation decreases, Kernza grain yield in the first year increases until it reaches an optimum and then declines with an asymmetrical slope, except for Arlington's spring seeded red clover treatment which had a linear decline. At Arlington, the fall and spring seeded red clover treatments reached the maximum yields of 463 kg ha^{-1} and 515 kg ha^{-1}, when the Kernza intermediate wheatgrass accumulated 721 and 972 GDD respectively (Figure 3A & supplemental table 1). At Lancaster, the fall and spring seeded red clover treatments reached the maximum yields of 278 kg ha^{-1} and 423 kg ha^{-1}, when the Kernza intermediate wheatgrass accumulated 630 and 664 GDD respectively (Figure 3A & supplemental table 1). The spring planted Kernza intermediate wheatgrass in the first year yielded 0 and 12 kg ha^{-1} at Arlington and Lancaster, respectively. There was no effect of planting date on the grain yield in the second year, leading to a horizontal line as the best model (Figure 3B & supplemental table 1). The mean yields for the Arlington fall and spring seeded red clover treatments were 164 kg ha^{-1}, a 65% decrease from the maximized yield from the previous year, and 91 kg ha^{-1} an 82% decrease from the maximized yield from the previous year respectively. The Lancaster fall and spring red clover seeded treatments were 291 kg ha^{-1}, a 5% increase from the maximized yield from the previous year, and 403 kg ha^{-1} a 5% decrease from the maximized yield from the previous year respectively. The spring planted Kernza intermediate wheatgrass in the second year yielded 64 and 205 kg ha^{-1} at Arlington and Lancaster respectively.

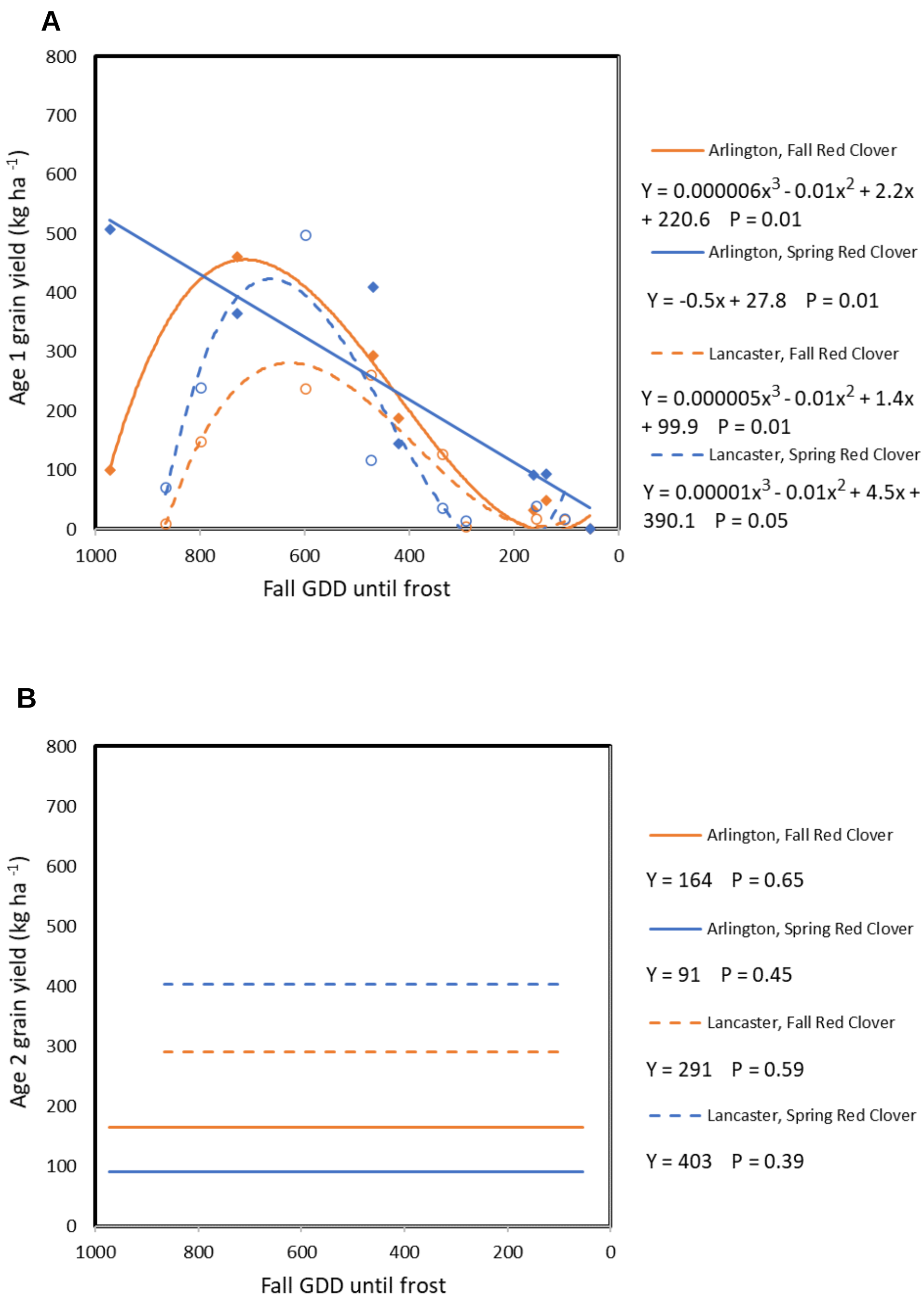
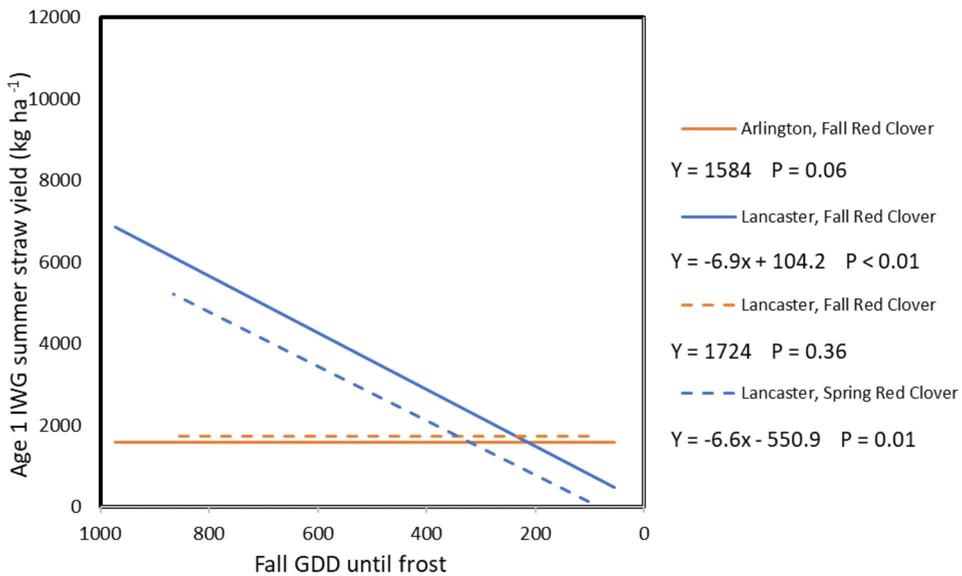


Figure 3. Kernza grain yield kg ha^{-1} in A) first year and B) second year at Arlington and Lancaster, WI with fall and spring planted red clover with regression equations and P-values.

IWG summer biomass

At Arlington, there was no effect of planting date on the fall red clover seeded treatment of intermediate wheatgrass summer biomass in the first year, leading to a horizontal line as the best model, with a mean yield of 1584 kg ha^{-1}. The red clover spring seeded treatment had a linear decline in the first year, and reached the maximum yield of 6811 kg ha^{-1} when the Kernza intermediate wheatgrass accumulated 972 GDD (Figure 4A, & supplemental table 2). At Lancaster, there was also no effect of planting date on the fall red clover seeded treatment of intermediate wheatgrass summer biomass in the first year, with a mean yield of 1724 kg ha^{-1}. The spring red clover seeded treatment had a linear decline in the first year, and reached the maximum yield of 5158 kg ha^{-1} when the Kernza intermediate wheatgrass accumulated 865 GDD (Figure 4A, & supplemental table 2). The spring planted Kernza intermediate wheatgrass in the first year yielded 0 and 25 kg ha^{-1} at Arlington and Lancaster respectively. There was no effect of planting date on the intermediate wheatgrass summer biomass yield in the second year, leading to a horizontal line as the best model (Figure 4B & supplemental table 2). The mean yields for the Arlington fall and spring red clover seeded treatments were 1728 kg ha^{-1}, a 9% increase from the previous year, and 1147 kg ha^{-1} an 83% decrease from the maximized yield from the previous year respectively. The Lancaster fall and spring red clover seeded treatments were 5762 kg ha^{-1}, a 234% increase from the previous year, and 4154 kg ha^{-1} a 19% decrease from the maximized yield from the previous year respectively. The spring planted Kernza intermediate wheatgrass in the second year summer yielded 526 and 2140 kg ha^{-1} at Arlington and Lancaster respectively.

A



B

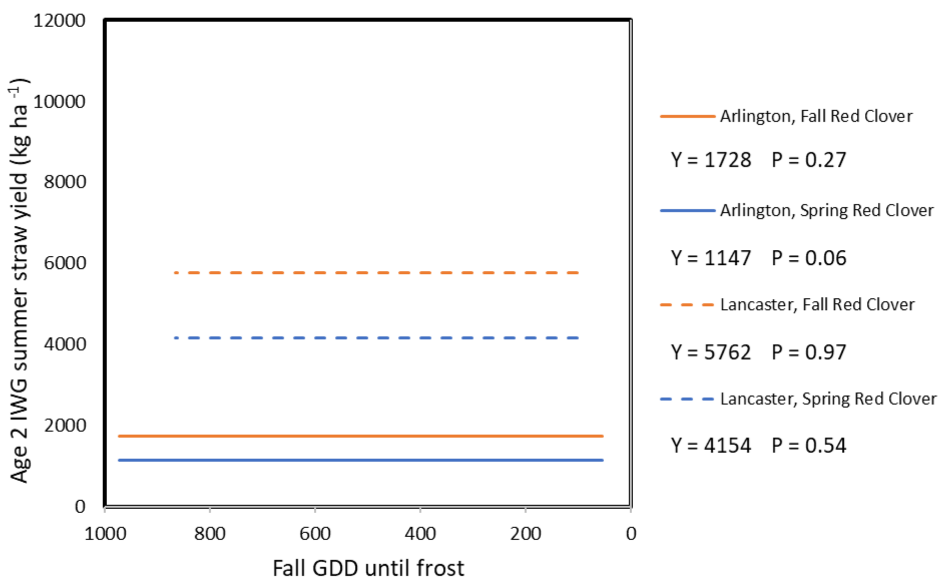


Figure 4. Intermediate wheatgrass summer biomass yield kg ha^{-1} in A) first year and B) second year at Arlington and Lancaster, WI with fall and spring planted red clover with regression equations and P-values.

IWG fall biomass

At both locations, there was no effect of planting date on the fall and spring red clover seeded treatments of intermediate wheatgrass fall biomass in the first year, leading to a horizontal line as the best model, with a mean yield of 256 kg ha^{-1} and 286 kg ha^{-1} for Arlington's fall and spring red clover seed treatments respectively. At Lancaster, the mean fall and spring red clover seed treatments were 744 kg ha^{-1} and 437 kg ha^{-1} respectively (Figure 5 & supplemental table 3).

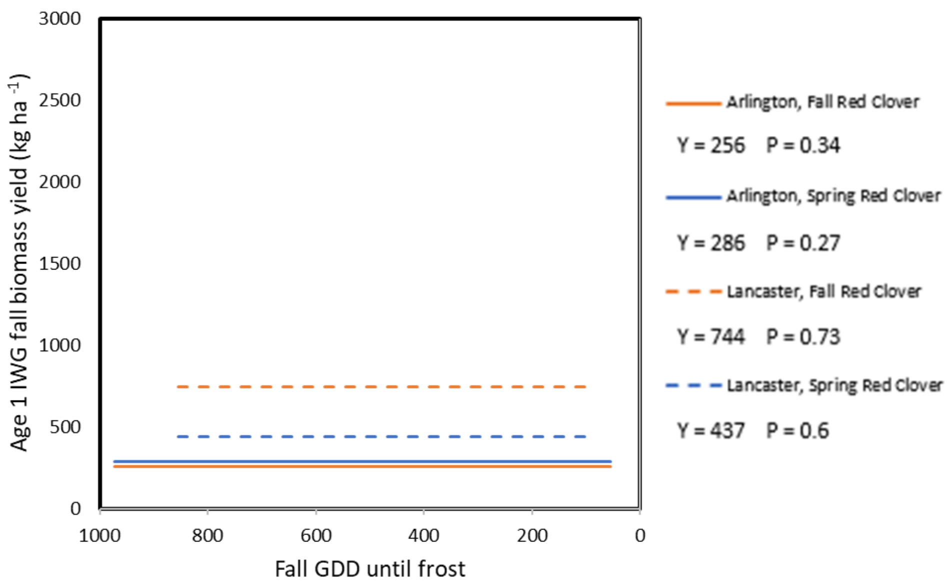


Figure 5. Intermediate wheatgrass fall biomass yield kg ha^{-1} in the first year at Arlington and Lancaster, WI with fall and spring planted red clover with regression equations and P-values.

Total IWG biomass

At Arlington, there was no effect of planting date on the fall red clover seeded treatment of total intermediate wheatgrass biomass in the first year, leading to a horizontal line as the best model, with a mean yield of 1999 kg ha^{-1}. The red clover spring seeded treatment had a linear decline in the first year, and reached the maximum yield of 7819 kg ha^{-1} when the Kernza intermediate wheatgrass accumulated 972 GDD (Figure 6). At Lancaster, there was also no effect of planting date on the fall and spring red clover seeded treatments of total intermediate wheatgrass biomass in the first year, with mean yields of 2668 kg ha^{-1} and 2646 kg ha^{-1} respectively (Figure 6 & supplemental table 4).

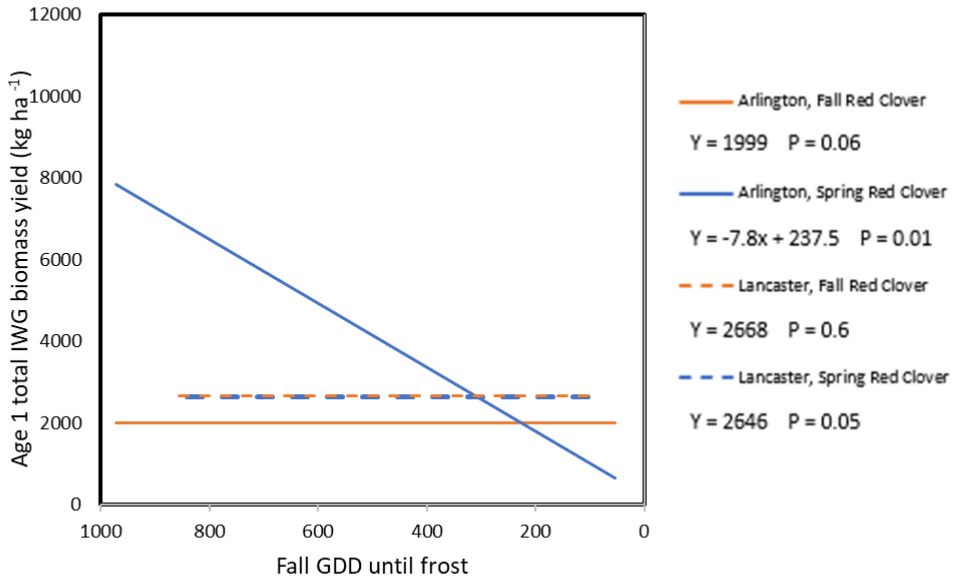


Figure 6. Total intermediate wheatgrass biomass (summer and fall) yield kg ha^{-1} in the first year at Arlington and Lancaster, WI with fall and spring planted red clover with regression equations and P-values.

Red clover summer biomass

At Arlington, there was no effect of planting date on the fall and spring red clover seeded treatments of red clover biomass in the first year, leading to a horizontal line as the best model, with mean yields of 655 kg ha^{-1} and 488 kg ha^{-1} respectively (Figure 7A & supplemental table 5). At Lancaster, there was also no effect of planting date on the fall red clover seeded treatment in the first year, with a mean yield of 8 kg ha^{-1}. The red clover spring seeded treatment had a linear increase in the first year, and was maximized at 705 kg ha^{-1} when the Kernza intermediate wheatgrass accumulated 103 GDD (Figure 7A). The spring planted Kernza intermediate wheatgrass in the first summer yielded 710 and 343 kg ha^{-1} at Arlington and Lancaster respectively. At both locations, there was no effect of planting date on the fall and spring red clover seeded treatments in the second year leading to mean yields for the Arlington fall and spring red clover seeded treatments of 1513 kg ha^{-1}, a 131% increase from the previous year, and 3450 kg ha^{-1}, a 607% increase from the previous year. The Lancaster fall and spring red clover seeded treatments were 359 kg ha^{-1}, a 1950% increase from the previous year, and 1701 kg ha^{-1} a 141% increase from the maximized yield from the previous year respectively (Figure 7B). The spring planted Kernza intermediate wheatgrass in the second year yielded 2861 and 1756 kg ha^{-1} at Arlington and Lancaster respectively.

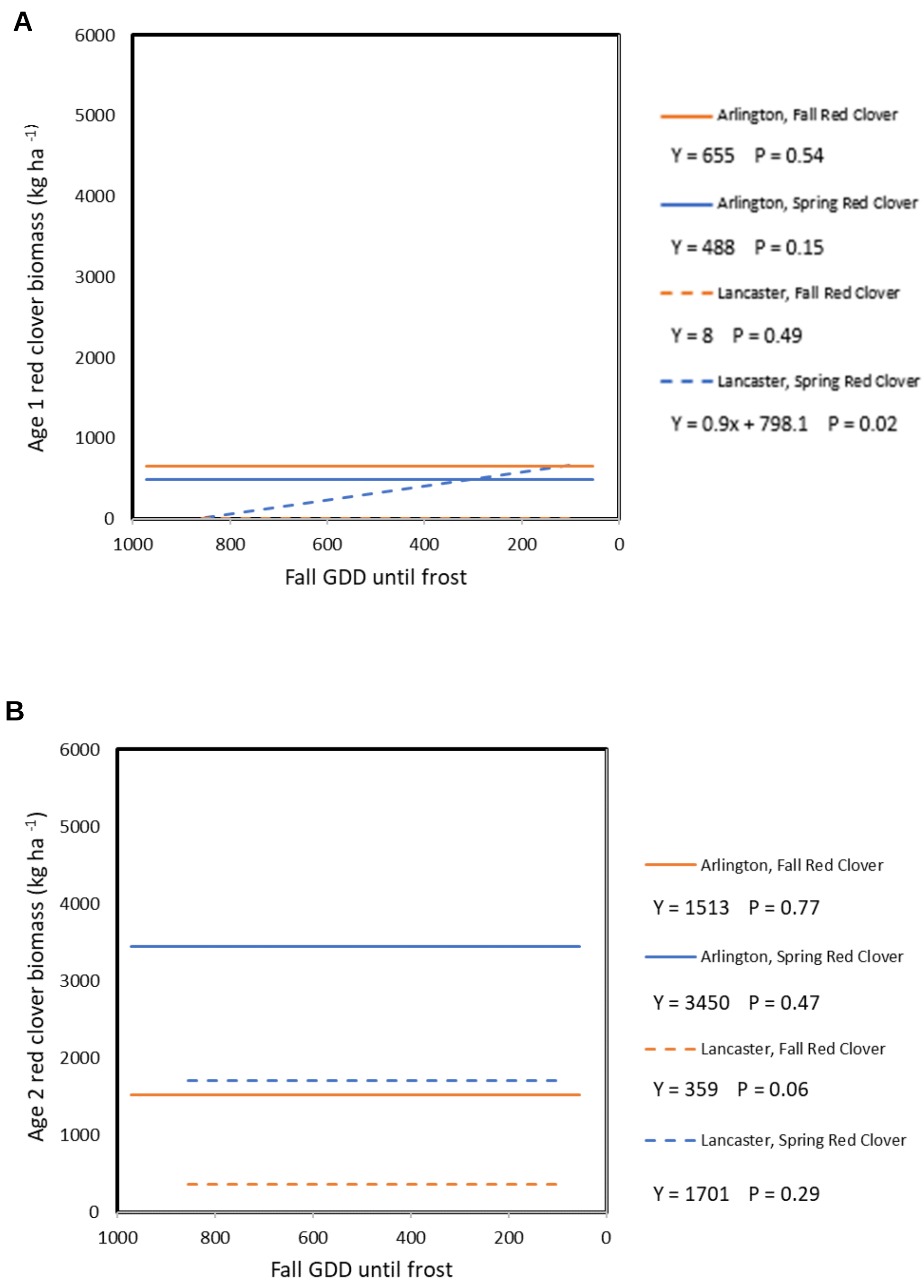
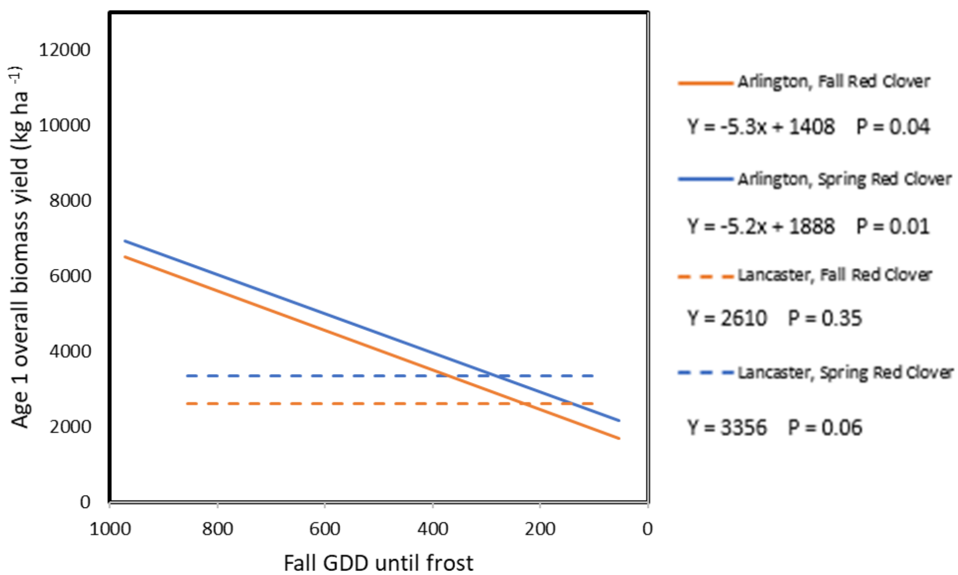


Figure 7. Red clover biomass kg ha^{-1} in A) first year and B) second year at Arlington and Lancaster, WI with fall and spring planted red clover with regression equations and P-values.

Overall (IWG+RC) biomass

At Arlington, the fall and spring seeded red clover treatments had a linear decline in the first year, and reached the maximum yields of 6560 kg ha^{-1} and 6942 kg ha^{-1} when the Kernza intermediate wheatgrass accumulated 972 GDD (Figure 8A & supplemental table 6). At Lancaster, there was no effect of planting date on the fall and spring red clover seeded treatments in the first year, with mean yields of 2610 kg ha^{-1} and 3356 kg ha^{-1} respectively. In the second year at Arlington, the fall seeded red clover treatment had a linear decline, and reached the maximum yield of 6303 kg ha^{-1} a decrease of 4% from the previous maximized yield when the Kernza intermediate wheatgrass accumulated 972 GDD (Figure 8B). There was no effect of planting date for the spring seeded red clover treatment at Arlington with a mean yield of 5679 kg ha^{-1}, an 18% decrease from the maximized yield from the previous year. The Lancaster fall and spring red clover seeded treatments were 7707 kg ha^{-1}, a 195% increase from the previous year, and 7361 kg ha^{-1} a 119% increase from the previous year respectively (Figure 8B).

A



B

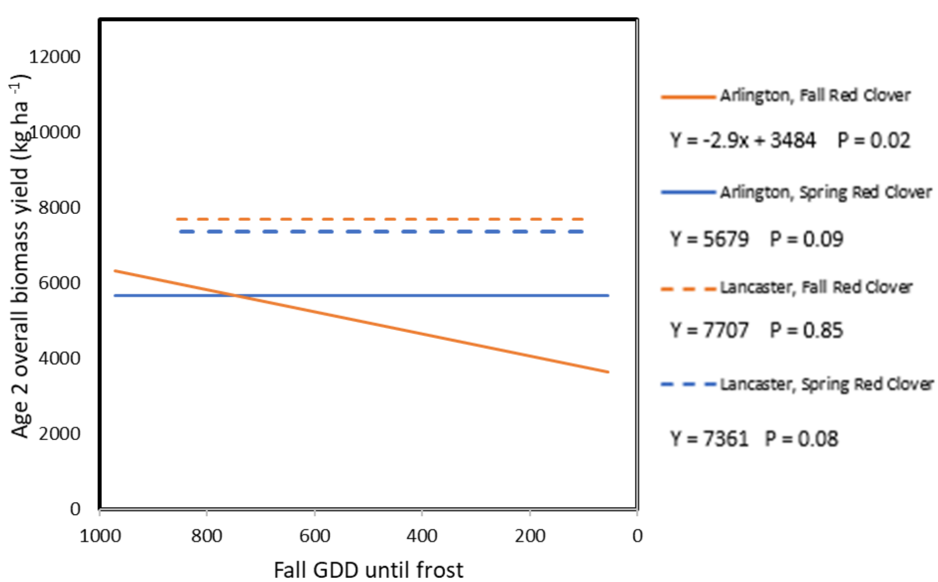
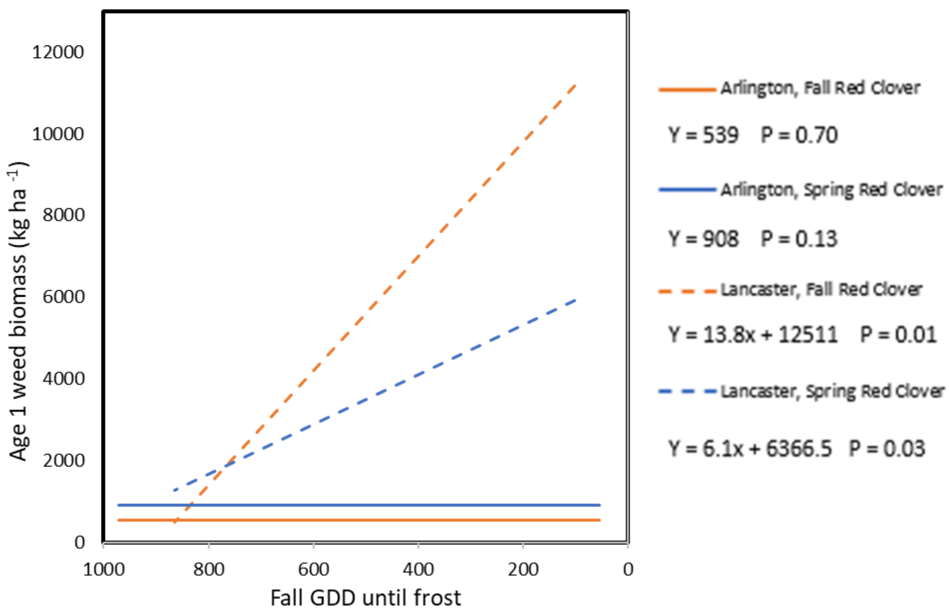


Figure 8. Overall biomass kg ha^{-1} in A) first year and B) second year at Arlington and Lancaster, WI with fall and spring planted red clover with regression equations and P-values.

Weed summer biomass

At Arlington, there was no effect of planting date on the fall and spring red clover seeded treatments in the first year, with means of 539 kg ha^{-1} and 908 kg ha^{-1} respectively. At Lancaster, the fall and spring seeded red clover treatments had a linear increase in the first year, and minimized weed biomass at 574 kg ha^{-1} and 1091 kg ha^{-1} respectively when the Kernza intermediate wheatgrass accumulated 865 GDD (Figure 9A & supplemental table 7). The spring planted Kernza intermediate wheatgrass in the first year amounted to 1481 and 2783 kg ha^{-1} at Arlington and Lancaster respectively. At both locations there was no effect of planting date on the weed summer biomass in the second year (Figure 9B & supplemental table 7). The mean for the Arlington fall and spring red clover seeded treatments were 256 kg ha^{-1}, a 53% decrease from the previous year, and 242 kg ha^{-1}, a 73% decrease from the previous year. The Lancaster fall and spring red clover seeded treatments were 506 kg ha^{-1}, a 12% decrease from the minimized value from the previous year and 319 kg ha^{-1}, a 71% decrease from the minimized value from the previous year respectively (Figure 9B). The summer weed biomass of the spring planted Kernza intermediate wheatgrass in the second year amounted to 355 and 466 kg ha^{-1} at Arlington and Lancaster respectively.

A



B

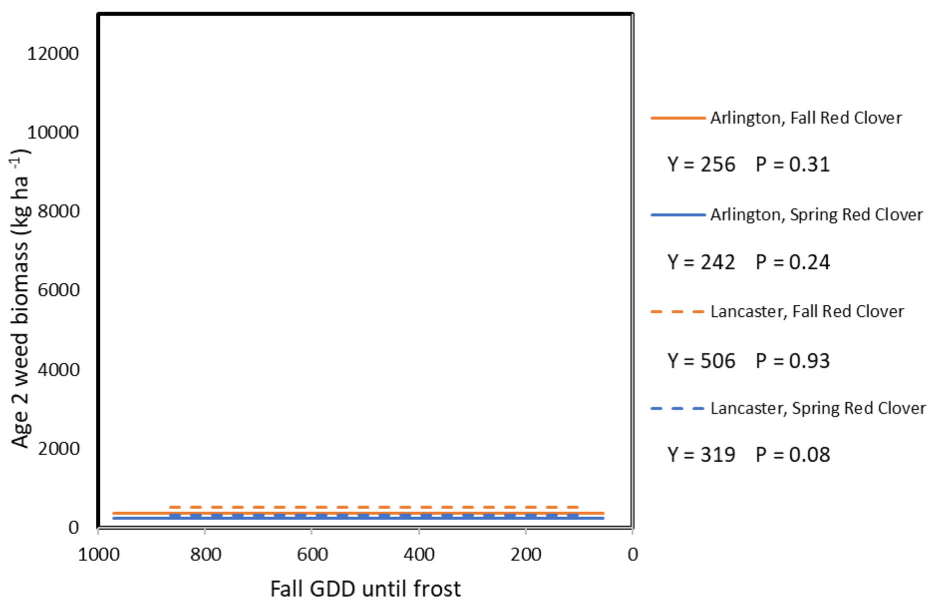


Figure 9. Weed biomass kg ha^{-1} in A) first year and B) second year at Arlington and Lancaster, WI with fall and spring planted red clover with regression equations and P-values.

Optimal planting dates

Table 4. Growing degree days, 30 year mean optimal planting date, and 90% probability date (the planting date that will optimize the yields 90% of the time) of Kernza grain yield, and the end of GDD accumulation for Arlington and Lancaster, WI.

		Arlington			Lancaster		
		GDD	**Avg date**	**90% date**	**GDD**	**Avg date**	**90% date**
Kernza grain yield	**Fall red clover**	721	8 Sep	1 Sep	630	15 Sep	8 Sep
	Spring red clover	972	26 Aug	19 Aug	664	13 Sep	6 Sep
End of GDD accumulation		0	28 Nov	12 Nov	0	29 Nov	13 Nov

Based on 30 year weather records, end of GDD accumulation falls on November 28 on average and by November 12 with a 90% probability at Arlington and at November 29 on average and by November 13 with a 90% probability at Lancaster.

Based on 30 year weather records, at Arlington, the optimal planting date of Kernza grain yield for fall seeded red clover is September 8 on average and on September 1 for a 90% probability of accumulating the required GDD, the spring seeded red clover date is August 26 on average and on August 19 for a 90% probability of accumulating the required GDD. At Lancaster, the optimal planting date of Kernza grain yield for fall seeded red clover is September 15 on average and on September 8 for a 90% probability of accumulating the required GDD, the spring seeded red clover date is September 13 on average and on September 6 for a 90% probability of accumulating the required GDD.

Discussion

Kernza planting date

The grain yield in the first year was consistent with a study on dual-use winter wheat in central Texas where the effect of planting date on grain yield had a third order polynomial trend, with yields first low then increasing and later decreasing (Darapuneni et al, 2016), which were similar trends observed in our findings. Early planting dates in winter wheat can lower yields due to a higher susceptibility to insects and diseases (Hunger et al., 2017; Lyon et al., 2001). Conley et al. (2015) reported that planting too early may lead to excessive fall growth that could potentially smother the crop. Late planting dates in our study had a shorter growing season available to them, and they did not accumulate enough growing degree days, thus the crop did not establish well in the fall, leading to low yields in the first harvest or no yield at all. However, for intermediate wheatgrass established as the sole crop in the fall (with red clover frost seeded in the next spring) there was a linear decline with planting date at Arlington, suggesting that when there is no competition from another crop, earlier dates may be preferred. However, we may not have had early enough planting dates to entirely capture the first section of the curve. Further research is needed to confirm this with earlier planting dates and also the susceptibility of intermediate wheatgrass to diseases that may occur when planted very early, there was no serious disease observed during the study.

It has been reported previously that Kernza grain yields tend to decline after the first year; grain yield components such as tiller number, spike number, spike weight and other could be limiting the yield (Jungers et al., 2017; Pinto et al., 2021). If number of spikes is reduced grain yield also decreases because grain yield is dependent on number of spikes

(Pinto et al., 2021). This is evident when looking at the Arlington grain yields, with the fall and spring red clover treatments on average decreased from their maximum yields by 65% and 82% respectively by the second year, although we did not test this statistically. At Lancaster, interestingly that was not the case. We do not know exactly why some of the grain yields increased in the second year, we hypothesize that it may be due to the higher N fertilizer rate that the Lancaster plots received. It should also be noted that while the ANOVA F-test showed significant effects from the interactions and main effects on the different variables, in the results using the Tukey test, there are a lot more tests and the threshold for significance is adjusted to avoid false positives, so those significant effects were not as widespread.

A previous study at Arlington and Lancaster showed that, cool season grasses such as smooth bromegrass (*Bromus inermis* Leyss.), timothy (*Phleum pretense* L.), reed canarygrass (*Phalaris arundinacea* L.) and others yielded the most from the first cutting dry matter yield when the planting was done in August through early September (Undersander and Greub, 2007). Interestingly the first cutting dry matter yield in our experiment produced similar results. Focusing on the intermediate wheatgrass biomass, at both locations the two treatments with similar linear trends both had spring seeded red clover. Suggesting that if cool season grasses grow with limited competition after planting, the later you plant the lower the yield is the following year. Looking at winter wheat, another dual-use crop, a study has shown that the planting date effect on the first harvest of forage yield had a linear trend, the later you plant the lower the yield (Darapuneni et al., 2016). We witnessed similar results, and at times there was no yield at all. The late planting date (30 October) at Arlington was not able to be established due to the low amount of growing degree days accumulated.

Regardless of planting date, majority of weed biomass decreased from the first year to the second year. This is reinforced by previous research which has shown that weed biomass decreases significantly in intermediate wheatgrass systems from 745 kg ha^{-1} in the first year stand to 87 kg ha^{-1} in the third year stand (Zimbric et al., 2020). Zimbric et al. (2020) observed that when Kernza intermediate wheatgrass is planted, annual weed density decreases while perennial weed density increases. This is seen with our first and second year weed biomass accumulation. The large presence of the annual weeds in the first year at Lancaster may also have affected the grain yield and with the weed biomass reduced in the second year it reduced the competition which may be one of the potential reasons for the higher grain yields in the second year at Lancaster.

Red clover planting season

Interestingly with intermediate wheatgrass biomass yield, both the spring seeded red clover treatments followed a linear trend in the first year. We speculate this happened due to the lack of competition in the fall for Kernza intermediate wheatgrass. This allowed it to establish and develop better in the fall, resulting in a good stand the next year for those that were planted earlier. The fall seeded red clover treatments led to more competition because red clover is well adapted to Wisconsin (Sheaffer & Evers, 2007) which leads to its aggressive establishment and in turn its competition with the Kernza intermediate wheatgrass, explaining why the planting date had little effect on the intermediate wheatgrass summer biomass yield. In a study of winter cereals intercropped with kura clover, tiller numbers were reduced due to competition (Kazula et al., 2019). In the second year, intermediate wheatgrass summer biomass increased overall on average, this could have been due to the plants being better established in the fall after the first year.

Red clover, already being well adapted to Wisconsin, also has a tolerance to shading (Singer et al., 2006), allowing continued growth even with low light intensity that may be caused by intermediate wheatgrass. In the first year, the Lancaster spring seeded red clover shows a linear trend for the red clover biomass. This could potentially be due to the late Kernza planting dates not establishing well giving the red clover more opportunities to establish. With less competition the red clover develops better. The fact that the highest red clover biomass came when the growing degree days was the lowest (103 GDD) reinforces the point.

When averaging across planting dates, the red clover planting season did not have an effect on the majority of the variables, except grain yield at Arlington and the red clover summer biomass at both locations. At Arlington, in the first year the grain yield of the fall seeded red clover treatment was maximized at 463 kg ha^{-1} with 721 GDD while the spring seeded red clover treatment was maximized at 515 kg ha^{-1} with 972 GDD.

At Arlington, in the second year the spring seeded red clover treatment (3450 kg ha^{-1}) accumulated more red clover summer biomass than the fall seeded red clover treatment (1513 kg ha^{-1}). At Lancaster, in the first year the spring seeded red clover treatment (maximized at 705 kg ha^{-1}) accumulated more red clover summer biomass than the fall seeded red clover treatment (8 kg ha^{-1}). This could be due to the spring seeded red clover competing less with the Kernza intermediate wheatgrass during establishment.

Spring planted intermediate wheatgrass

Major differences for the spring planted intermediate wheatgrass came between locations. The spring planted intermediate wheatgrass by their nature just have a spring red clover planting, and Lancaster yielded more grain and forage than Arlington, but overall lower than the fall plantings dates. Therefore, to maximize Kernza yield it would be beneficial to plant in the following fall if the original planting was delayed, but not in the spring. In the first year the spring planted intermediate wheatgrass essentially did not produce any grain due to not having a vernalization period to induce flowering. In the second year the grain yield of the spring planted intermediate wheatgrass at Arlington and Lancaster was 64 kg ha^{-1} and 205 kg ha^{-1} respectively.

Conclusion

Delaying fall planting date of Kernza intermediate wheatgrass reduced grain yields in the first year but not the second year. The optimal planting date for grain yield in the first year would be by September 8 50% of the time and by September 1 90% of the time at Arlington and by September 15 50% of the time and by September 8 90% of the time at Lancaster. We recommend planting sometime before those dates to optimize summer intermediate wheatgrass biomass and increase weed suppression in the first year. In the following year intermediate wheatgrass biomass should increase and weed biomass decrease overall.

Red clover planting season did not have much of an effect on yields but the few times it did, the spring seeded red clover allowed higher intermediate wheatgrass biomass yields and red clover biomass depending on the planting date and location. We recommend that red clover intercrop should be planted in the spring.

If farmers must delay fall Kernza intermediate wheatgrass planting, we recommend waiting till the following fall to maximize forage or grain yield, instead of planting in the spring.

Looking forward, more research is still needed in this area. Understanding the effects of intermediate wheatgrass planting date with other legumes such as alfalfa or an annual clover such as crimson clover (*Trifolium incarnatum* L.) and as a monoculture would be beneficial. The intermediate wheatgrass-legume competition we observed could be different when another legume is utilized. Looking at the planting date, testing even earlier planting dates across more locations would also be beneficial.

References

Ashworth, A., Keyser, P., Allen, F., Bates, G., & Harper, C. (2015). Intercropping legumes with native warm-season grasses for livestock forage production in the mid-south. *Center for Native Grasslands Management*. University of Tennessee. https://extension.tennessee.edu/publications/Documents/SP731-G.pdf

Blue, E. N., Mason, S. C., & Sander, D. H. (1990). Influence of planting date, seeding rate, and phosphorus rate on wheat yield. *Agronomy Journal*, *82*(4), 762–768. https://doi.org/10.2134/agronj1990.00021962008200040022x

Conley S., Gaska, J., Marburger, D., & Smith, D. (2015). Top 8 recommendations for winter wheat establishment in 2015*. Integrated Pest and Crop Management*. https://ipcm.wisc.edu/blog/2015/08/top-8-recommendations-for-winter-wheat-establishment-in-2015/

Dahlke, B. J., Oplinger, E. S., Gaska, J. M., & Martinka, M. J. (1993). Influence of planting date and seeding rate on winter wheat grain yield and yield components. *Journal of Production Agriculture*, *6*(3), 408. https://doi.org/10.2134/jpa1993.0408

Darapuneni, M. K., Morgan, G. D., Shaffer, O. J., & Dodla, S. (2016). Impact of planting date and seeding rate on forage and grain yields of dual-purpose wheat in central Texas. *Crop, Forage & Turfgrass Management*, *2*(1), 1–8. https://doi.org/10.2134/cftm2016.06.0045

DeHaan, L. R., Wang, S., Larson, S. R., Cattani, D. J., Zhang, X., & Kantarski, T. (2014). Current efforts to develop perennial wheat and domesticate *Thinopyrum intermedium* as a perennial grain. *Perennial Crops for Food Security Proceedings of the FAO Expert Workshop*, 72–89.

Duchene, O., Dumont, B., Cattani, D.J., Fagnant, L., Schlautman, B., DeHaan, L., Barriball, S., Jungers, J.M., Picasso, V.D., David C., Celette, F. (2021). Process-based analysis of *Thinopyrum intermedium* phenological development across various environments highlights the importance of dual induction for reproductive growth and agronomic performance. *Agricultural and Forest Meteorology, 301-302*, 108341. https://doi.org/10.1016/j.agrformet.2021.108341

Favre, J. R., Castiblanco, T. M., Combs, D. K., Wattiaux, M. A., & Picasso, V. D. (2019). Forage nutritive value and predicted fiber digestibility of Kernza intermediate wheatgrass in monoculture and in mixture with red clover during the first production year. *Animal Feed Science and Technology*, *258*. https://doi.org/10.1016/j.anifeedsci.2019.114298

Hunger, B., De Oliveira Silva, A., Royer, T., Giles, K. (2017). Effect of planting date and seed treatment on diseases and insect pests of wheat. Retrieved from https://extension.okstate.edu/fact-sheets/effect-of-planting-date-and-seed-treatment-on-diseases-and-insect-pests-of-wheat.html

Ivancic, K. A., Locatelli, A., Tracy, W, F., & Picasso. V, D. (2021). Kernza intermediate wheatgrass (*Thinopyrum intermedium*) response to a range of vernalization conditions. *Canadian Journal of Plant Science*. https://doi.org/10.1139/CJPS-2020-0251

Jensen, E. S., Carlsson, G., & Hauggaard-Nielsen, H. (2020). Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: A global-scale analysis. *Agronomy for Sustainable Development*, *40*(1). https://doi.org/10.1007/s13593-020-0607-x

Jungers, J.M., DeHaan, L.R., Betts, K.J., Sheaffer, C.C., Wyse, D.L. (2017). Intermediate wheatgrass grain and forage yield responses to nitrogen fertilization. *Agronomy Journal 109*(2), 462–472. https://doi.org/10.2134/agronj2016.07.0438

Jungers, J. M., Frahm, C. S., Tautges, N. E., Ehlke, N. J., Wells, M. S., Wyse, D. L., & Sheaffer, C. C. (2018). Growth, development, and biomass partitioning of the perennial grain crop *Thinopyrum intermedium*. *Annals of Applied Biology*, *172*(3), 346–354. https://doi.org/10.1111/aab.12425

Kazula, M. J., Andrzejewska, J., Conley, S. P., & Albrecht, K. A. (2019). Intercropping winter cereals in kura clover for spring forage production. *Canadian Journal of Plant Science*, *99*(5), 740–750. https://doi.org/10.1139/cjps-2019-0097

Lanker, M., Bell, M., & Picasso, V. D. (2019). Farmer perspectives and experiences introducing the novel perennial grain Kernza intermediate wheatgrass in the US midwest. *Renewable Agriculture and Food Systems*, *35*(6), 653–662. https://doi.org/10.1017/S1742170519000310

Lyon, D. J., Baltensperger, D. D., & Siles, M. (2001). Wheat grain and forage yields are affected by planting and harvest dates in the central great plains. *Crop Science, 41(2)*, 488-492 https://doi.org/10.2135/cropsci2001.412488x

McMaster, G. S., & Wilhelm, W. W. (1997). Growing degree-days: one equation, two interpretations. *Agricultural and Forest Meteorology, 87*(1), 291-300. https://doi.org/10.1016/S0168-1923(97)00027-0

National Weather Service, 2021. National weather service. Online weather data. National weather service Silver Spring, MD. https://www.ncdc.noaa.gov/cdo-web/

Picasso, V.D., Brummer, E.C., Liebman, M., Dixon, P.M. and Wilsey, B.J. (2008). Crop species diversity affects productivity and weed suppression in perennial polycultures under two management strategies. *Crop Science*, *48*(1), 331-342. https://doi.org/10.2135/cropsci2007.04.0225

Pinto, P., DeHaan, L., & Picasso, V. (2021). Post-harvest management practices impact on light penetration and Kernza intermediate wheatgrass yield components. *Agronomy*, *11*(3), 442. https://doi.org/10.3390/agronomy11030442

SAS Institute. 2021. The SAS system for windows. Release 9.4. Sas Inst., Cary, NC.

Sheaffer, C.C & Evers, G.W. (2007). Cool-season legumes for humid areas. In R.F. Barnes, C.J. Nelson, K.J. Moore, M. Collins. (Ed). Forages, Volume 2: The science of grassland agriculture. (Sixth edition, 808). Ames, Iowa: Blackwell.

Singer, J. W., Casler, M. D., & Kohler, K. A. (2006). Wheat effect on frost-seeded red clover cultivar establishment and yield. *Agronomy Journal*, *98*(2), 265–269. https://doi.org/10.2134/agronj2005.0037

Undersander, D. J., & Greub, L. J. (2007). Summer-fall seeding dates for six cool-season grasses in the Midwest United States. *Agronomy Journal*, *99*(6), 1579–1586. https://doi.org/10.2134/agronj2006.0194

Web Soil Survey, 2021. USDA-Natural Resources Conservation Service. https://websoilsurvey.sc.egov.usda.gov/

Zimbric, J. W., Stoltenberg, D. E., & Picasso, V. D. (2020). Effective weed suppression in dual-use intermediate wheatgrass systems. *Agronomy Journal*, *112*(3), 2164–2175. https://doi.org/10.1002/agj2.20194

Chapter 3 - Performance of breeding populations of Kernza intermediate wheatgrass in Wisconsin, USA

Introduction

Kernza, a new cool season perennial grain, has been developed through conventional breeding for increased seed production of intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) at The Land Institute; Salina, KS, USA (DeHaan & Van Tassel, 2014). There are also other breeding programs across the United States (University of Minnesota) and in Canada (University of Manitoba) (Hybner et al., 2012; Cattani, 2016; Bajgain et al., 2020).

In most crops, cultivar or “variety” trials are conducted annually to provide data on how different breeding populations or lines perform at different locations in different years (Conley et al., 2019; Conley et al., 2020; Gutiérrez et al., 2019; Gutiérrez et al., 2020). Dual-purpose winter wheat (*Triticum aestivum* L.) has been researched for selecting the right population(s) for different traits such as nitrogen content and fall biomass (MacKown, 2005). There are also variety trials on winter wheat, oat (*Avena sativa* L.), and barley (*Hordeum vulgare* L., Conley et al., 2019; Conley et al., 2020; Gutiérrez et al., 2019; Gutiérrez et al., 2020). These trials are conducted annually to serve Wisconsin farmers by providing data to help the farmers choose which variety to plant. As a new emerging crop in Wisconsin, a breeding population performance trial of dual-purpose Kernza intermediate wheatgrass can provide information needed when choosing an appropriate breeding population for grain production, forage production, or one that maximizes both grain and forage production.

This intermediate wheatgrass breeding population performance trial was conducted over three years with two seedings at University of Wisconsin Arlington Agricultural Research Station. The 2017 planting was intercropped with alfalfa while the 2018 planting was intercropped with red clover. Different breeding populations from different breeding programs were planted to determine which is best suited for Wisconsin.

The first objective of this trial was to determine the breeding population(s) that maximized Kernza intermediate wheatgrass grain yield, forage biomass, and legume biomass. The second objective was to determine if differences existed among the breeding programs for Kernza intermediate wheatgrass grain yield, forage biomass, and legume biomass. This information can be used to help farmers in Wisconsin select the best breeding population to plant, and help breeders assess their current program.

Material and Methods

Experimental design

The experiment was established at University of Wisconsin Arlington Agricultural Research Station: (43°18′6.66″N, 89°19′41.55″W) in the fall of 2017 and 2018 and performance evaluations were collected for three subsequent years. None of the experiments were irrigated. The treatment design was a single-factor experiment with 16 and 15 breeding population treatments (Table 1) in the 2017 and 2018 plantings respectively. All plots were intercropped with alfalfa in 2017, and red clover in 2018, respectively. The field plot design was complete randomized blocks with 4 replications for eacplanting. The 2017 Kernza intermediate wheatgrass established planting date was September 29, except the Canada originated breeding populations because we received the seeds late and planted in the spring on April 30 2018. The 2018 Kernza intermediate wheatgrass established planting date was September 21. 439 and 420 GDD were accumulated for the 2017 and 2018 plantings respectively. From the previous topic, it is now known that these were both planted about a month past the optimal dates. Seeding rate was 13.5 kg ha^{-1} (12 lbs/acre) of pure live seeds (PLS) with 30.5 cm (12 inches) row-spacings. The 2017 and 2018 seeding plot sizes were 1.2 by 3 and 1.7 by 3 m^2 (4 by 10 and 5.5 by 10 ft) respectively. Alfalfa seed variety was unnamed and red clover seed was variety "FF 9615" from LaCrosse Seeds. Alfalfa and red clover were frost seeded at 16.8 and 10.1 kg ha^{-1} (15 and 9 lbs/acre) PLS on 9 March 2018 and 8 April 2019 respectively.

Soils were Plano silt loam with 2 to 6% slope (PaB – Web Soil Survey, 2021) at Arlington. In 2017 the land was disked in late July and cultipacked in late September just before planting. In 2018 rototilling was done prior to planting. The 2017 seeding had no fertilizer applied in 2017 and 2018, it received 44.8 kg/ha of N in mid-June 2019. In 2020 42 kg/ha of N was applied in mid-June. The 2018 planting had no fertilizer applied in 2018 and 2019. In 2020 42 kg/ha of N was applied in mid-June.

Breeding program descriptions

Canada (CA): Led by Dr. Doug Cattani. The Canada program started in 2011, Carman, MB. Intermediate wheatgrass seeds were obtained from United States Department of Agriculture-Natural Resources Conservation Service Germplasm Resources

Information Network (USDA-NRCS GRIN) and The Land Institute's Cycle 3 and 4 germplasm. All were transplanted by 2012 into Ian N. Morrison Research Farm of the University of Manitoba located at Carman, MB. Seed yield stability, and relative yield were calculated for each plant over three harvests. There were the top two plants with consistent relative yields compared to other plants. (Cattani, 2016). Two breeding populations from this program were included in our trial.

Kansas (KS): Led by Dr. Lee DeHaan. The Kansas program started in 2003 at the Land Institute, Salina, KS, USA. Obtained seeds from the Rodale Institute, Kutztown, PA, USA. This breeding program focused on long term seed yield. Reproductive traits data was collected in 2005 during the first harvest. Selections were made continuously after evaluating the top individuals and new cycles were produced (Dehaan et al., 2018), and in the year 2021 there are field evaluations up to the tenth cycle focused on grain yield (Crain et al., 2021). Seeds from this program were used to start other breeding programs (Bajgain et al., 2020; Cattani, 2016). Three breeding populations were included in this trial.

Minnesota (MN): Led by Dr. James Anderson. The Minnesota program started in 2012, St. Paul MN. The Land Institutes third cycle germplasm was used for recurrent selections. The breeding program's goal was to produce varieties with high grain yields and ecosystem services. For our study we used the fourth cycle from the University of Minnesota's breeding program (Forever Green) as well as synthetic cultivars. After a 2 year evaluation, 24 genets (a genetically unique organism) received from The Land Institute were chosen based on agronomic performance to be utilized in different combinations as parents of five synthetic cultivar candidates (MN1501, MN1502, MN1503, MN1504, MN1505) (Bajgain et al., 2020). MN1504 was the experimental designation of "MN-Clearwater", which was released by the University of Minnesota and is the first Kernza intermediate wheatgrass cultivar that produces grain approved for sale as Kernza perennial grain (Bajgain et al., 2020). 5 synthetic cultivars and one breeding population cycle were used in this trial.

Forage: Two forage varieties of intermediate wheatgrass were used. **Oahe**, a selected Russian originated seed, was released in 1961, South Dakota. It is adapted for conservation purposes, hay, and pasture. It was selected for its traits such as drought tolerance, vigor, and high seed yields (Hybner et al., 2012). **Rush**, a selected German originated seed, was released in 1994, Idaho. It was selected for its vigor, seedling emergence, unvarying seedheads, wide leaves, and high seed and forage production. It is adapted for soil erosion control, hayland, pastureland, and forage for wildlife (Hybner et al., 2012).

Forage and grain sampling

Experiment 1 (2017 seeding with alfalfa) was harvested with combines only. The first and second harvest were done with a "Wintersteiger plot master" combine. The third harvest was done with the "ZÜRN 150" plot combine harvester. Experiment 2 (2018 seeding with red clover) was harvested with 50-by-50 cm quadrats only. All other variables (intermediate wheatgrass biomass, legume biomass, weed biomass, and harvest index) were only measured in experiment 2. Harvest index has been described as the ability and physiological efficiency of crops converting economic yield from total dry matter, it is the ratio of grain to total shoot dry matter (Sharifi et al., 2009). The quadrat harvesting protocol was hand-harvesting at the soil level with sickles, using one 50-by-50 cm quadrat to include two Kernza intermediate wheatgrass rows. The grain and the forage were harvested from the same quadrat area. All species in the quadrat (IWG, Red clover, weeds) were harvested and placed in bags and were separated manually in the lab. Forage samples were then placed in a forced-air dryer at 52 °C for at least five days. The seed heads from the quadrat samples were cut and dried at 29 °C for at least five days then threshed manually to estimate grain yield. Dry matter yields per hectare were then extrapolated from the quadrat data on an area basis. In 2018, the combine Kernza grain harvest was on August 8. In 2019 all hand harvests were done on August 8 and the combine harvest was on August 21. In 2020, all hand harvests were done on July 31 and the combine harvest was on July 28.

Table 1. Breeding program, breeding population, and seed origin for each population used in the 2017 and 2018 seeding. 16 and 15 different populations in the 2017 and 2018 seeding respectively.

Breeding Program	Population	Seed origin	Seeding year
Kansas	KS Cycle 4- HK1	Wisconsin	Both
Kansas	KS Cycle 4 - AK1 /AK5	Wisconsin	Both
Kansas	KS Cycle 4 - AK2	Wisconsin	Both
Kansas	KS Cycle 3	Kansas	Both
Kansas	KS Cycle 4	Kansas	Both
Kansas	KS Cycle 5	Kansas	Both
Minnesota	MN Cycle 4	Minnesota	2017 only
Minnesota	MN Cycle 4 - LK1	Wisconsin	2017 only
Minnesota	MN Cycle 4 - PK1	Wisconsin	2017 only
Minnesota	MN: 1501	Minnesota	Both
Minnesota	MN: 1502	Minnesota	Both
Minnesota	MN: 1503	Minnesota	Both
Minnesota	MN: 1504	Minnesota	Both
Minnesota	MN: 1505	Minnesota	Both
Canada	CA: Early lines	Canada	Both
Canada	CA: Late lines	Canada	Both
Forage	Forage: Oahe		2018 only
Forage	Forage: Rush		2018 only

Temperature and Precipitation

Temperature and precipitation records were obtained from the online database of the National Weather Service (NWS, 2021). In 2018 the average temperature warmed up earlier than any other year after winter. Also in 2018 average temperature after planting started to cool earlier than the previous year (Figure 1). Average temperature did not vary a lot between years with the majority of the months sharing similar temperatures. Although 2019 was the wettest year, August 2018 was recorded as the wettest month in all four years with just over 200 mm of precipitation (Figure 2). The accumulated precipitation for each growing season (April-August) from 2018-2020 were 681, 614, and 483 mm respectively.

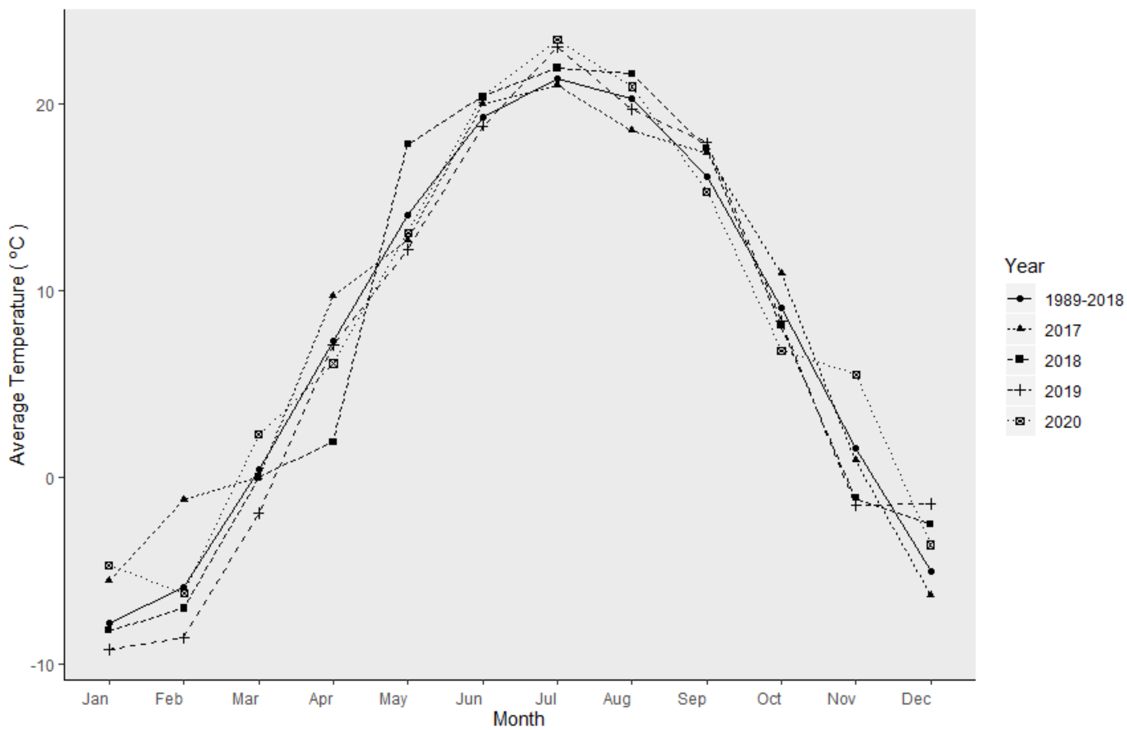


Figure 1. Average monthly temperature (°C) at Arlington for 2017-2020 and 30 year average 1989-2018 (Data from NWS)

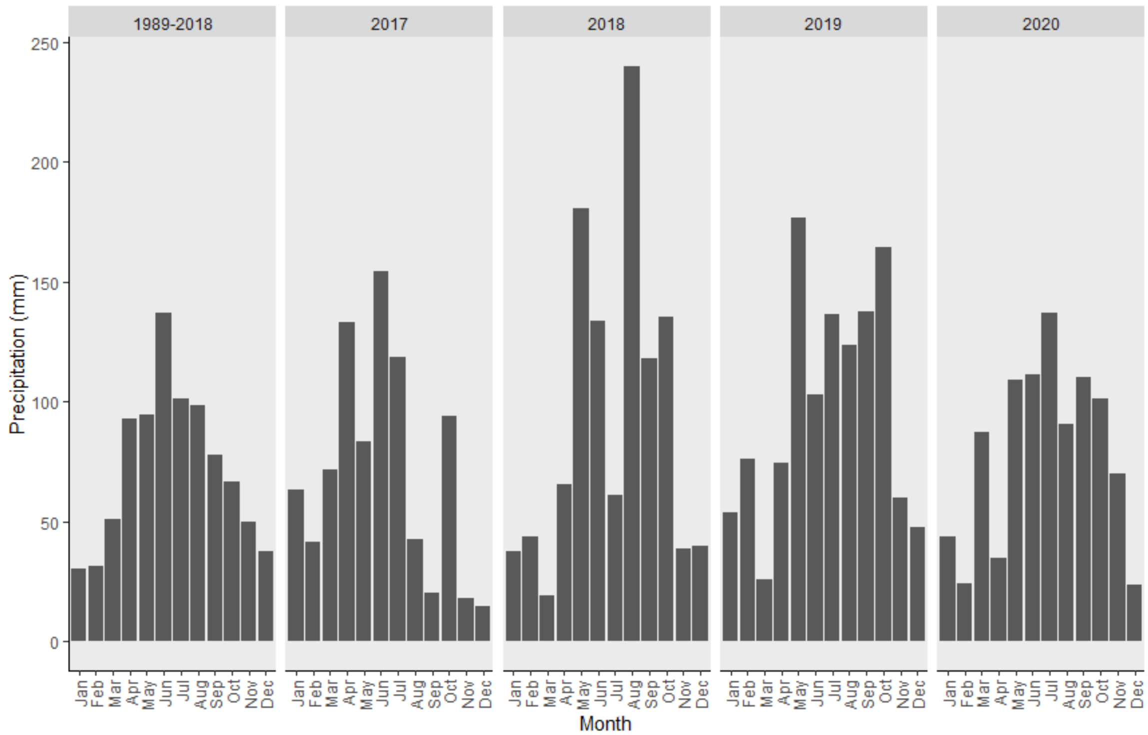


Figure 2. Monthly precipitation (mm) at Arlington for 2107-2020 and 30 year average 1989-2018 (Data from NWS)

Statistical Analyses

Statistical analyses were conducted using PROC MIXED procedure in SAS 9.4 (SAS Institute, 2021) software. The first analysis was focused on each individual breeding population, and the second analysis was focused on the different breeding programs. Analysis of variance were conducted by experiment which is a combination of seeding year confounded with legume intercropping species.

By breeding population, we used a model for a randomized complete block design:

$$Y_{mno} = \boldsymbol{\mu} + P_m + A_n + B_o + P^*A_{mn} + E_{mno}$$

where Y_{mno} = Combine grain yield, hand harvested grain yield, hand harvested intermediate wheatgrass summer biomass yield, hand harvested legume biomass, hand harvested weed biomass, or the intermediate wheatgrass harvest index; μ = the overall mean; P_m = effect of intermediate wheatgrass populations planted; A_n = effect of age of stand, as repeated measure; B_o = Blocking effect; $P*A_{mn}$, = effect of the two way interaction and E_{mno} = random residual. All effects and interactions were considered fixed. When significant, differences among treatments were further investigated with a Tukey test and considered significant at $p < 0.05$.

By breeding program, we used a model for a randomized complete block design:

$$Y_{qrst} = \boldsymbol{\mu} + P_{q(s)} + A_r + Bp_s + Bl_t + Bp*A_{sr} + E_{qrst}$$

where Y_{qrst} = Combine grain yield, hand harvested grain yield, hand harvested intermediate wheatgrass summer biomass yield, hand harvested legume biomass, hand harvested weed biomass, or the intermediate wheatgrass harvest index; μ = the overall mean; $P_{q(s)}$ = effect of intermediate wheatgrass populations planted nested within breeding program; A_r = effect of age of stand; Bp_s = effect of breeding program; Bl_t = Blocking effect; $Bp*A_{sr}$, = effect of the two way interaction and E_{qrst} = random residual. All effects and interactions were considered fixed. When significant, differences among treatments were further investigated with a Tukey test and considered significant at $p < 0.05$.

Results and Discussion

Table 2. P-values from the analysis of variance by experiment (2017-Alfalfa and 2018-Red clover) for the effect of block, population (16 and 15 different populations for experiment 1 and 2, respectively), age of stand, breeding program (from Canada, Kansas, Minnesota, and forage) and their respective interaction on combine grain yield, hand harvested (HH) grain yield, hand harvested intermediate wheatgrass (HH IWG) summer biomass, hand harvested legume summer biomass, hand harvested weed summer biomass, and intermediate wheatgrass harvest index. P-values with * significant at < 0.05 and ** significant at < 0.01.

	Experiment 1 (2017-Alfalfa)	**Experiment 2 (2018-Red clover)**				
	Combine grain	**HH grain**	**HH IWG**	**HH legume**	**HH weed**	**Harvest index**
Block	0.13	0.77	0.08	**<0.01****	**<0.01****	**0.02***
Breeding program	**<0.01****	0.13	0.15	0.06	0.38	0.17
Age	**<0.01****	**<0.01****	**<0.01****	0.09	**<0.01****	0.07
Population	**0.02***	0.6	0.14	0.28	0.22	0.1
Breeding program * age	**<0.01****	0.52	0.66	0.82	**0.05***	0.43
Number of years	3	2	2	2	2	2

Population

The hand harvested intermediate wheatgrass grain and biomass yield in the second experiment showed no differences between breeding programs in either the first or second year (Figure 4 & 5). The first and second year hand harvested grain yield had a mean value of 120 and 57 kg ha^{-1} respectively, while the first and second year hand harvested biomass yield had a mean value of 2198 and 1110 kg ha^{-1} respectively.

Breeding population had an effect on combine grain yield in the first year only (Table 2). The first year combine grain yield had differences between breeding populations unlike the second or third year (Table 3), “MN Cycle 4-LK1” the highest yielding population (928 kg ha^{-1}) was only significantly different from “MN 1502”, “MN 1504”, “MN 1505”, and both breeding populations from the Canada breeding program (Table 3). The two Canada breeding populations in the first experiment, harvested with combines did not produce any grain in the first year because the seeds were received late and planting was delayed till the spring, so the Canada breeding populations are confounded with planting date.

In the second experiment that was hand harvested, the “KS Cycle 4 - AK5” population had the highest intermediate wheatgrass biomass (3970 kg ha^{-1}) in the first year, however it was not significantly different from any of the other breeding populations apart from “KS Cycle 4 - AK2” which had the lowest intermediate wheatgrass biomass at 972 kg ha^{-1} (Table 4). Interestingly, “KS Cycle 4 - AK2” also had the highest harvest index value (0.13) in the first year, and it was significantly different from “CA Late lines”, “Kansas Cycle 3”, “MN 1502”, and “Rush forage” being the lowest with a value of 0.02 (Table 4). In the second year there were no differences between breeding populations on hand harvested grain yield, intermediate wheatgrass biomass, red clover biomass, weed biomass, or harvest index (Table 4).

Table 3. Populations of the 2017 seeding by age on combine grain yield. Within columns, means followed by the same letter are not significantly different according to Tukey (.05), means with no letter signify no differences between means.

	2018 (age 1)	2019 (age 2)	2020 (age 3)
Population	**Combine grain**		
		Kg ha $^{-1}$	
KS Cycle 4 - HK1	829 abc	263	360
KS Cycle 4 - AK1	780 abc	201	421
KS Cycle 4 - AK2	606 abcd	289	413
Kansas Cycle 3	911 ab	242	403
Kansas Cycle 4	585 abcd	262	429
Kansas Cycle 5	815 abc	298	440
MN Cycle 4	832 abc	283	429
MN Cycle 4 - LK1	928 a	255	412
MN Cycle 4 - PK1	743 abc	228	415
MN: 1501	530 abcd	242	380
MN: 1502	203 de	239	447
MN: 1503	536 abcd	277	366
MN: 1504	394 bcde	261	472
MN: 1505	339 cde	265	392
CA Early lines	0 e	283	408
CA Late lines	0 e	236	463

Table 4. Populations of the 2018 seeding by age on hand harvested grain yield, hand harvested intermediate wheatgrass summer biomass (HH IWG) , hand harvested legume summer biomass, hand harvested weed summer biomass, and intermediate wheatgrass harvest index. Within columns, means followed by the same letter are not significantly different according to Tukey (.05), means with no letter signify no differences between means.

	2019 (age 1)					2020 (age 2)				
Population	HH grain	HH IWG	HH Red clover	HH weed	Harvest index	HH grain	HH IWG	HH Red clover	HH weed	Harvest index
					kg ha^{-1}					
KS Cycle 4 - HK1	137	2480 ab	1000	486	0.06 ab	56	1310	1600	679	0.04
KS Cycle 4 - AK5	228	3970 a	1960	628	0.06 ab	79	1241	1800	60	0.06
KS Cycle 4 - AK2	115	972 b	1900	894	0.13 a	84	1454	1780	120	0.05
Kansas Cycle 3	91	2140 ab	1920	473	0.04 b	56	1560	1240	-	0.03
Kansas Cycle 4	165	2252 ab	1640	698	0.07 ab	60	1182	1940	195	0.03
Kansas Cycle 5	184	2363 ab	1530	770	0.07 ab	61	1578	2040	100	0.03
MN: 1501	161	1989 ab	1520	905	0.06 ab	15	605	2080	30	0.02
MN: 1502	150	3330 ab	1950	969	0.04 b	21	980	1300	-	0.03
MN: 1503	83	1840 ab	2100	822	0.05 ab	97	1380	2870	63	0.05
MN: 1504	55	1359 ab	1910	1496	0.04 ab	47	1155	2410	205	0.04
MN: 1505	88	1385 ab	1980	893	0.06 ab	52	688	2250	153	0.05
CA Early lines	150	2639 ab	1720	667	0.06 ab	90	1288	2440	17	0.06
CA Late lines	95	2280 ab	2150	1115	0.04 b	51	980	2280	124	0.05
Oahe forage	109	1670 ab	1560	975	0.06 ab	61	1420	1880	72	0.04
Rush forage	67	2818 ab	2230	1275	0.02 b	49	1021	2320	71	0.03

Breeding program

Breeding program showed a significant effect in experiment 1 (combine grain yield) only (Table 2) the combine grain yield of all three breeding programs were different from each other. Combine grain yield in the first year revealed the Kansas breeding program produced the highest yields on average reaching 749 kg ha^{-1}, the Minnesota breeding program came next with 567 kg ha^{-1} (Fig. 3). The Canada breeding program had no yield because the seeds were planted in the spring, and due to intermediate wheatgrass needing a vernalization period to flower (Ivancic et al., 2021) it did not produce any grain. It was able to vernalize the following winter and produced grain the following year.

Interestingly, the first combine grain harvest of the Canada program was not similar to the first combine grain harvest of the other programs and more similar to the other programs' second combine grain harvest.

Each breeding program's combine grain yields in the second and third year were not significantly different from each other, the second and third year had a mean value of 258 and 420 kg ha^{-1} respectively. Intermediate wheatgrass research, still being new, has numerous knowledge gaps, such as harvest management i.e. the best settings for combine harvest. Different settings and combines were utilized during this trial so differences between years cannot be directly compared.

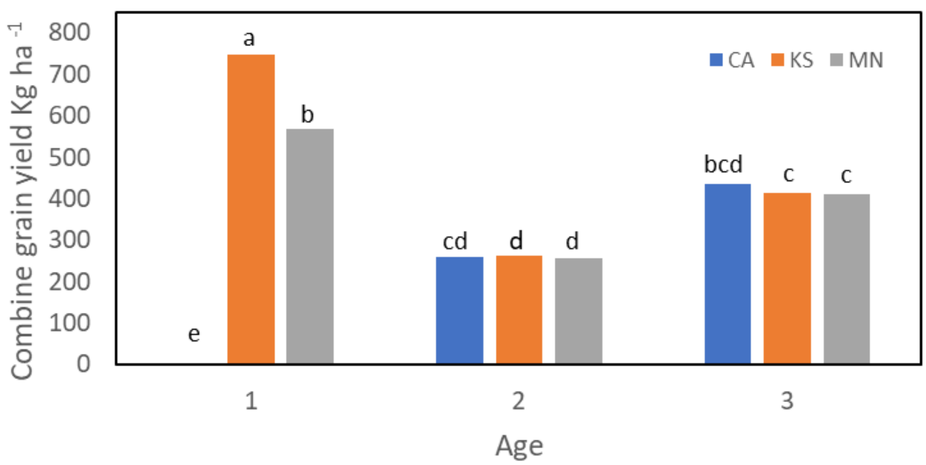


Figure 3. Kernza combine grain yield by age for the breeding programs in the experiment 1 (2017 seeding). If the letters above the bar are the same letter the bars are not significantly different according to Tukey (.05).

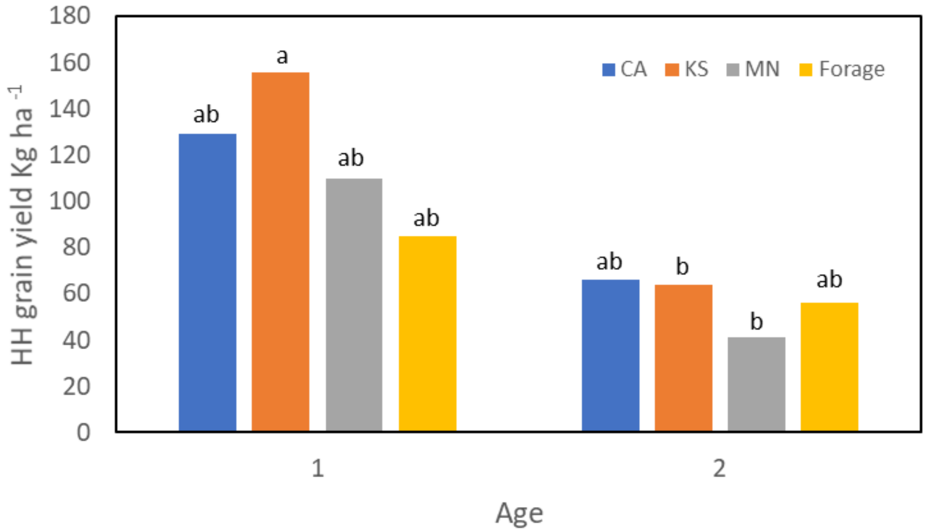


Figure 4. Hand harvested Kernza grain yield by age for the breeding programs in the experiment 2 (2018 seeding). If the letters above the bar are the same letter the bars are not significantly different according to Tukey (.05).

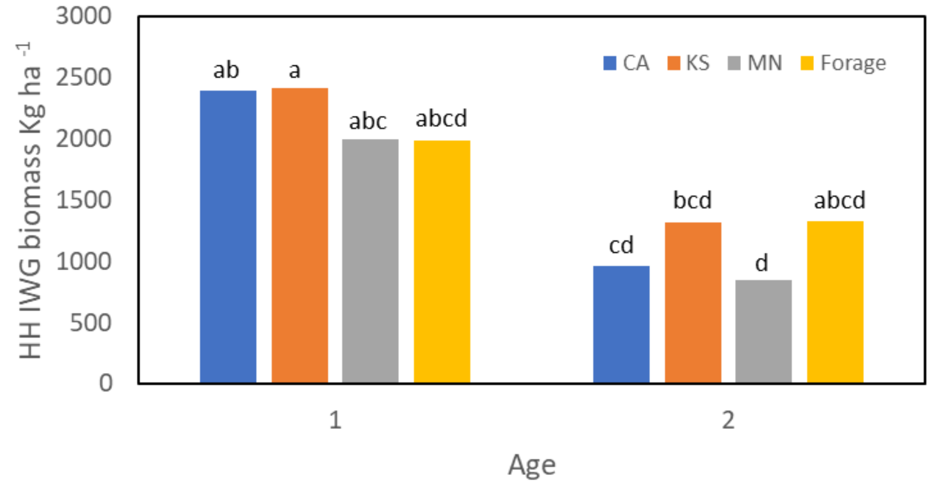


Figure 5. Hand harvested intermediate wheatgrass biomass yield by age for the breeding programs in the 2018 seeding. If the letters above the bar are the same letter the bars are not significantly different according to Tukey (.05).

The importance of harvest index in this trial is because breeders can utilize it as a selection criterion to improve yields, it provides data on the reproductive efficiency of crops (Asefa, 2019). A study by Unkovich et al., (2010) in Australia observed that small grains, such as wheat (*Triticum aestivum),* barley (*Hordeum vulgare*), oat (*Avena sativa*), and triticale (*Triticum durum* × *Secale cereale*) each had on average a harvest index of 0.37, 0.38, 0.21, and 0.34 respectively. The intermediate wheatgrass harvest index in the 2018 seeding showed no differences between breeding programs and had an average value of 0.05. Future research could look into the effect of Kernza-legume intercrops on harvest index.

Conclusion

Combine grain yield differences between breeding programs and populations occurred only in the first year, with the Land Institutes breeding program at Kansas developing breeding populations that currently yield the most in Wisconsin so far with a mean combine grain yield of 749 kg ha^{-1}. There was no effect of the breeding program on intermediate wheatgrass summer biomass, legume summer biomass, since most of the breeding efforts are being focused on grain. There was no effect of breeding population on legume biomass in the hand harvested second experiment. Combine grain yield differences occurred only in the first year with the highest yield at 928 kg ha^{-1} from “MN Cycle 4 - LK1”. Intermediate wheatgrass biomass in the first year of the hand harvested experiment 2 had its highest yield at 3970 kg ha^{-1} from “KS Cycle 4 - AK5”. Harvest index highest value by breeding population came at 0.13 from KS Cycle 4 - AK2” from the first year in experiment 2. Research is still ongoing, with additional data collected in 2021, long term yields of the different breeding populations can be discovered, and intermediate wheatgrass still has the potential room to improve its reproductive efficiency. Next steps for breeding efforts could focus on the improvement of intermediate wheatgrass-legume intercrop system yields.

References

Asefa, G. (2019). The role of harvest index in improving crop productivity: A review. *Journal of Natural Sciences Research*, *9*, https://doi.org/10.7176/JNSR/9-6-04

Bajgain, P., Zhang, X., Jungers, J. M., DeHaan, L. R., Heim, B., Sheaffer, C. C., Wyse, D. L., & Anderson, J. A. (2020). 'MN- Clear-water', the first food-grade intermediate wheatgrass (Kernza perennial grain) cultivar. *Journal of Plant Registrations*, *14*(3), 288–297. https://doi.org/10.1002/plr2.20042.

Cattani D.J. (2016). Selection of a perennial grain for seed productivity across years: intermediate wheatgrass as a test species. *Canadian Journal of Plant Science*, *97*(3), 516-524. https://doi.org/10.1139/cjps-2016-0280

Conley, S., Roth, A., Gaska, J., Mueller, B., & Smith, D. (2019). Wisconsin winter wheat performance trials 2019. University of Wisconsin-Madison.

Conley, S., Roth, A., Gaska, J., Mueller, B., & Smith, D. (2020). Wisconsin winter wheat performance trials 2020. University of Wisconsin-Madison.

Crain, J., DeHaan, L., & Poland, J. (2021). Genomic prediction enables rapid selection of high-performing genets in an intermediate wheatgrass breeding program. *The Plant Genome*, *14*(2), 1–17. https://doi.org/10.1002/tpg2.20080

DeHaan, L. R., Christians, M., Crain, J., & Poland, J. (2018). Development and evolution of an intermediate wheatgrass domestication program. *Sustainability*, *10*(5), https://doi.org/10.3390/su10051499

Dehaan, L. R., & Van Tassel, D. L. (2014). Useful insights from evolutionary biology for developing perennial grain crops. *American Journal of Botany*, *101*(10), 1801–1819. https://doi.org/10.3732/ajb.1400084

Forever Green. (n.d.) Intermediate Wheatgrass (Kernza®). https://www.forevergreen.umn.edu/crops-systems/perennial-grains-oilseeds-pulses/kernza

Gutiérrez, L., Sandro, P., Conley, S. (2019). 2019 Oat and barley performance tests. University of Wisconsin-Madison.

Gutiérrez, L., Sandro, P., Conley, S. (2020). 2020 Oat and barley performance tests. University of Wisconsin-Madison.

Hybner, R. M. (2012). Intermediate wheatgrass (*Thinopyrum intermedium* L.): An introduced conservation grass for use in Montana and Wyoming. *United States Department of Agriculture Natural Resources Conservation Service Plant Materials Technical Note No. MT-80.* https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/mtpmctn11288.pdf

Ivancic, K. A., Locatelli, A., Tracy, W, F., & Picasso. V, D. (2021). Kernza intermediate wheatgrass (*Thinopyrum intermedium*) response to a range of vernalization conditions. *Canadian Journal of Plant Science*. https://doi.org/10.1139/CJPS-2020-0251

MacKown, C.T., & Carver, B.F. (2005). Fall forage biomass and nitrogen composition of winter wheat populations selected from grain-only and dual-purpose environments. *Crop Science*, *45*(1), 322-328. https://doi.org/10.2135/cropsci2005.0322

National Weather Service, 2021 National weather service. Online weather data. National Weather Service Silver Spring, MD. https://www.ncdc.noaa.gov/cdo-web/

SAS Institute. 2021. The SAS system for windows. Release 9.4. Sas Inst., Cary, NC.

Sharifi, R.S., Sedghi M., & Gholipouri, A. (2009). Effect of population density on yield and yield attributes of maize hybrids. *Research Journal of Biological Sciences*, *4*(4), 375-379, https://medwelljournals.com/abstract/?doi=rjbsci.2009.375.379

Unkovich, M., Baldock, J., & Forbes, M. (2010). Variability in harvest index of grain crops and potential significance for carbon accounting: Examples from Australian agriculture. *Advances in Agronomy, 105,* 173–219. https://doi.org/10.1016/S0065-2113(10)05005-4

Web Soil Survey, 2021. USDA-Natural Resources Conservation Service. https://websoilsurvey.sc.egov.usda.gov/

Supplemental table 1 - Simple linear regression parameters, AIC (Akaike information criterion, smaller is best) and P-values for linear, quadratic, and third order equations for Kernza grain yield against accumulation of fall GDD until end of GDD accumulation (base temperature of 0°C) for Arlington and Lancaster, red clover planted in fall (R1) or spring (R2) for first and second years. Bolded models are best fit.

Age	Location	Clover	Equation	AIC	P-Value
1	Arlington	R1	$Y = -0.2x + 76.9$	62.9	0.37
1	Arlington	R1	$Y = 0.002x^2 - 1.9x - 223.8$	57.7	0.12
1	**Arlington**	**R1**	$\mathbf{Y = 0.000006x^3 - 0.01x^2 + 2.2x + 220.6}$	**39.8**	**0.01**
1	**Arlington**	**R2**	$\mathbf{Y = -0.5x + 27.8}$	**55.3**	**0.01**
1	Arlington	R2	$Y = 0.0002x^2 - 0.7x - 6.1$	57.2	0.08
1	Arlington	R2	$Y = -0.0000005x^3 + 0.001x^2 - 1.1x - 47.7$	59.1	0.26
1	Lancaster	R1	$Y = -0.1x + 44.7$	76.7	0.42
1	Lancaster	R1	$Y = 0.001x^2 - 1.4x - 171.3$	71.3	0.07
1	**Lancaster**	**R1**	$\mathbf{Y = 0.000005x^3 - 0.01x^2 + 1.4x + 99.9}$	**63.4**	**0.02**
1	Lancaster	R2	$Y = -0.3x + 0.9$	82.5	0.22
1	Lancaster	R2	$Y = 0.001x^2 - 1.3x - 179.1$	82.8	0.29
1	**Lancaster**	**R2**	$\mathbf{Y = 0.00001x^3 - 0.01x^2 + 4.5x + 390.1}$	**74.2**	**0.05**
2	Arlington	R1	$Y = -0.1x + 214.5$	64.1	0.65
2	Arlington	R1	$Y = 0.0005x^2 - 0.6x + 122.1$	65.8	0.84
2	Arlington	R1	$Y = 0.000004x^3 - 0.01x^2 + 2.3x + 441.7$	67	0.89
2	Arlington	R2	$Y = -0.1x + 78.1$	53.8	0.45
2	Arlington	R2	$Y = 0.0005x^2 - 0.6x - 24.8$	53	0.39
2	Arlington	R2	$Y = 0.000003x^3 - 0.005x^2 + 1.6x + 224.4$	48.3	0.25
2	Lancaster	R1	$Y = 0.3x + 511.5$	96.3	0.59
2	Lancaster	R1	$Y = -0.0008x^2 + 1.1x + 644.7$	98.1	0.83
2	Lancaster	R1	$Y = 0.00001x^3 - 0.01x^2 + 6.2x + 1147.7$	99.4	0.87
2	Lancaster	R2	$Y = 0.3x + 586.5$	89.2	0.39
2	Lancaster	R2	$Y = 0.00009x^2 + 0.2x + 572.2$	91.2	0.72
2	Lancaster	R2	$Y = -0.00001x^3 + 0.02x^2 - 5.9x - 21.5$	90.7	0.57

Supplemental table 2 - Simple linear regression parameters, AIC (Akaike information criterion, smaller is best) and P-values for linear, quadratic, and third order equations for intermediate wheatgrass summer biomass yield against accumulation of fall GDD until end of GDD accumulation (base temperature of 0°C) for Arlington and Lancaster, red clover planted in fall (R1) or spring (R2) for first and second years. Bolded models are best fit.

Age	Location	Clover	Equation	AIC	P-Value
1	Arlington	R1	$Y = -5.7x + 218.4$	107.3	0.06
1	Arlington	R1	$Y = 0.01x^2 - 19.4x - 1785.9$	104.5	0.05
1	Arlington	R1	$Y = 0.00005x^3 - 0.06x^2 + 11.7x + 734.6$	100.9	0.05
1	**Arlington**	**R2**	**$Y = -6.9x + 104.2$**	**102.8**	**< 0.01**
1	Arlington	R2	$Y = 0.006x^2 - 13.2x - 811.5$	103.3	**0.03**
1	Arlington	R2	$Y = 0.000007x^3 - 0.005x^2 - 8.8x - 452.8$	105.2	0.12
1	Lancaster	R1	$Y = -2.4x + 1167.6$	121.5	0.36
1	Lancaster	R1	$Y = 0.007x^2 - 9.6x - 58.9$	123	0.57
1	Lancaster	R1	$Y = 0.00006x^3 - 0.1x^2 + 26.7x + 3485.5$	123.3	0.59
1	**Lancaster**	**R2**	**$Y = -6.6x - 550.9$**	**117.8**	**0.01**
1	Lancaster	R2	$Y = 0.002x^2 - 8.4x - 847.5$	119.8	0.06
1	Lancaster	R2	$Y = 0.00007x^3 - 0.1x^2 + 33.5x + 3248.3$	118.1	0.07
2	Arlington	R1	$Y = -3.0x + 1384$	108.3	0.27
2	Arlington	R1	$Y = 0.01x^2 - 10.9x + 235.9$	109.3	0.44
2	Arlington	R1	$Y = 0.000002x^3 + 0.005x^2 - 9.8x + 320.4$	111.3	0.7
2	Arlington	R2	$Y = -3.3x + 209.7$	100.1	0.06
2	Arlington	R2	$Y = 0.003x^2 - 6.6x - 261.2$	101.6	0.18
2	Arlington	R2	$Y = 0.00004x^3 - 0.05x^2 + 16.5x + 1612.6$	99.1	0.17
2	Lancaster	R1	$Y = 0.2x + 6165.4$	130.6	0.97
2	Lancaster	R1	$Y = 0.01x^2 + 10.5x + 7938.8$	132.2	0.88
2	Lancaster	R1	$Y = 0.0002x^3 - 0.3x^2 + 112.8x + 17938$	129.1	0.39
2	Lancaster	R2	$Y = 1.7x + 5072.9$	122.5	0.54
2	Lancaster	R2	$Y = 0.001x^2 + 0.4x + 4855.9$	124.5	0.84
2	Lancaster	R2	$Y = -0.000001x^3 + 0.003x^2 - 0.4x + 4776.5$	126.5	0.96

Supplemental table 3 - Simple linear regression parameters, AIC (Akaike information criterion, smaller is best) and P-values for linear, quadratic, and third order equations for IWG fall biomass against accumulation of fall GDD until end of GDD accumulation (base temperature of 0°C) for Arlington and Lancaster, red clover planted in fall (R1) or spring (R2) for first and second years. Bolded models are best fit.

Age	Location	Clover	Equation	AIC	P-Value
1	Arlington	R1	$Y = -0.7x + 208.7$	76.1	0.34
1	Arlington	R1	$Y = 0.003x^2 - 4x - 395.2$	75.8	0.39
1	Arlington	R1	$Y = 0.00002x^3 - 0.03x^2 + 8.9x + 1008.1$	74	0.39
1	Arlington	R2	$Y = -0.7x + 199.4$	73.1	0.27
1	Arlington	R2	$Y = 0.001x^2 - 2.7x - 170.9$	73.8	0.44
1	Arlington	R2	$Y = 0.00002x^3 - 0.02x^2 + 8.4x + 1036.6$	71.7	0.41
1	Lancaster	R1	$Y = -0.5x + 841.3$	112.1	0.73
1	Lancaster	R1	$Y = 0.001x^2 - 1.9x + 592.3$	114	0.93
1	Lancaster	R1	$Y = 0.00002x^3 - 0.03x^2 + 10.9x + 1848.8$	115.4	0.93
1	Lancaster	R2	$Y = -0.7x + 572.7$	111.5	0.6
1	Lancaster	R2	$Y = 0.002x^2 - 2.5x + 266.1$	113.3	0.85
1	Lancaster	R2	$Y = 0.00003x^3 - 0.04x^2 + 14.5x + 1925.6$	114.2	0.82

Supplemental table 4 - Simple linear regression parameters, AIC (Akaike information criterion, smaller is best) and P-values for linear, quadratic, and third order equations for total IWG biomass (summer+fall) against accumulation of fall GDD until end of GDD accumulation (base temperature of 0°C) for Arlington and Lancaster, red clover planted in fall (R1) or spring (R2) for first and second years. Bolded models are best fit.

Age	Location	Clover	Equation	AIC	P-Value
1	Arlington	R1	$Y = -6.6x + 355$	110.2	0.07
1	Arlington	R1	$Y = 0.02x^2 - 23.2x - 2065.7$	107.7	0.06
1	Arlington	R1	$Y = 0.00006x^3 - 0.08x^2 + 14.7x + 1005$	104.5	0.07
1	**Arlington**	**R2**	$\mathbf{Y = -7.8x + 237.5}$	**105.4**	**0.01**
1	Arlington	R2	$Y = 0.008x^2 - 16x - 947.2$	105.6	0.04
1	Arlington	R2	$Y = 0.00001x^3 -0.01x^2 - 7.1x - 232.5$	107.3	0.13
1	Lancaster	R1	$Y = -1.9x + 2374.8$	127.6	0.6
1	Lancaster	R1	$Y = 0.01x^2 - 14.5x + 229.4$	128.7	0.68
1	Lancaster	R1	$Y = 0.0001x^3 - 0.1x^2 + 41.6x + 5712.8$	128.8	0.62
1	Lancaster	R2	$Y = -8.4x - 354.5$	127.4	0.05
1	Lancaster	R2	$Y = 0.007x^2 - 15.6x -1582.3$	129.1	0.16
1	Lancaster	R2	$Y = 0.0001x^3 - 0.2x^2 + 55.1x + 5328.9$	127.9	0.18

Supplemental table 5 - Simple linear regression parameters, AIC (Akaike information criterion, smaller is best) and P-values for linear, quadratic, and third order equations for red clover summer biomass against accumulation of fall GDD until end of GDD accumulation (base temperature of 0°C) for Arlington and Lancaster, red clover planted in fall (R1) or spring (R2) for first and second years. Bolded models are best fit.

Age	Location	Clover	Equation	AIC	P-Value
1	Arlington	R1	$Y = 0.7x + 1165$	96.5	0.54
1	Arlington	R1	$Y = -0.004x^2 + 4.3x + 1690.5$	97.3	0.6
1	Arlington	R1	$Y = -0.000005x^3 + 0.004x^2 + 1.4x + 1453.3$	99.2	0.82
1	Arlington	R2	$Y = 1.8x + 1472.9$	96.2	0.15
1	Arlington	R2	$Y = -0.002x^2 + 4.0x + 1799.9$	97.7	0.35
1	Arlington	R2	$Y = 0.00001x^3 - 0.02x^2 + 11.3x + 2394.2$	99.2	0.56
1	Lancaster	R1	$Y = 0.1x + 54.1$	65.7	0.49
1	Lancaster	R1	$Y = 0.0002x^2 - 0.1x + 23.1$	67.3	0.71
1	Lancaster	R1	$Y = -0.000002x^3 + 0.003x^2 - 1.2x - 82.7$	67.7	0.69
1	**Lancaster**	**R2**	**$Y = 0.9x + 798.1$**	**86.9**	**0.02**
1	Lancaster	R2	$Y = -0.001x^2 + 2.1x + 1008.1$	87.6	0.05
1	Lancaster	R2	$Y = 0.000006x^3 - 0.01x^2 + 5.6x + 1346$	88.3	0.12
2	Arlington	R1	$Y = 0.6x + 1956.8$	104.9	0.77
2	Arlington	R1	$Y = -0.01x^2 + 8.1x + 3055.5$	105.2	0.6
2	Arlington	R1	$Y = 0.000003x^3 - 0.01x^2 + 10x + 3211.9$	107.2	0.83
2	Arlington	R2	$Y = 1.0x + 3939.9$	99.6	0.47
2	Arlington	R2	$Y = 0.002x^2 - 1.3x + 3597.7$	101.3	0.73
2	Arlington	R2	$Y = -0.00003x^3 + 0.1x^2 - 20.5x + 2041$	100.5	0.59
2	Lancaster	R1	$Y = -1.0x + 34.5$	94.5	0.06
2	Lancaster	R1	$Y = -0.0004x^2 - 0.6x + 98.2$	96.5	0.21
2	Lancaster	R1	$Y = 0.00002x^3 - 0.03x^2 + 12.5x + 1385.8$	89.6	0.05
2	Lancaster	R2	$Y = 2.3x + 3072.1$	118.7	0.29
2	Lancaster	R2	$Y = -0.003x^2 + 5.2x + 3559.3$	120.6	0.58
2	Lancaster	R2	$Y = 0.00004x^3 - 0.05x^2 + 25.3x + 5528$	121.9	0.72

Supplemental table 6 - Simple linear regression parameters, AIC (Akaike information criterion, smaller is best) and P-values for linear, quadratic, and third order equations for overall biomass (IWG+RC) against accumulation of fall GDD until end of GDD accumulation (base temperature of 0°C) for Arlington and Lancaster, red clover planted in fall (R1) or spring (R2) for first and second years. Bolded models are best fit.

Age	Location	Clover	Equation	AIC	P-Value
1	**Arlington**	**R1**	**$Y = -5.3x + 1408$**	**104.9**	**0.04**
1	Arlington	R1	$Y = 0.01x^2 - 15x - 11.4$	103.8	0.07
1	Arlington	R1	$Y = 0.00005x^3 - 0.06x^2 + 13.5x + 2294.3$	100.8	0.07
1	**Arlington**	**R2**	**$Y = -5.2x + 1887.8$**	**100.4**	**0.01**
1	Arlington	R2	$Y = 0.003x^2 - 8.3x + 1427.6$	101.9	0.06
1	Arlington	R2	$Y = 0.00001x^3 -0.02x^2 + 0.3x + 2128.3$	103.5	0.18
1	Lancaster	R1	$Y = -2.4x + 1525.9$	121.3	0.35
1	Lancaster	R1	$Y = 0.01x^2 - 10.3x + 182.4$	122.6	0.54
1	Lancaster	R1	$Y = 0.00006x^3 - 0.07x^2 + 21x + 3236$	123.4	0.62
1	Lancaster	R2	$Y = -4.4x + 1349.7$	118.4	0.06
1	Lancaster	R2	$Y = 0.001x^2 - 5.3x + 1203.1$	120.4	0.21
1	Lancaster	R2	$Y = 0.00008x^3 - 0.1x^2 + 41.6x + 5792$	117.9	0.16
2	**Arlington**	**R1**	**$Y = -2.9x + 3484.3$**	**93.9**	**0.02**
2	Arlington	R1	$Y = 0.0004x^2 - 3.3x + 3428.7$	95.9	0.1
2	Arlington	R1	$Y = 0.000008x^3 - 0.01x^2 + 1.4x + 3806.9$	97.6	0.25
2	Arlington	R2	$Y = -3.4x + 4466.8$	102.1	0.09
2	Arlington	R2	$Y = 0.004x^2 - 7.6x + 3841.1$	103.4	0.22
2	Arlington	R2	$Y = -0.000003x^3 + 0.01x^2 - 9.3x + 3706.7$	105.4	0.47
2	Lancaster	R1	$Y = -0.8x + 7325.8$	131.1	0.85
2	Lancaster	R1	$Y = -0.02x^2 + 22x + 11228$	131.1	0.54
2	Lancaster	R1	$Y = 0.0002x^3 - 0.3x^2 + 134.1x + 22185$	124.9	0.13
2	Lancaster	R2	$Y = 3.4x + 8892$	115.5	0.08
2	Lancaster	R2	$Y = 0.005x^2 - 1.2x +8114.4$	117	0.22
2	Lancaster	R2	$Y = -0.000003x^3 + 0.01x^2 - 2.7x + 7961.8$	119	0.45

Supplemental table 7 - Simple linear regression parameters, AIC (Akaike information criterion, smaller is best) and P-values for linear, quadratic, and third order equations for weed summer biomass against accumulation of fall GDD until end of GDD accumulation (base temperature of 0°C) for Arlington and Lancaster, red clover planted in fall (R1) or spring (R2) for first and second years. Bolded models are best fit.

Age	Location	Clover	Equation	AIC	P-Value
1	Arlington	R1	$Y = 1.6x + 2594.7$	114.2	0.7
1	Arlington	R1	$Y = -0.01x^2 + 13.9x + 4393.5$	115.1	0.68
1	Arlington	R1	$Y = -0.00006x^3 + 0.1x^2 - 24.8x + 1254.8$	115.6	0.71
1	Arlington	R2	$Y = 3.6x + 3205.9$	105.4	0.13
1	Arlington	R2	$Y = -0.004x^2 + 7.1x + 3718.4$	107.1	0.34
1	Arlington	R2	$Y = -0.00002x^3 + 0.02x^2 - 2.2x + 2962.1$	108.9	0.58
1	**Lancaster**	**R1**	**$Y = 13.8x + 12511$**	**130**	**0.01**
1	Lancaster	R1	$Y = -0.01x^2 + 22.3x + 13974$	131.4	0.06
1	Lancaster	R1	$Y = -0.00006x^3 + 0.1x^2 - 12.5x + 10568$	132.9	0.15
1	**Lancaster**	**R2**	**$Y = 6.1x + 6366.5$**	**120.3**	**0.03**
1	Lancaster	R2	$Y = 0.0003x^2 + 5.8x + 6322$	122.3	0.13
1	Lancaster	R2	$Y = 0.00003x^3 - 0.04x^2 + 21x + 7802.4$	124	0.29
2	Arlington	R1	$Y = 0.4x + 597.6$	81.5	0.31
2	Arlington	R1	$Y = -0.001x^2 + 1.5x + 751.7$	82.6	0.49
2	Arlington	R1	$Y = -0.000006x^3 + 0.008x^2 - 2.3x + 449.7$	83.2	0.59
2	Arlington	R2	$Y = 0.4x + 503.5$	67.6	0.24
2	Arlington	R2	$Y = 0.001x^2 - 0.5x + 378.9$	68.8	0.45
2	Arlington	R2	$Y = -0.000006x^3 + 0.01x^2 - 4.4x + 64.7$	68.3	0.52
2	Lancaster	R1	$Y = -0.1x +715.4$	82.7	0.93
2	Lancaster	R1	$Y = 0.003x^2 - 3.6x + 144.5$	84.1	0.87
2	Lancaster	R1	$Y = -0.00007x^3 + 0.1x^2 - 37.2x - 2879.8$	82.1	0.62
2	Lancaster	R2	$Y = -1.3x -15.4$	85.3	0.08
2	Lancaster	R2	$Y = 0.00003x^2 - 1.4x - 20.3$	87.3	0.26
2	Lancaster	R2	$Y = 0.00002x^3 - 0.02x^2 + 7.4x + 836.4$	87.5	0.36