Title: Identifying optimal alfalfa germplasm types and characteristics for compatibility and performance in mixed cropping systems

PI Brandon Schlautman
Lead Scientist, Perennial Legumes
The Land Institute
2440 E. Water Well Rd.
Salina, KS 67456
schlautman@landinstitute.org, 785-823-5376

Co-PIs Craig Sheaffer
Professor, Agronomy Dept.
University of Minnesota
411 Borlaug hall, 1991 Upper Buford Circle, St. Paul, MN 55108
sheaf001@umn.edu, 612-625-7224

Tim Crews
Director of Research, Biogeochemist
The Land Institute
2440 E. Water Well Rd.
Salina, KS 67456.
crews@landinstitute.org, 785-823-5376

Valentin Picasso
Assistant Professor, Agronomy Dept., University of Wisconsin-Madison
Associate Professor, Universidad de la Rep República, Uruguay
455 Moore Hall, 1575 Linden Dr.
Madison, WI, 68501
picassorisso@wisc.edu, 608 422 961

Abstract

Growing alfalfa between rows of annual or perennial grain crops in mixed cropping systems that produce both grains for human consumption and forage for livestock is a promising alternative use for alfalfa. Besides providing saleable forage for livestock, alfalfa also provides numerous ecological services to the agroecosystem - nitrogen fixation, continuous vegetative cover, and improved soil organic nitrogen and carbon cycling. In this project, we evaluate the potential to develop intermediate wheatgrass (IWG) + alfalfa mixtures for the production of Kernza® perennial grain and improved spring, summer, and fall forage yield and quality, with a specific interest in exploring whether underutilized alfalfa germplasm types (e.g., falcata alfalfa, grazing sativa types, and spreading sativa types) might prove well-adapted to the system. Our initial data

from a single year suggests that including alfalfa in mixtures with IWG increases CP in summer residue following Kernza grain harvest and can increase the RFV and gross value of fall hay/forage compared to monoculture IWG systems. Including alfalfa did not significantly decrease Kernza grain yields in any location. Additional years of data collection will be needed to determine long-term yield potential of IWG+alfalfa systems and to identify optimal germplasm types for IWG+alfalfa mixtures.

Introduction

New perennial grain crops are being domesticated in recognition of the ecosystem benefits provided by perennial crop plants, like alfalfa (Randall et al., 1997; DeHaan et al., 2018). Kernza®, the perennial grain from domesticated intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey), is the first of such perennial grains to enter the commercial marketplace with active interest and investment from US farmers, bakeries, and food companies like Patagonia Provisions and General Mills. However, Kernza grain yields remain below those of wheat and conventional cereal grains (Bajgain et al., 2019), and research and innovative agronomic strategies are needed to ensure that farmers can grow Kernza profitably at scale while bringing its environmental benefits to consumers, farmers, and agricultural landscapes.

One possible way to achieve greater ecological intensification and profitability in Kernza perennial grain production is to grow it in mixtures with alfalfa. Like other perennial grass seed production, IWG biomass must be removed in the summer after grain harvest to ensure good yields in subsequent years (Jungers et al., 2017; Pugliese et al., 2019). While leaf and stem biomass remaining after IWG grain harvest can exceed 10 Mg ha^{-1}, the low quality of the straw (crude protein (CP) < 6% and relative feed value (RFV) < 70) limits its utility and value as forage (Favre et al., 2019; Hunter et al., 2020). Planting IWG in mixtures with alfalfa (*Medicago sativa* L.) could improve the forage quality of IWG straw after grain harvest and create additional high-value grazing opportunities in the spring and fall. In addition to improving forage quality, IWG-alfalfa crop mixtures might also help maintain stable IWG grain yields over multiple years and require less synthetic nitrogen (N) inputs (Tautges et al., 2018).

The success of alfalfa+IWG mixtures depends on maximizing alfalfa's compatibility with IWG to optimize the benefits of biological nitrogen fixation (BNF) and increased forage quality without decreasing forage and Kernza grain yields. Ongoing research by Grabber (2016) has observed substantial variation in compatibility between alfalfa lines and corn when grown in mixtures for silage production during the alfalfa establishment year. This research posits that similar variation in compatibility exists for alfalfa in mixtures with IWG and other grains. Some underutilized alfalfa germplasm types (e.g., falcata alfalfa, grazing sativa types, and spreading sativa types) might prove well-adapted to the system. Our research aimed to compare grain yields, forage yields, and forage quality of alfalfa+IWG mixtures planted with multiple alfalfa varieties at multiple locations and managed as a dual-purpose forage and grain production system. Additional control plots of IWG monocultures with and without nitrogen fertilization and IWG mixtures were included for comparison purposes.

Materials and Methods

This research was conducted at four locations in Kansas (1 site), Wisconsin (2 sites), and Minnesota (1 site) during 2019. Each site was planted in the fall of 2017 and included up to 30 treatments replicated four times in a randomized complete block design. Plots were 3.7 m long by 3.7 m wide with six IWG rows planted 60 cm apart. The treatments included wide-spaced (60 cm rows) monoculture IWG with (IWGw+) and without (IWGw) synthetic nitrogen fertilization; narrow-spaced (30 cm rows) IWG monocultures with (IWGn+) and without (IWGn) nitrogen fertilization; and alfalfa+IWG mixtures with IWG and alfalfa planted in alternating 30 cm rows (Figure 1). Each site tested 10 to 25 total diverse alfalfa varieties, including germplasm developed for grazing, haying, *Medicago sativa* subsp. *falcata* type alfalfa, hybrid alfalfa, and alfalfa bred for biofuels production. Six of the treatments - the four IWG monoculture treatments and two alfalfa varieties (i.e., HYB1 and GRZ1) – were used as control treatments in Objectives 1,2, and 5.

Figure 1. Spring forage in an intermediate wheatgrass + alfalfa plot in KS as part of this study. Intermediate wheatgrass and alfalfa are planted in alternating 30 cm rows.

Summer Kernza grain and IWG straw + alfalfa yields and quality

Kernza grain was harvested and weighed at physiological maturity from each plot in late July or early August depending on location using plot combines or quadrats. Total biomass yield of IWG straw or IWG straw + alfalfa was measured using plot forage harvesters and quadrats. The forage in the quadrats was separated into IWG straw biomass and alfalfa forage biomass, which were weighed separately. Subsamples of the IWG straw and alfalfa biomass were dried and sent to the University of Wisconsin, ground to pass through a 1mm screen, and scanned using NIRS to estimate forage quality. Wet chemistry (crude protein, NDF, and ADF) was performed with ten percent of the samples harvested from each site to improve the NIRs models.

Soil nutrient supply rates

Timely understanding of soil properties and nutrient mobility is hard to achieve through standard soil tests. This study measured soil nutrient supply rates in the control treatments during the final 30 days of grain development (anthesis through grain fill) using Western Ag Plant Root Simulator (PRS®) Probes, which measure cation and anion exchange in the soil. Measures of nitrogen (N), phosphorus (P), and potassium (P) supply rates were used to determine whether alfalfa biological nitrogen fixation or synthetic fertilizers could improve nutrient availability for Kernza grain production.

Spring, summer, and fall IWG + alfalfa forage yields, quality, and gross value in Kansas

Dual-purpose management of winter wheat (*Triticum aestivum* L.) is common during late fall, winter, and spring in Kansas and the High Plains (Lollato et al., 2017). Grazing has little effect on wheat yield if fertility is adequate and livestock are removed before stem elongation (Holman et al., 2009). Grazing or haying IWG or IWG + alfalfa forage in fall and early spring could increase net returns and extend the spring grazing season for grazers waiting to move livestock to warm-season native pasture. Though it is uncertain how spring or fall grazing or haying would impact Kernza grain yields, this study harvested IWG and IWG + biomass and measured forage quality from the Kansas location in spring and fall 2019. Subsequent analyses grouped alfalfa varieties by similar type to compare the performance of underutilized alfalfa germplasm types (e.g., grazing types and falcata types) with conventional and hybrid alfalfa germplasm.

The potential gross return of IWG and IWG+alfalfa spring and fall forage and summer straw was estimated for the control treatments in KS during 2019. We used a simple linear model previously reported by Hunter et al. (Hunter et al., (2020) to estimate the potential forage price of IWG and IWG + alfalfa biomass based on relative forage values calculated using NIRS data:

$$\text{Forage price } (\$ \text{ Mg}^{-1}) = 34.0+1.02\text{RFV}$$

Potential forage value ha^{-1} was calculated by multiplying the estimated forage price by the measured yield in each plot. This calculation fails to capture other quality parameters (e.g., crude protein), and straw is not normally sold based on RFV. Still, estimated straw prices were similar to 2018 straw auction prices, ensuring that prices were reasonable (Hunter et al., 2020). Ultimately these data provide an opportunity for preliminary comparisons of the relative economic value of harvested forage under the two cropping systems (i.e., IWG monoculture vs. IWG + alfalfa mixtures) used for Kernza grain production.

Project Objectives and Corresponding Results

Objective	Corresponding Result
1. Determine expected forage yields, forage quality, and Kernza grain yields of IWG wheatgrass + alfalfa mixtures vs. IWG monocultures.	Kernza perennial grain yields did not differ between monoculture IWG and alfalfa + IWG mixtures. Including alfalfa in IWG mixtures improved summer straw/forage yields in some but not all locations. Including alfalfa in IWG mixtures improved crude protein content of summer straw/forage.
2. Determine soil nutrient supply rates in IWG monocultures vs. IWG+alfalfa mixtures.	Differences in soil nutrient supply rates were not observed between the IWG and IWG+alfalfa mixtures.
3. Explore genetic variation among alfalfa varieties for forage yield and quality in IWG+alfalfa mixtures.	We did not observe significant genetic variance for IWG straw + alfalfa forage yield or alfalfa forage yield among diverse alfalfa varieties in the four test locations. We observed a small amount of genetic variance in alfalfa for Kernza grain yield and crude protein of IWG straw + alfalfa summer forage. We observed greater levels of genetic variance and genotype x environment variance in alfalfa CP, ADF, and NDF.
4. Explore the potential for underutilized alfalfa germplasm in IWG + alfalfa mixtures.	There were no differences in Kernza grain yield, summer IWG straw + alfalfa forage yield, or summer alfalfa forage yield between alfalfa germplasm types in mixtures with IWG. There were no differences between alfalfa germplasm types in mixtures with IWG for summer IWG straw + alfalfa forage crude protein or alfalfa crude protein.

5. Determine expected yield, quality, and gross value of forage available in the spring and fall in IWG monocultures vs. IWG + alfalfa mixtures in KS.	Alfalfa improved fall biomass yield and gross forage value in IWG+alfalfa mixtures compared to widely spaced and unfertilized monoculture IWG. IWG + alfalfa mixtures had higher relative forage values than IWG monocultures in the spring and fall, but did not improve total annual forage yield or total gross forage value.

Results and Discussion

OBJECTIVE 1. Determine and compare Kernza grain yields and summer forage (straw) yields and quality in IWG monocultures and IWG + alfalfa.

Kernza perennial grain yields did not differ between monoculture IWG and alfalfa + IWG mixtures. While there were differences in Kernza grain or spike yields across locations, there were no observed differences in Kernza grain yield between IWG monoculture and IWG + alfalfa mixtures within locations (Tables 1-4). Chemical nitrogen fertilizer only increased grain yields at the West Salem, WI location when compared to unfertilized monocultures (Table 2). This preliminary data from a single year of study suggests that planting IWG in mixtures with alfalfa may not decrease Kernza grain yields. However, a previous study observed reductions in Kernza grain yield in alfalfa + IWG mixtures compared to IWG monocultures when grown in locations where alfalfa growth is more favored (Tautges et al., 2018). We expect that properly pairing IWG with compatible alfalfa germplasm (e.g., appropriate fall dormancy and branching patterns) will be necessary.

Including alfalfa in IWG mixtures improved summer straw yields in some but not all locations. Summer straw/ forage yields were highest in Kansas, but no significant differences were observed among treatments at the KS location (Table 4). Similarly, no differences among treatments were observed at the Arlington, WI location (Table 3). Intermediate wheatgrass+alfalfa straw/forage yields were equivalent to IWGn, IWGn+, and IWGw+ and higher than the IWGw yield at the Minnesota location (Table 1). Narrow-spaced (30 cm row) IWG out yielded wide-spaced (60 cm) IWG, which has been previously observed in MN (Hunter et al., 2020) (Table 1). Summer straw and forage yields were only higher in alfalfa + IWG mixtures than narrow-spaced IWG monocultures at the West Salem, WI location (Table 2).

Including alfalfa in mixtures with IWG improved crude protein content of summer straw in all locations with available data. Crude protein (CP) only exceeded 7% of dry matter in IWG monoculture straw or IWG+alfalfa straw/forage after Kernza grain harvest for the IWG+HYB1 alfalfa treatment at the MN location (Table 1). Neither the summer IWG straw nor the IWG + alfalfa straw/forage from any treatment or location had sufficient CP concentrations to allow for its use as feed without some form of additional protein supplementation. However, including alfalfa in mixtures with IWG did increase CP in straw/forage compared to one or more IWG monoculture treatments in each of the MN, Arlington, WI, and KS locations (Tables 1, 3, & 4). At the KS location, IWG+HYB1 alfalfa had significantly higher CP concentrations than all four of the IWG monoculture treatments, and the IWG+GRZ1 alfalfa had higher CP concentrations than both unfertilized monoculture IWG treatments (Table 4). In Kansas, Kernza growers observed CP ranging from 8.1 to 8.9 percent of dry matter in bales of IWG+alfalfa straw/forage in 2019 and 2020 harvested from IWG planted in 76 cm rows with two rows of alfalfa between each pair of IWG rows (Kaufman, personal communication). Future studies should explore other strategies (e.g., increased alfalfa population sizes or inter-row alfalfa mowing early in the season) to increase the proportion of alfalfa biomass in the summer straw/forage or improve the quality of the alfalfa biomass to increase CP percentages of dry matter to palatable levels, while ensuring that Kernza grain yields are not compromised. No differences in neutral detergent fiber (aNDF) or acid detergent fiber (ADF) were observed among this study's treatments or locations. Overly mature IWG straw/alfalfa resulted in forage that was low in energy and of poor quality (ADF > 45 and aNDF > 60).

Table 1. Mean Kernza grain yields, straw/forage yields, crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (aNDF) observed in summer 2019 at Rosemount, MN for intermediate wheatgrass (IWG) monocultures planted in narrowly spaced 30 cm rows (IWG) with (IWGn+) and without (IWGn) nitrogen fertilization, IWG monocultures planted in widely spaced 60 cm rows with (IWGw+) and without (IWGw) nitrogen fertilization, and IWG+alfalfa mixtures planted with a hybrid (HYB1) or grazing type (GRZ1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

Treatment	Kernza yield (kg ha^{-1})	Straw/forage (kg ha^{-1})	CP	ADF	aNDF
GRZ1	151.2	2848.4 ab	4.1 a	49.6	73.6
HYB1	124.4	2771.3 ab	4.2 a	48.1	70.8
IWGn	85.7	3255.7 a	3 ab	47.2	72.3
IWGn+	86.9	2893.4 a	2.9 ab	47.1	71.3
IWGw	76.7	1119.7 c	2.4 b	48.1	73.3
IWGw+	114.0	1423.8 bc	2.7 b	48.1	72

Table 2. Mean Kernza grain yields and straw/forage yields observed in summer 2019 at West Salem, WI for intermediate wheatgrass (IWG) monocultures planted in narrowly spaced 30 cm rows (IWG) with (IWGn+) and without (IWGn) nitrogen fertilization and IWG+alfalfa mixtures planted with a hybrid (HYB1) or grazing type (GRZ1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

Treatment	**Kernza yield (kg ha^{-1})**	**Straw/forage (kg ha^{-1})**
GRZ1	291.2 ab	4572.9 a
HYB1	323.4 ab	4766.6 a
IWGn	124.2 b	1099.3 c
IWGn+	403.7 a	2978.0 b

Table 3. Mean Kernza grain yields, straw/forage yields, crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (aNDF) observed in summer 2019 at Arlington, WI for intermediate wheatgrass (IWG) monocultures planted in narrowly spaced 30 cm rows (IWG) with (IWGn+) and without (IWGn) nitrogen fertilization, IWG monocultures planted in widely spaced 60 cm rows with (IWGw+) and without (IWGw) nitrogen fertilization, and IWG+alfalfa mixtures planted with a hybrid (HYB1) or grazing type (GRZ1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

Treatment	**Kernza yield (kg ha^{-1})**	**Straw/forage (kg ha^{-1})**	**CP**	**ADF**	**aNDF**
GRZ1	1327.9	3524.6	6.2 a	45.3	63.7
HYB1	1082.0	3786.9	7.1 a	45.5	63.4
IWGn	721.337	2483.6	3.9 ab	42.2	62.7
IWGn+	1213.1	4098.4	2.5 b	44.5	66.2
IWGw	811.5	2237.7	4.8 ab	43.9	64.9
IWGw+	1426.2	3582.0	4.2 ab	45.2	66.1

Table 4. Mean Kernza grain yields, straw/forage yields, crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (aNDF) observed in summer 2019 at Salina, KS for intermediate wheatgrass (IWG) monocultures planted in narrowly spaced 30 cm rows (IWG) with (IWGn+) and without (IWGn) nitrogen fertilization, IWG monocultures planted in widely spaced 60 cm rows with (IWGw+) and without (IWGw) nitrogen fertilization, and IWG+alfalfa mixtures planted with a hybrid (HYB1) or grazing type (GRZ1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

Treatment	**Kernza yield (kg ha^{-1})**	**Straw/forage (kg ha^{-1})**	**CP**	**ADF**
GRZ1	475.1	7567.7	5.4 ab	45.1
HYB1	454.7	7203.0	6.0 a	46.7
IWGn	653.3	8927.0	3.1 bc	47.6
IWGn+	873.5	10264.8	2.4 c	48.0
IWGw	793.0	9089.3	2.6 bc	47.0
IWGw+	515.2	6813.1	2.5 c	48.3

OBJECTIVE 2. Explore changes in soil nutrient supply rates in IWG monocultures vs. IWG + alfalfa mixtures.

Differences in soil nutrient supply rates were not observed between the IWG and IWG+alfalfa mixtures. Although we observed differences in soil potassium (K) supply rates between the Kansas and Arlington, WI locations, we did not observe significant differences in nitrogen (N), phosphorus (P), or K supply rates between treatments within locations (Figure 2). The Western Ag Plant Root Simulator (PRS®) Probes used in this study measure cation and anion exchange in the soil. The PRS probes mimic the processes by which roots adsorb nutrients from the soil solution, and like roots, soil moisture has a large effect on nutrient availability. Variation in soil water content within and among plots could have reduced our ability to detect differences between treatments. Just as a root needs moisture to absorb ions, these films also need consistent soil contact through a water film. We utilized the PRS probes during the Kernza grain fill period in July, which is typically one of the warmest (and in the case of KS) driest periods of the year. This was also only a single year of data in the second year of production; previous research has demonstrated that measurable differences in N-mineralization and transfer in grass-legume mixtures are more easily detected later in the rotation (3+ years) (Louarn et al., 2015).

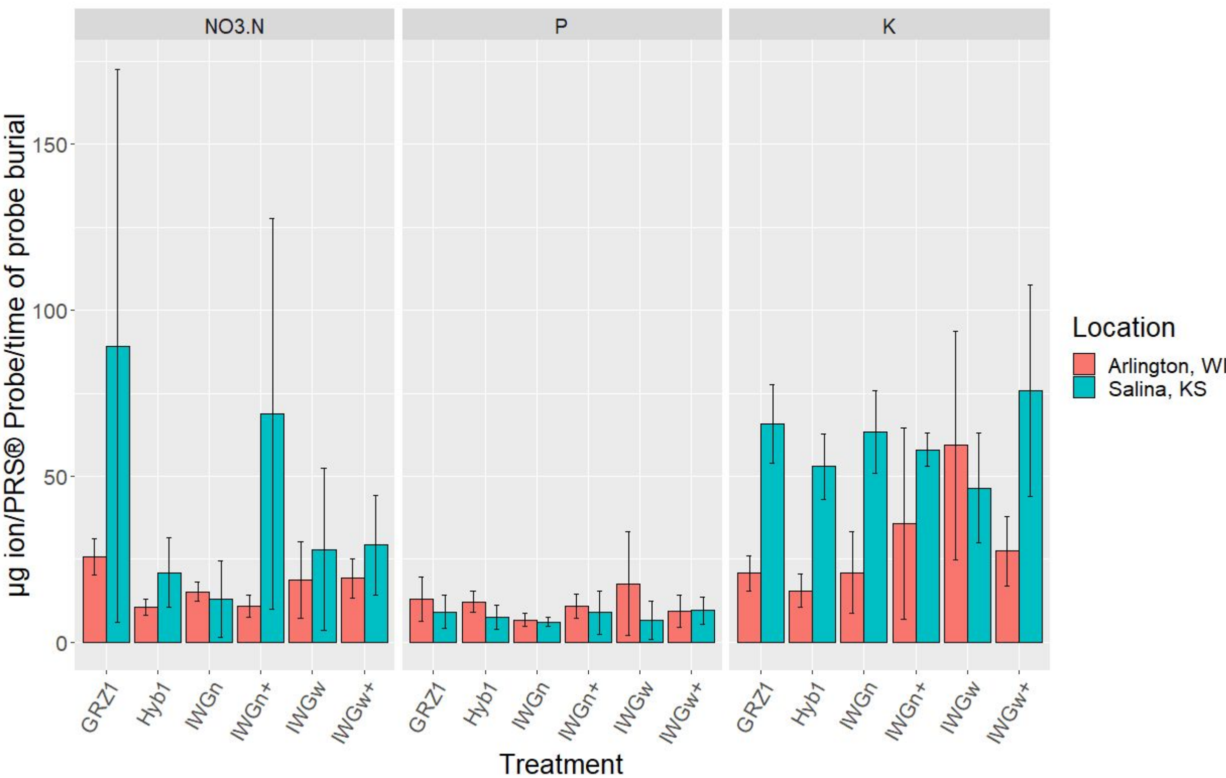

Figure 2. Mean NO3 nitrogen (N), phosphorus (P), and potassium (K) soil nutrient supply rate measured as the rate of nutrient ion adsorption by Plant Root Simulator (PRS®) Probes during Kernza grain fill ® at the Arlington, WI and Salina, KS locations. Bars indicate standard deviations. Treatments included monoculture intermediate wheatgrass spaced in 30 cm rows with (IWGn+) and without (IWGn) spring urea fertilizer application; monoculture intermediate wheatgrass spaced in 60 cm rows with (IWGw+) and without (IWGw) urea fertilizer application; IWG planted in alternating, 30 cm rows with a grazing type alfalfa variety (GRZ1); and IWG planted in alternating, 30 cm rows with a quick recovering hybrid type variety (HYB1).

<u>OBJECTIVE 3</u>. Explore genetic variation among alfalfa varieties for forage yield and quality in IWG + alfalfa mixtures.

We did not observe significant genetic variance for IWG straw + alfalfa forage yield or alfalfa forage yield among diverse alfalfa varieties in the four test locations. Initial screens in 2019 of a panel of 10 to 25 alfalfa varieties planted in mixtures with IWG in Salina, KS, Rosemount, MN, Arlington, WI, and West Salem, WI did not observe significant variation for summer IWG straw + alfalfa forage yield following Kernza grain harvest; environmental and error variance made up most of the variance for each measured characteristic (Figure 3). This may be primarily explained by the competitiveness of IWG in some locations, which made up a high proportion (>75%) of the total summer biomass; however, significant genetic variance was not observed for alfalfa summer forage yield within or among the locations either (Figure 3). Competition between alfalfa and IWG for resources within IWG + alfalfa mixtures, and lack of observed genetic variance for yield, suggests that additional follow-up study is needed to improve strategies for screening alfalfa germplasm in these systems that reduce error variance (Asay et al., 1999) or that focus on other aspects of the cropping system, besides improving summer straw yield or quality, where alfalfa can contribute additional ecological or economic benefits and where genetic variance in alfalfa may more likely be observed.

We observed a small amount of genetic variance in alfalfa for Kernza grain yield and crude protein of IWG straw + alfalfa summer forage. We observed greater levels of genetic variance and genotype x environment variance in alfalfa CP, ADF, and NDF. We observed small but significant amounts of genetic and genotype x environment variance for forage nutritive characteristics of alfalfa summer forage (Figure 3). There was also slight genetic variance for CP of the IWG straw + alfalfa forage due to differential contributions of improved CP by alfalfa varieties in IWG + alfalfa mixtures (Figure 3). These results suggest that gains in improving alfalfa nutritive in the near-term within the system could be made, especially on a location-by-location basis, by choosing an appropriate adapted variety that retains forage nutritive quality later during the season. Long-term, because alfalfa has set mature seed, is senescing leaves, and is generally low in nutritive quality by the time Kernza grain is harvested from the IWG, agronomic strategies that mow or harvest alfalfa between rows of IWG earlier in the season so that higher quality regrowth is present at the time of IWG straw harvest might provide additional gains in nutritive quality. Such practices may require wider IWG row-spacing, allowing for higher alfalfa densities, higher alfalfa yields per hectare, and increased nutritive quality of the IWG straw + alfalfa forage. From a long-term breeding perspective, strategies to develop or incorporate the *STAY-GREEN* green gene and low-lignin traits into alfalfa germplasm may prolong alfalfa forage nutritive quality in alfalfa+row-crop systems when alfalfa cannot be harvested within peak forage quality windows (Zhou et al., 2011).

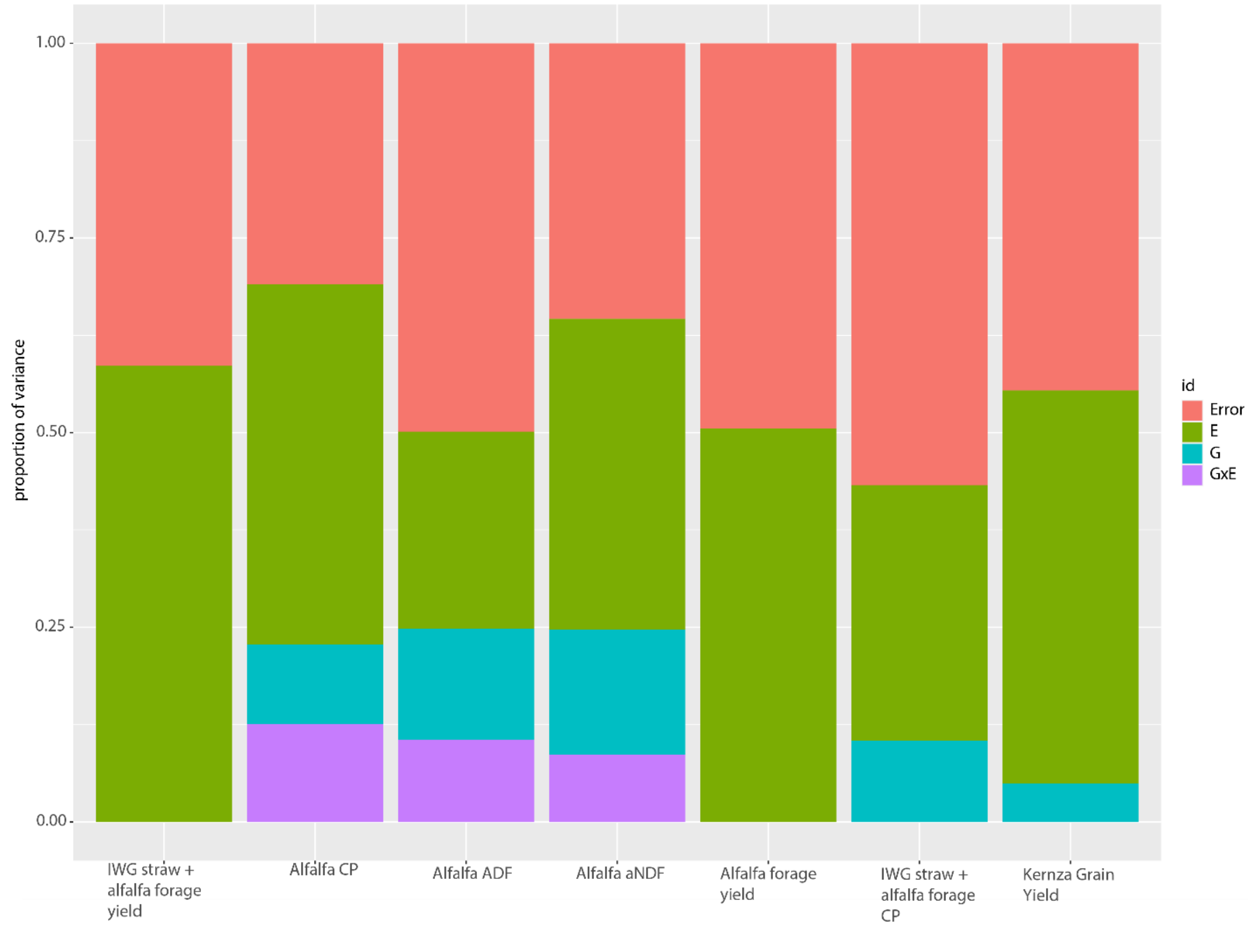

Figure 3. Proportions of genotype (G), environment (E), genotype x environment (GxE), and error variance in a panel of 10 to 25 alfalfa varieties for IWG straw + forage yields, IWG straw + alfalfa forage crude protein (CP), Kernza grain yield, alfalfa forage yield, and CP, ADF, and aNDF of the alfalfa forage following summer Kernza grain yields in four locations: Salina, KS, Rosemount, MN, Arlington, WI, and West Salem, WI.

<u>OBJECTIVE 4</u>. Explore the potential for underutilized alfalfa germplasm in IWG+alfalfa mixtures.

There were no differences in Kernza grain yield, summer IWG straw + alfalfa forage yield, or summer alfalfa forage yield between alfalfa germplasm types in mixtures with IWG. Mean Kernza perennial grain yields and summer IWG straw + alfalfa forage yields did not differ between alfalfa germplasm types in mixtures with IWG during summer 2019 in KS (Figure 4). The alfalfa made up a small proportion (~20%) of the overall summer IWG straw + alfalfa forage biomass, and no significant differences were observed in alfalfa summer forage yield, which varied widely within the alfalfa germplasm types (Figure 4). These observations highlight the competitiveness of intermediate wheatgrass with alfalfa in KS, especially in the summer, and suggests that improvements in the IWG + alfalfa cropping system should focus on IWG genetic improvement or focus on improving alfalfa yield and nutritive value at other times during the season (e.g., spring or fall).

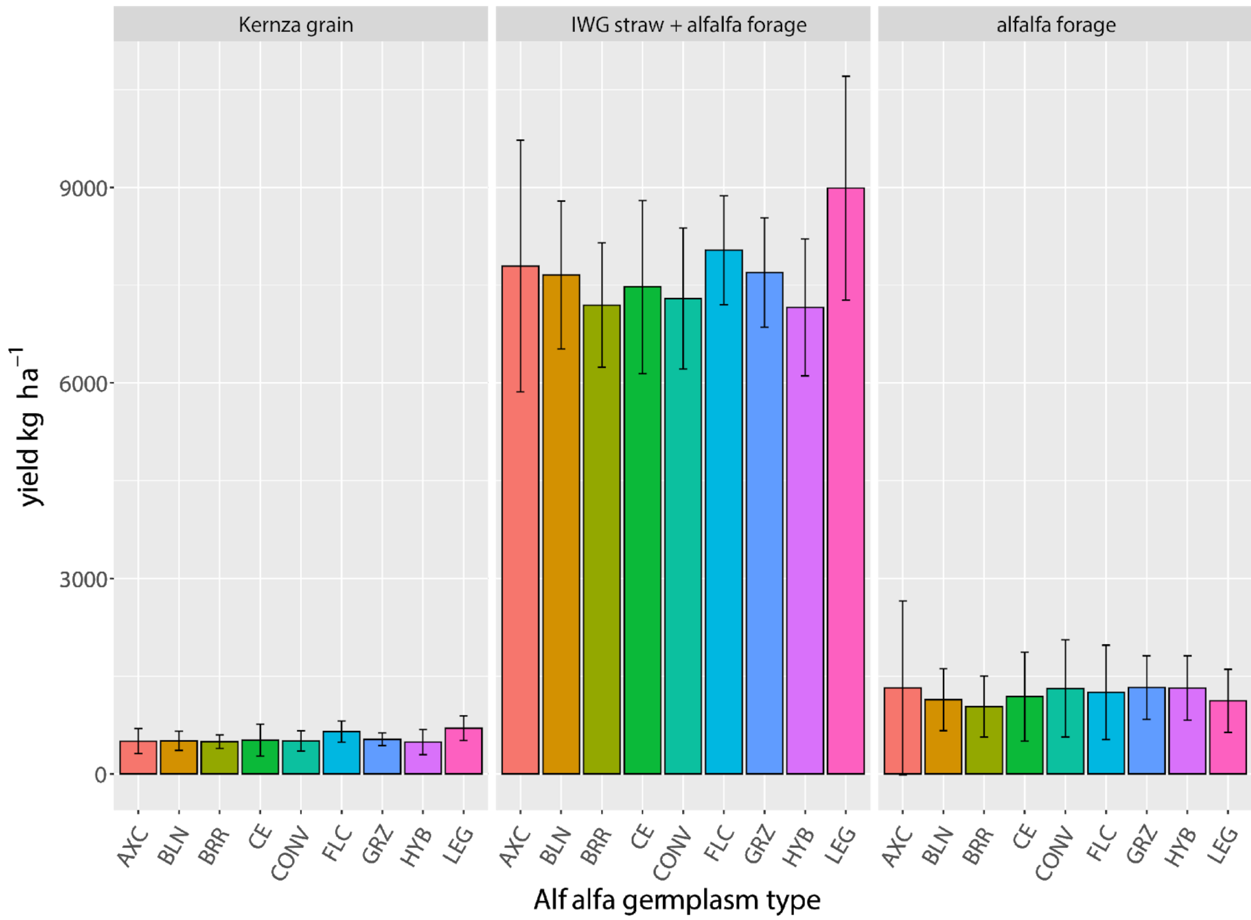

Figure 4. Mean Kernza perennial grain yields, summer intermediate wheatgrass (IWG) straw + alfalfa forage yield, and summer alfalfa forage yields for alfalfa germplasm types in IWG+alfalfa mixtures in KS. Bars indicate standard deviations. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

There were no differences between alfalfa germplasm types in mixtures with IWG for summer IWG straw + alfalfa forage crude protein or alfalfa crude protein. There were no differences between the alfalfa germplasm types for crude protein (CP) during the summer when planted in mixtures with IWG, but alfalfa CP still averaged ~ 18% across the germplasm types (Figure 5). However, none of the alfalfa germplasm types improved overall crude protein (CP) of the IWG straw + summer forage; CP of the IWG straw + alfalfa summer forage was less than (7%) across all germplasm (Figure 5). There were slight differences in ADF and aNDF among the alfalfa germplasm types; the legacy (LEG) germplasm – including older public varieties like 'Ladak' and 'Ranger' had poorer nutritive value than the grazing (GRZ) and alfalfa hybrid varieties created using male sterility systems (AXC) (Figure 5).

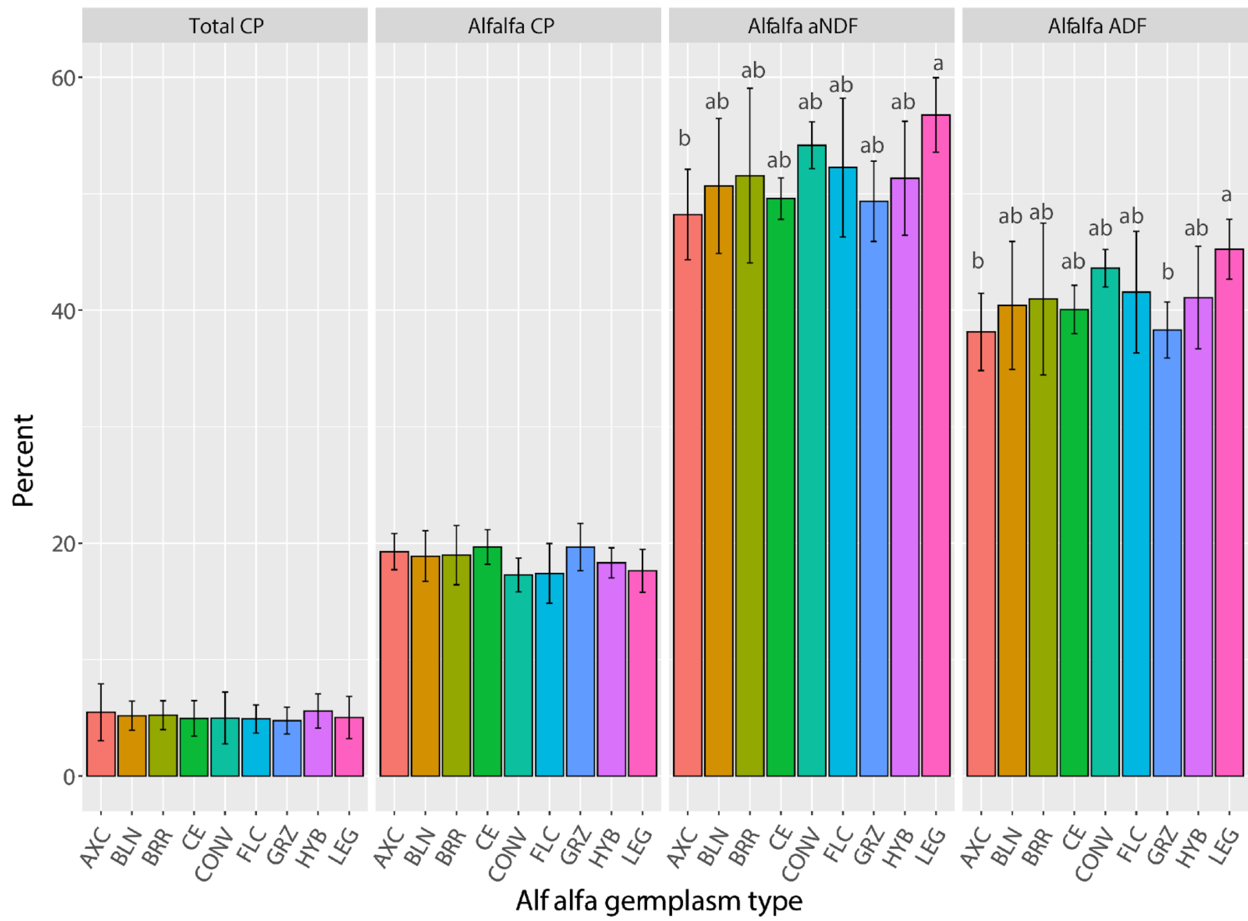

Figure 5. Mean summer intermediate wheatgrass (IWG) straw + alfalfa forage yield crude protein (CP) and summer alfalfa CP, neutral detergent fiber (aNDF), and acid detergent fiber (ADF) for alfalfa germplasm types in IWG+alfalfa mixtures in KS. Bars indicate standard deviations. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

<u>OBJECTIVE 5.</u> Determine expected yield, quality, and gross value of forage available in the spring and fall in IWG monocultures vs. IWG + alfalfa mixtures in KS.

Alfalfa improved fall forage yield and gross fall forage value in IWG+alfalfa mixtures in KS compared to widely spaced and unfertilized IWG monocultures. In fall 2019, alfalfa contributed significantly to regrowth following the summer straw/residue harvest in KS, which improved fall forage yield and quality (Figure 6). The hybrid type alfalfa (HYB1) planted in mixtures with IWG had greater fall forage yield than all the IWG monoculture treatments except for fertilized, narrow row spaced IWG (IWGn+); the other grazing alfalfa type (GRZ1) had higher biomass yield than IWGw+ (Figure 6). Mean forage yields in the fall were significantly lower than in the spring for the IWG monoculture treatments, but spring and fall forage yields were not significantly different for the IWG+alfalfa treatments. The increased fall yield provided by alfalfa, combined with additional improvements in nutritive value of the forage, resulted in the gross value of the fall forage in the IWG+HYB1 treatment ($527.20 USD ha^{-1}), more than double that of the four monoculture IWG treatments ($194.40 USD ha^{-1}) (Figure 7). Mean gross value of the fall forage for the other alfalfa type (GRZ1) trended higher but was not significantly different from the narrow-spaced IWG treatments (Figure 7). No differences in forage yield or gross value were observed between treatments in the spring or summer. Costs of production were not included in this economic analysis. The relatively low spring and forage yields observed in this study may make grazing a more economical option than haying to utilize spring and fall/winter forage in the system.

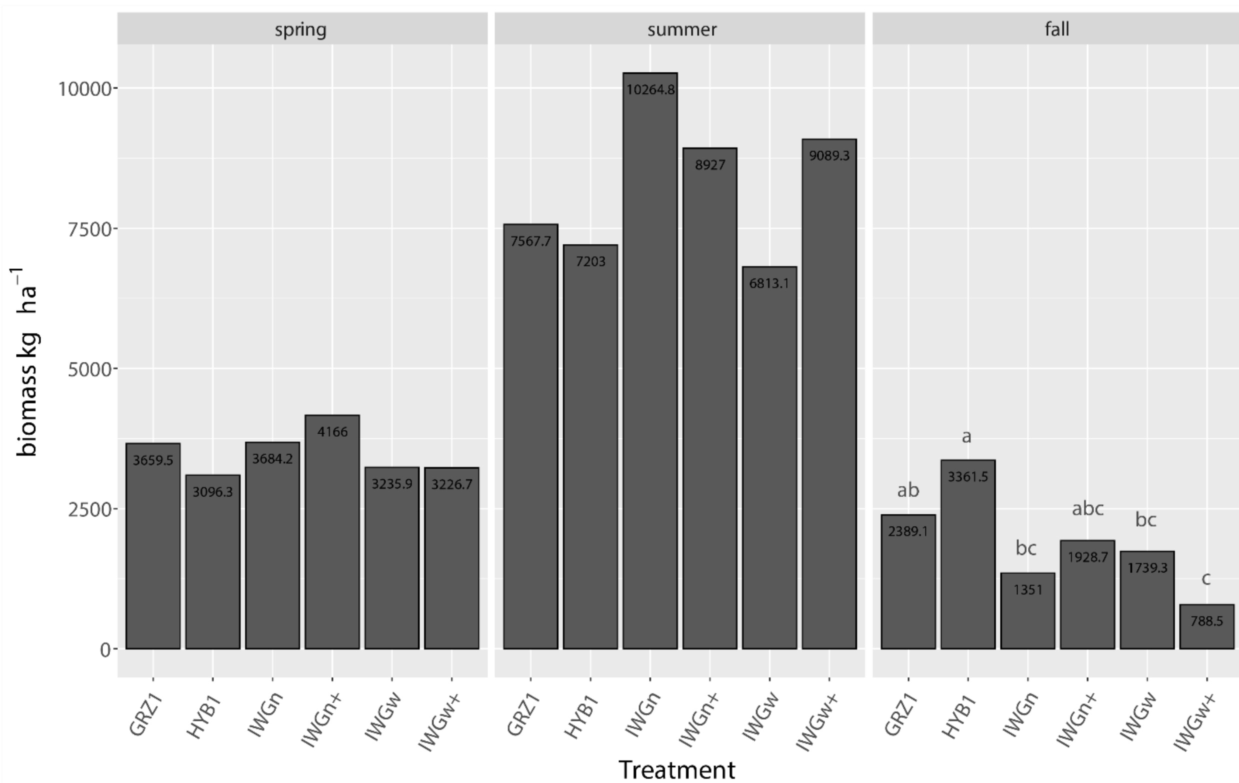

Figure 6. Spring and fall hay/forage and summer straw yields in KS harvested from intermediate wheatgrass monocultures spaced in 30 cm rows with (IWGn+) and without (IWGn) spring nitrogen fertilizer application; monoculture intermediate wheatgrass spaced in 60 cm rows with (IWGw+) and without (IWGw) spring nitrogen fertilizer application; and IWG planted in alternating 30 cm rows with a grazing type alfalfa hybrid (HYB1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

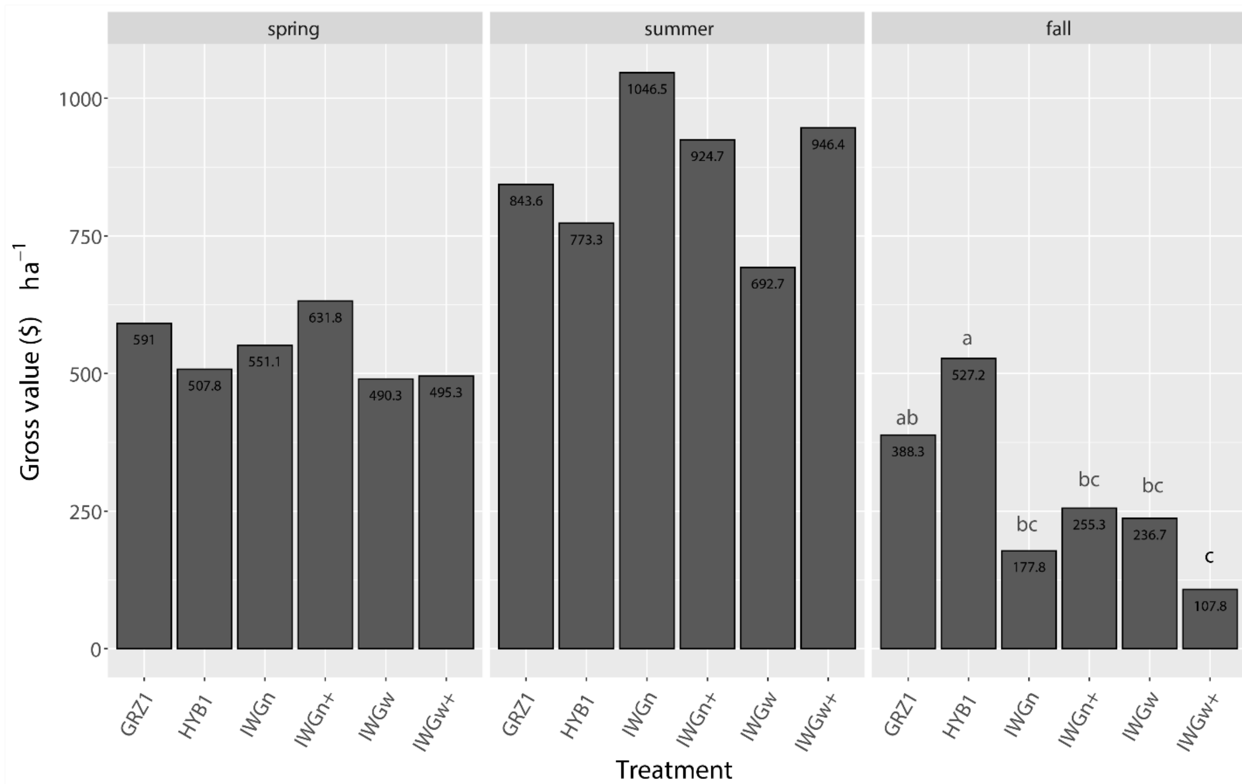

Figure 7. Gross value of spring and fall hay/forage and summer straw yields in KS harvested from intermediate wheatgrass monocultures spaced in 30 cm rows with (IWGn+) and without (IWGn) spring nitrogen fertilizer application; monoculture intermediate wheatgrass spaced in 60 cm rows with (IWGw+) and without (IWGw) spring nitrogen fertilizer application; and IWG planted in alternating30 cm rows with a grazing type alfalfa hybrid (HYB1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

IWG+alfalfa mixtures had higher relative forage values than IWG monocultures in the spring and fall in KS but did not improve total annual forage yield or total gross forage value. Including alfalfa in mixtures with intermediate wheatgrass managed as a dual-purpose grain (Kernza) and forage crop increased the nutritive value of both the spring and fall forage. Relative forage value (RFV) for IWG mixtures with the HYB1 type alfalfa variety was significantly higher for both the spring and forage than the four IWG monoculture treatments, and RFV of IWG mixtures with the GRZ1 grazing type alfalfa variety was higher than all the IWG monoculture treatments except IWGw+ in the spring and IWGw and IWGw+ in the fall (Figure 8).

Despite the increased fall forage yields and spring and fall forage quality and fall gross value of the IWG+alfalfa mixtures compared to IWG monocultures, no differences were observed in the total annual biomass yield or gross value (Figure 9). This is likely because the bulk of the annual biomass production occurs in the summer when the IWG straw and alfalfa forage are baled after grain harvest. No differences were observed in summer forage yields, quality, or gross value among the treatments. The total mean annual forage yield of IWG and IWG+alfalfa biomass in KS in 2019 (13,680 kg ha^{-1}) was higher than previously reported in WI and MN (10,466 kg ha^{-1}) (Favre et al., 2019; Hunter et al., 2020).

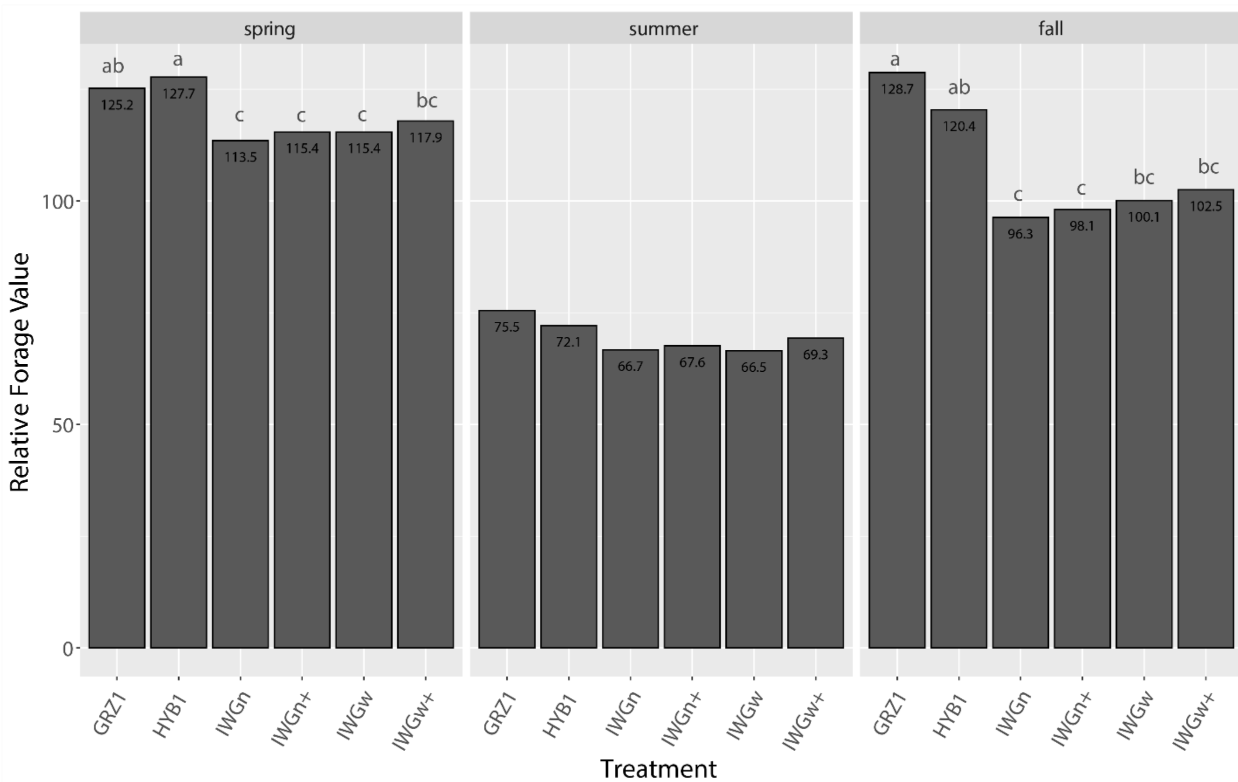

Figure 8. Relative forage value of spring and fall hay/forage and summer straw in KS harvested from intermediate wheatgrass monocultures spaced in 30 cm rows with (IWGn+) and without (IWGn) spring nitrogen fertilizer application; monoculture intermediate wheatgrass spaced in 60 cm rows with (IWGw+) and without (IWGw) spring nitrogen fertilizer application; and IWG planted in alternating30 cm rows with a grazing type alfalfa hybrid (HYB1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

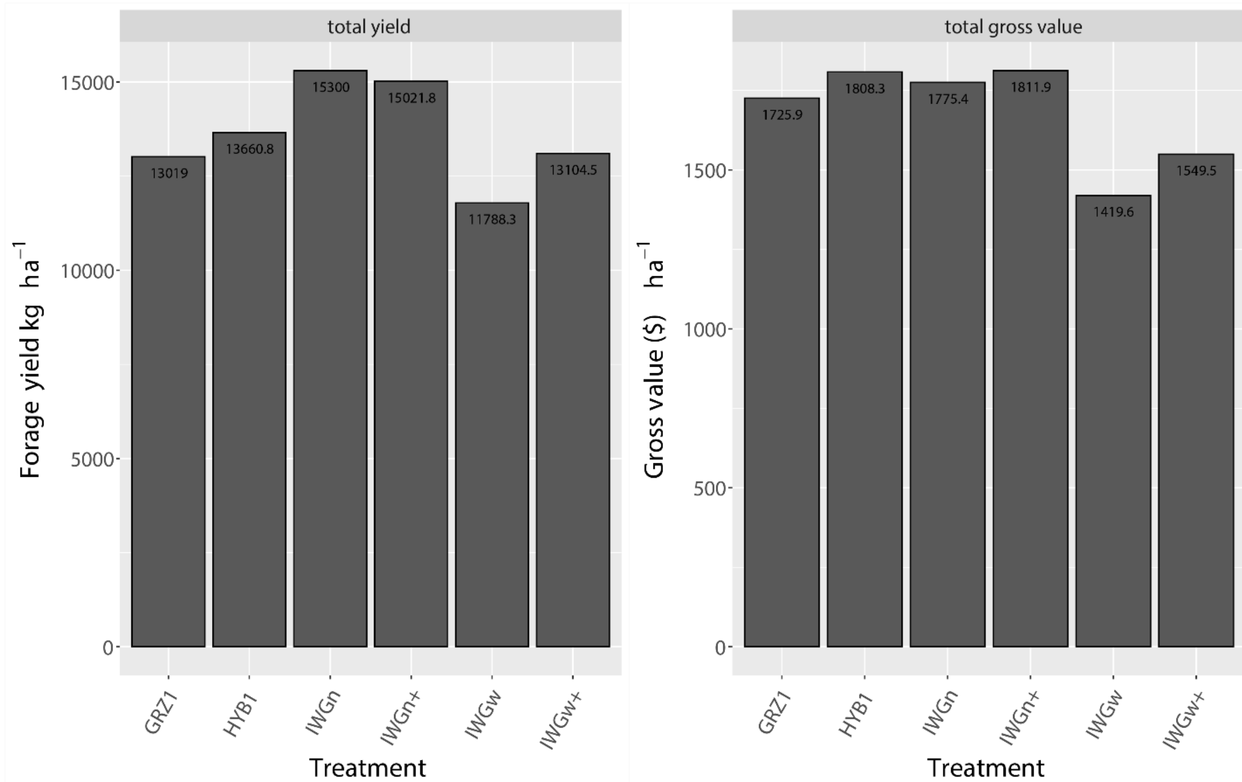

Figure 9. Total forage yield and forage gross value of intermediate wheatgrass monocultures spaced in 30 cm rows with (IWGn+) and without (IWGn) spring nitrogen fertilizer application; monoculture intermediate wheatgrass spaced in 60 cm rows with (IWGw+) and without (IWGw) spring nitrogen fertilizer application; and IWG planted in alternating 30 cm rows with a grazing type alfalfa hybrid (HYB1) alfalfa variety. Means followed by a common letter are not significantly different at the alpha = 0.05% level after Bonferroni correction.

Conclusions

Emerging row-crop grain + alfalfa mixed cropping strategies that focus on long-term economic and agronomic stability while producing high-value products may represent untapped market potential for alfalfa and previously underutilized alfalfa germplasm. However, the trade-offs between forage and grain production in row-crop + grain mixtures must be assessed over multiple years and locations to identify and assemble compatible varieties. We did not observe superior performance of any alfalfa variety or germplasm type in this study; instead, we observed substantial environmental and genotype x environment variation, suggesting pairing appropriate alfalfa and IWG varieties in mixtures will likely need to be pursued on a location-by-location basis. The relatively small and negligent genetic variance observed in this study for total summer forage yield and quality suggests that breeders and agronomists should work together to reevaluate plot management and data collection methods to reduce error in measurements and set shared cropping system goals (e.g., maximizing seasonal distribution of forage yield and quality vs. improving summer straw/forage quality). This study, which collected data from IWG+alfalfa mixtures from four locations in 2019, did not observe significant differences or reductions in

Kernza grain yields, total forage yield, and total gross value of the forage in IWG + alfalfa mixtures compared to IWG monocultures. These observations and the added benefits of including alfalfa in the cropping system – reduced N-fertilization requirements, improved CP of forage, additional ecosystem services – suggest that development of IWG + alfalfa mixtures merits further future research.

Acknowledgments
Funding for this study was provided by the U.S. Alfalfa Farmer Research Initiative of the National Alfalfa & Forage Alliance.

Keywords: Intermediate wheatgrass, forage mixtures, compatibility

References

Asay, K.H., D.A. Johnson, and M.D. Rumbaugh. 1999. Genotype by competition level interactions in alfalfa (*Medicago sativa* L.). Int. J. Plant Sci. 160(1): 129–134.

Bajgain, P., X. Zhang, and J.A. Anderson. 2019. Genome-wide association study of yield component traits in intermediate wheatgrass and implications in genomic selection and breeding. G3 9(8): 2429–2439.

DeHaan, L., M. Christians, J. Crain, and J. Poland. 2018. Development and evolution of an intermediate wheatgrass domestication program. Sustain. Sci. Pract. Policy 10(5): 1499.

Favre, J.R., T.M. Castiblanco, D.K. Combs, M.A. Wattiaux, and V.D. Picasso. 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. Anim. Feed Sci. Technol. 258: 114298.

Grabber, J.H. 2016. Prohexadione–Calcium improves stand density and yield of alfalfa interseeded into silage corn. Agronomy Journal 108: 726–735.

Holman, J.D., C.R. Thompson, R.L. Hale, and A.J. Schlegel. 2009. Grazing effects on yield and quality of hard red and hard white winter wheat. Agron. J. 101(4): 775–788.

Hunter, M.C., C.C. Sheaffer, S.W. Culman, W.F. Lazarus, and J.M. Jungers. 2020. Effects of defoliation and row spacing on intermediate wheatgrass II: Forage yield and economics. Agron. J. 112(3): 1862–1880.

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

Lollato, R.P., J.T. Edwards, and T.E. Ochsner. 2017. Meteorological limits to winter wheat productivity in the U.S. southern Great Plains. Field Crops Res. 203: 212–226.

Louarn, G., E. Pereira-Lopès, J. Fustec, B. Mary, A.-S. Voisin, et al. 2015. The amounts and dynamics of nitrogen transfer to grasses differ in alfalfa and white clover-based grass-legume mixtures as a result of rooting strategies and rhizodeposit quality. Plant Soil 389(1): 289–305.

Pugliese, J.Y., S.W. Culman, and C.D. Sprunger. 2019. Harvesting forage of the perennial grain crop kernza (*Thinopyrum intermedium*) increases root biomass and soil nitrogen cycling. Plant Soil 437(1): 241–254.

Randall, G.W., D.R. Huggins, M.P. Russelle, D.J. Fuchs, W.W. Nelson, et al. 1997. Nitrate losses through subsurface tile drainage in Conservation Reserve Program, alfalfa, and row crop systems. Journal of Environmental Quality 26(5): 1240–1247.

Tautges, N.E., J.M. Jungers, L.R. DeHaan, D.L. Wyse, and C.C. Sheaffer. 2018. Maintaining grain yields of the perennial cereal intermediate wheatgrass in monoculture v. bi-culture with alfalfa in the Upper Midwestern USA. J. Agric. Sci. 156(6): 758–773.

Zhou, C., L. Han, C. Pislariu, J. Nakashima, C. Fu, et al. 2011. From model to crop: functional analysis of a STAY-GREEN gene in the model legume *Medicago truncatula* and effective use of the gene for alfalfa improvement. Plant Physiol. 157(3): 1483–1496.