Agronomic practices to manage competition in dual-use Kernza intermediate wheatgrass (*Thinopyrum intermedium*) to sustain grain yield over time

by

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Dedication

To my littlest love, Ivie Jean bean

In memory of my Pappy, the sweetest man I have ever known

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Chapter 1: Intermediate wheatgrass opportunities and challenges

Grains, the seeds that we harvest from grasses, are a vital part of our diet. On average, people get 48% of their calories from annual grains — rice, corn, and wheat being the most common (Boudreau et al., 2011). Unfortunately, the annual cropping systems that we depend on for most grain calories also cause a host of environmental disservices. Annual grain crops require planting and replanting each growing season which means that the soil is regularly disturbed or left bare when the annual crop is harvested. Bare soil can cause nutrients in the soil and soil particles to runoff into water causing nutrient pollution (Zuazo et al., 2011). This has cascading effects on surface water ecosystems as it alters food webs and oxygen availability (Ribaudo & Johansson, 2007). Further, regular soil disturbance through tillage and continuous planting causes stored carbon in the soil to be released into the atmosphere as carbon dioxide, a common greenhouse gas that contributes to climate change (Reicosky et al., 1997). Shifting the dominant annual-based agricultural systems to perennial-based systems has the potential to mitigate many of these issues resulting from annual cropping systems.

Perennial crops can live for many years continually providing food for people and animals while contributing ecosystem services such as sequestering soil carbon and improving soil health and water quality (DeHaan and Ismail, 2017; Culman et al., 2010; de Oliveira et al., 2020). Perennial plants have robust root systems and continuous soil cover that reduces soil erosion by holding the soil in place and moving carbon from the atmosphere into the soil, storing carbon and forming richer soils through increasing soil organic matter and soil fungal diversity (DeHaan and Ismail, 2017; Ryan et al., 2018; Culman et al., 2013; Jungers et al., 2019; Pimentel et al., 2012). This means that there is less soil sediment and nutrient pollution that moves from the fields to watersheds polluting groundwater and aquatic ecosystems (Culman et al., 2013). Further, perennials establish ground cover year round which increases evapotranspiration rates compared to annuals (de Oliveira et al., 2020). Increased evapotranspiration rates can have a regional cooling effect buffering a region from experiencing full impacts of global warming (Georgescu et al., 2011). Perennial crops can do all of this while decreasing the need for fuel consumption, tillage, and labor (Pimentel et al, 2012).

Besides the environmental benefits, perennial crops have considerable potential to provide food for humans and forage for animals. A promising perennial grain crop comes from the plant species intermediate wheatgrass [IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey]. Intermediate wheatgrass is a cool season perennial grass originally from Eurasia and introduced to North America in 1932 as forage for cattle (DeHaan and Ismail, 2017). This grass is competitive with weeds, provides high yielding and good quality forage suitable for lactating beef and dairy cows as well as growing heifers, and is highly palatable to all types of livestock (Zimbric et al., 2020; Hybner, 2012; Favre et at., 2019). Because of the qualities of this grass, The Land Institute in Kansas has bred IWG to increase its grain yield and seed size to cultivate a dual-purpose grain and forage crop. They trademarked the IWG seed as Kernza®. The Land Institute continues to breed this grass with the goal of having grain yields similar to annual wheat while selecting for increased grain yield and larger, free-threshing kernels (Cox et al., 2010). The Land Institute predicted there to be an additional 17 breeding cycles before reaching yields similar to annual wheat. From 2019 to 2021, Kernza IWG harvested grain yield more than doubled to over 400 lbs per acre (The Land Institute, 2022).

Figure 1. Annual wheat (right) roots extend 1 m while Kernza IWG roots extend 3-4 m. Image by The Land Institute.

Figure 2. Kernza IWG roots extend deep into the soil in 2021 at the Arlington Agricultural Research Station in WI. Image by Erica Shoenberger.

Kernza grain flour can be incorporated in products such as breads, pasta, cereals, baked goods and more. It can be malted and or mixed into whisky and beer. It has a nutty and sweet flavor and superior nutritional quality. Compared to 100 grams of all-purpose white wheat flour, Kernza refined flour has similar calories, fats, and carbohydrates but has 7.2 grams more protein, 1.6 grams more fiber, 35 mg more calcium, 2.53 mg more iron, and 33 mg more potassium (AURI, 2020). Because this perennial grain can be incorporated into products so easily, it is a viable market substitute for wheat flour that promotes more sustainable agricultural and food systems.

Commercial interest in Kernza has grown in recent years outpacing supply largely as a result of the publicity of the General Mills' Cascadian Farm Kernza Cereal limited edition fundraiser (Charles, 2019). Food industry stakeholders such as General Mills, Patagonia Provisions, Perennial Pantry, Madison Sourdough and more are interested in incorporating Kernza in their product lines; but demand is greater than the farmer's capacity to grow the perennial grain which is why supporting farmers with continued studies on optimal practices for production is imperative.

Figure 3. Kernza grain products from right to left: 1. Cascadian Farm Climate Smart Kernza Grains cereal 2. Patagonia Provisions Kernza Fusilli pasta 3. Patagonia Provisions and Hopworks Long Root Ale. Image by the Land Institute.

In addition to cultivating the Kernza grain, farmers have the option of two high quality forage harvests in the spring and fall and a low-quality forage harvest in the summer after the grain harvest. This dual-purpose reduces the economic risk for farmers because it provides multiple avenues of income. Harvesting multiple forage harvests has been shown to stimulate root, forage, and grain production in Kernza IWG showing that using it as a dual purpose crop increases total productivity (Pugliese et al., 2019).

Figure 4. Stages of Kernza IWG growth in WI. A. IWG Vegetative growth (late May) B. IWG Flowering (early July) C. Mature Kernza grain (early August) D. Harvested Kernza grain (early August).

Though farmers are interested in growing Kernza for its ecological and economic benefits, there still are many factors to optimize production and minimize risk for farmers that are not well understood. Specifically, farmers have asked for information on how to better maintain grain yield overtime, a paramount issue for the economic viability of Kernza IWG systems (Lanker et al., 2020). Currently, grain yield tends to peak in the first production year and subsequently decline leading to farmers rotating or replanting every 3-5 years (Jungers et al., 2017; Law et al., 2020; Zimbric et al., 2020, Pinto et al., 2021). Yield decline overtime is thought to be related to intraspecific competition among the IWG that reduces reproductive tiller initiation in the fall thereby reducing grain yield the following summer (Pinto et al., 2021; Fernandez et al., 2020). It is also thought to be related to the increase in belowground competition for water, nutrients, and space throughout the life of the IWG stand (Fernandez et al, 2020; Hunter et al., 2020a; Tautges et al. 2018).

In addition to maintaining grain yield overtime, farmers have requested more information on weed management in their Kernza IWG systems (Lanker et al., 2020). As of 2019, herbicides effects have not been studied nor have any herbicides been approved for use on IWG for Kernza grain production. Without herbicides, recommended weed management practices include 1. planting in fields with low weed pressure from perennial and highly competitive weeds, (DeHann et al. 2019) 2. timely mowing before stem elongation occurs in the spring, (Zimbric et al., 2020) and 3. inter-row cultivation to reduce weeds between rows if row spacing is wide enough for the equipment (DeHaan et al. 2019; Zimbric et al., 2019). But these practices are not always practical or possible for farmers, so to give farmers more tools for weed management in Kernza IWG stands, it is imperative to study selected herbicide effects on grain yield and crop injury.

1.1 | Research goals and objectives

The goal of this thesis is to address two major farmer concerns with growing dual-use Kernza IWG. Particularly, research was conducted to: 1. determine optimal agronomic management practices to maintain Kernza grain yield overtime, and 2. to address the knowledge gap of synthetic auxin herbicide effects on IWG injury, grain yield, and weed efficacy in order to have additional weed management options to produce Kernza IWG for human consumption.

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Chapter 1: Managing stand thinning and nitrogen fertility in dual-use Kernza intermediate wheatgrass to sustain grain yield over time

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Abstract

Kernza intermediate wheatgrass (IWG; *Thinopyrum intermedium*) is a dual-purpose perennial crop that can provide both forage and grain harvests in the same year. A problem for the long term production and success of this novel crop is the grain yield decline overtime. To address this concern, two management practices were studied: applying nitrogen (N) fertilizer and thinning aging Kernza IWG stands. One experiment was established in four environments, combinations of two locations (Lancaster and West Madison, WI) and two years (2021 and 2022). Each environment had a randomized complete block design with 4 replications. A full factorial design of treatments with two factors was applied: 1) spring N fertilizer application at 0, 75, 150 kg N ha^{-1} and 2) stand thinning in the spring using a broad spectrum herbicide with 0, 25, 38, and 50% of the stand thinned. The objective was to determine the effect of the treatment factors and their interaction on grain and forage yield, weed pressure, harvest index (HI), and seed mass. Data were collected the year that thinning treatments were applied at all sites, and the year after thinning treatments were applied at one site. There was no interaction between N fertilization and thinning. Grain yield, forage yield, HI, and seed mass were 110, 70, 60, and 19% greater, respectively, with N fertilization compared to no fertilization, and not different between 75 and 150 kg N ha^{-1}. In the treatment year, grain yield was not affected by thinning. Forage yield remained the same or was reduced with thinning whereas HI and seed mass were not affected. In contrast, grain yield the year after thinning was greater (47%) for the 50% thinning compared to no thinning, while forage yield, HI, and seed mass were not affected. Weed biomass was not affected by N fertilization and was only affected by thinning at one site where weed biomass increased with increased thinning. These results provide evidence that moderate N fertilization (75 kg ha^{-1}) coupled with aggressive row thinning (50%) in the spring can increase Kernza grain yields the year after thinning, but can come with the potential tradeoff of decreased forage yields in the year of thinning.

1 | Introduction

1.1 The problem of grain yield decline in Kernza

Two leading issues for adoption of Kernza IWG for dual-use grain and forage is relatively low grain yield compared to annual cereals and its grain yield decline over time (Dick et al., 2019; Hunter et al., 2020a; Jungers et al., 2018, 2017; Lanker et al., 2021; Tautges et al., 2018; Zimbric et al., 2020). Several studies show that grain yield declines from the first to the third production year. For example, yield declined from first to third production years from 880 to 418 kg ha^{-1}(Hunter et al., 2020a), 961 to 153 kg ha^{-1} (Jungers et al., 2017), and 763 to 371 kg ha^{-1} (Zimbric et al., 2020). Franco et al., 2021 reviewed Kernza IWG yields across the US. Because of grain yield decline, farmers rotate or replant their Kernza IWG stand after 3-5 years (Law et al., 2020). It is essential to maintain yields in the years after establishment as this dual-purpose crop already has comparatively low yields at 10-20% of annual wheat in the same region (DeHaan and Ismail, 2017). Breeding efforts mainly have focused on increasing seed mass and seed number per head, boosting shatter resistance, and improving free-threshing seeds, so both agronomic management and continued genetic improvement is needed to increase and maintain grain yields over time (DeHaan et al., 2018).

1.2 | Reasons for grain yield decline overtime

Kernza IWG has an optimal vernalization requirement of 4-5 ℃, similar to other cool season forage grasses and annual grain crops (Duchene et al., 2021, Locatelli et al., 2021). Primary induction in Kernza IWG occurs in the winter when reproductive tillers are produced, and secondary induction begins in the spring when reproductive tillers develop leading into flowering and seed development (Cooper & Calder, 1964; Duchene et al., 2021, Heide, 1994, Ivancic et al., 2021). The first year of grain production tends to be the highest because as perennial plants age, they favor vegetative growth and asexual reproduction under stable conditions rather than grain-producing sexual reproduction (Garnier, 1992). Additionally, as IWG ages, the resources allocated shift from competitive to stress tolerant strategies (Jaikumar et al., 2016). In Kernza IWG, fertile tillers have been shown to be the prime predictor of grain yield, and the decline in fertile tiller production in years following establishment suggests that the plant shifts from reproductive to vegetative growth (Fernandez et al., 2020). Increasing the number of fertile tillers per area in perennial grasses can also increase seed yield (Deleuran et al., 2009 and 2010; Han et al., 2013).

It has been proposed that Kernza IWG grain yield declines over time because light penetration to the crown of the plant is limited during the fall which reduces fertile tiller growth (Pinto et al., 2021; Fernandez et al., 2020). Light quantity and quality, specifically red light, are key factors for fertile tillers yield in grasses (Casal et al., 1985; Deregibus et al., 1985). Because Kernza IWG canopies become thicker in the years after planting, the light needed for fertile tiller production may not be reaching the crown, reducing tillering and consequently decreasing grain yield (Fernandez et al, 2020). Further, grain number per tiller, proportion of high yielding tillers, and seed mass per tiller tend to decline with stand age in perennial crops which also could contribute to grain yield decline overtime (Hunter et al., 2020a; Canode & Law., 1995).

Another hypothesis for grain yield decline over time is an increase in belowground competition for water and nutrients. This has been proposed to explain why lower planting densities tend to have higher grain yields in the years following establishment (Fernandez et al, 2020). Perennial grasses are extremely competitive, so when planting densities are high, they distribute considerable carbon to belowground biomass production increasing competition in the inner row spaces and for access to the nutrient pools (DeHaan et al., 2005; Garnier, 1992; Sakiroglu et al., 2020). So it follows that increased root competition for space and nutrients is a possible reason for grain yield decline overtime (Fernandez et al, 2020; Hunter et al., 2020a; Tautges et al. 2018).

1.2 | Effects of N on grain yield

Determining the ideal N fertilizer rate for a perennial grain is more difficult than for an annual grain because perennials have substantially larger root systems and can access more nutrients deeper in the soil profile and store them for years (Ryan et al., 2018; Sprunger et al., 2018). Though the agronomic optimum N rate for Kernza IWG has been reported for the first three years of Kernza IWG production, from 61-96 kg N ha^{-1}, little is known about N requirements in older perennial plants to sustain grain production (Jungers et al., 2017; Rebesquini et al., 2022; Tautges et al. 2018).

Nitrogen applied to forage grasses has been shown to improve leaf elongation rate and leaf area which could further impact nutrient assimilation, plant growth rate and tillering (Gastal and Durand, 2000; Mitchell et al., 1998; Simon and Lemaire, 1987). Specifically, some studies have shown that N fertilizer applied in the agronomically optimum range had no impact on Kernza IWG grain yield in the first year, likely because soil N was not yet a limiting growth factor (Fernandez et al., 2020; Jungers et al., 2017). But, in the second year, the same N application of 80 kg N ha^{-1} increased grain yields by 217% in year 2 and 240% in year 3 of production (Fernandez et al., 2020). This suggests that as root biomass increases and reduces available soil inorganic N in years after establishment, N applications are needed to recharge soil nutrient pools to maintain grain yields (Pugliese et al., 2019; Fernandez et al., 2020). Applications of N higher than the agronomically optimum range have been shown sometimes to increase stand lodging in perennial grasses and subsequently decrease grain yield, especially in post-establishment years as the canopy becomes more dense (Fernandez et al., 2020; Jungers et al., 2017; Koeritz et al., 2015). However, Zimbric et al. (2020) reported no lodging on high N Kernza stands in Wisconsin.

Although N fertilizer can ameliorate grain yield decline in years following establishment, these increases do not fully make up for the overall grain yield decline from the establishment year (Fernandez et al., 2020). Therefore, the management practices of N fertilization may not be enough to maintain grain yields overtime alone, but coupling the practice with an agronomic management practice to increase light penetration to the crown may make maintaining grain yield overtime more likely (Pinto et al., 2021). It is also possible that other nutrients like P and K may be depleted overtime due to removal of grain and forage, however, research is ongoing to address the role of limiting P and K in long-term Kernza yields (Kernza®CAP, 2022).

1.3 | Strategies for increasing Kernza IWG grain yield

Researchers have explored several management strategies that cause canopy disturbance to increase light penetration to the crown of Kernza IWG to stimulate fertile tiller production. Strategies include: 1. planting at wider row spaces (Hunter et al., 2020a; Fernandez et al., 2020), 2. defoliating the stand throughout the growing season (Pugliese et al, 2019; Pinto et al., 2021), 3. thinning already established stands to stimulate grain production (Law et al., 2020; Pinto et al., 2021), and 4. post-harvest burning Pinto et al., 2021). Planting in 30 cm row spaces compared to typical 15 cm row spaces can lead to higher grain yields because of an increase in fertile tiller production if planting density is reduced along with the wider row spacing (Canode, 1964; Fernandez et al., 2020; Han et al., 2013). Some row spacing comparisons have maintained the same planting density per area while increasing row spacing, leading to higher density in each row, thus more competition (Pinto et al., 2022). Though planting at a lower density has been shown to increase yield components in the establishment year, the increase did not account for the overall decrease in planting at a lower density. In year 2, grain yields were higher with the lower planting density, again, because of the positive effect on fertile tiller production (Fernandez et al., 2020).

Another strategy for reducing yield decline overtime is defoliation which increases the quantity and quality of red light and consequently stimulates tiller production (Aamlid et al., 1997; Deregibus et al., 1983; Ugarte et al., 2010; Youngner, 1972). In some cases, defoliation in the form of summer and/or fall forage harvests have been shown to stimulate grain production and forage and root biomass following defoliation (Hunter et al., 2020a; Pugliese et al, 2019; Sakiroglu et al., 2020). In a different case, defoliation in the form of summer, and/or fall, and/or spring forage harvests did not affect grain yield (Zimbric et al., 2020). Again in a 2021 in WI, defoliation treatments such as chopping, burning, and mechanical and chemical thinning had no effect on grain yield (Pinto et al., 2021). In another case, defoliation in the form of spring grazing actually reduced grain yields compared to a no-graze control in the first study year and had no effect in the second study year (Picasso et al., 2020). In this study, researchers proposed that grain yield reduction was a result of poor grazing timing that damaged the Kernza flower.

In cases where defoliation did stimulate grain production, researchers identified that stimulation occurred because of increased nutrient cycling and availability, reduced intraspecific competition ascribed to disturbance from defoliation, and/or increase in light penetration from biomass removal (Pugliese et al, 2019; Knapp and Seastedt 1986). In particular, the timing of defoliation has not been shown to affect the proportion of *fertile* tillers, though spring more so than fall defoliation has been shown to stimulate tiller production (Hunter et al., 2020a).

Alternatively, researchers also have used thinning practices on aging Kernza stands to stimulate grain production (Pinto et al., 2021; Law et al., 2020). Thinning practices can be chemical or mechanical ways to reduce Kernza IWG stand density. One example of mechanical thinning practice is mechanical thinning using strip-tillage (Figure 1). When applied in the fall, before the Kernza IWG enters winter dormancy, the strip tillage has been shown to increase grain yield by 61% attributed to increase in the amount of fertile tillers per area (Law et al., 2020). While strip-tillage applied in the spring has been shown to reduce competition among fertile tillers, there was no difference in overall grain yield regardless of lower stand density compared to the control (Law et al., 2020).

Figure 1. Kernza IWG field after the mechanical thinning practice of strip-tillage using a Unverferth Zone Builder Subsoiler Model 122. Image from Law et al., 2020.

Other thinning practices that reduce stand density include inter-row cultivation, chopping, burning, and banded herbicide applications in Kernza IWG stands have been explored (Bergquist, 2019; Ensign et al., 1983; Pinto et al., 2021). Berquist (2019) used banded herbicide applications (Figure 2), a form of chemical thinning, to reduce stand density . In this study, highest grain yields occurred in fall inter-row cultivation and spring banded herbicide applications in years 2 and 3, though these yields did not statistically differ from the nontreated control. Pinto et al. (2021) showed that thinning practices used (chopping, burning, mowing) increased light penetration and the proportion of fertile tillers row^{-1}, but reduced overall yield because the increase was not significant enough to make up for the decrease in rows per area . It follows that because thinning can increase fertile tillers per row but not always per area, it is crucial to apply treatments that are not so extreme to reduce yield while being significant enough to increase light penetration to effect grain yield (Pinto et al., 2021).

Figure 2. Chemical thinning in the form of spring banded herbicide applications reduces Kernza IWG stand density at the West Madison Agricultural Research Station in WI. Image by Erica Shoenberger.

1.4 | Objectives and hypotheses

The first objective is to determine the effect of 0, 75, and150 kg ha^{-1} N fertilizer on grain and forage yield and weed pressure. We expect that a moderate fertilizer rate, 75 kg N ha^{-1}, has the most favorable effect on grain yield because it falls within the agronomically optimum range (AOR) of N fertilization (Jungers et al., 2017). The highest rate, 150 kg N ha^{-1}, is expected to have similar or reduced overall grain yields because of the higher chance of lodging (Fernandez et al., 2020; Jungers et al., 2017; Koeritz et al., 2015). The 150 kg N ha^{-1} treatment is expected to lead to the greatest weed pressure because N will not be a limiting factor for growth and competition among vigorous perennial weeds often found in Kernza IWG systems (Zimbric et al., 2020).

The second objective is to determine the effect of various levels of chemical thinning in the spring on grain, forage, and weed yield in a Kernza IWG system. We hypothesize that grain and forage yield is highest in the treatments that are moderately thinned (25 and 38% stand reduction). We expect that moderate thinning treatments result in the greatest grain yield. It will reduce stand density enough to decrease intraspecific competition allowing for greater fertile tiller growth while maintaining enough of the stand so that overall yield is not reduced. We also expect weed pressure is greatest with maximum thinning (50% stand reduction) because the IWG stand is competitive with weeds and will be greatly reduced in this treatment, allowing the most space for weed competition.

Ultimately we want to determine the interaction between thinning and N fertilization in Kernza IWG systems. We predict that high N fertilization coupled with no thinning will have lower yields than when coupled with moderate or maximum thinning. The high N fertilization will increase intraspecific competition among tillers. This will lead to higher grain yields in the moderately and maximally thinned treatments, because the tillers have light and space to grow and produce grain. In the no thinning treatment, intraspecific competition will increase and result in lower grain yields. Conversely, with moderate N fertilization, we expect that moderate thinning will have the highest grain yield because it will have enough N and reduced stand density to encourage the most fertile tiller production. With moderate N fertilization, we also expect that no thinning will still have too much intraspecific competition and maximum thinning will have reduced stand density that is too great to result in the highest grain yields. Finally, we expect that with no fertilization, the no thinning treatment will be greatest because N will be a limiting resource. Therefore, the stands with reduced density may not be able to produce enough fertile tillers to account for what was removed.

2 | Materials and Methods

2.1 | Site characterization

The study was conducted at two locations from the University of Wisconsin-Madison Agricultural Research Stations (ARS): Lancaster (42°49'48.5" N, 90°47'19.1" W) and West Madison (43°03'42.2"N 89°31'54.1"W). The soil at Lancaster was a Fayette silt loam, mildly eroded phase (Fine-silty, mixed, superactive, mesic Typic Hapludalfs) and at West Madison was Plano silt loam, gravelly substratum (Fine-silty, mixed, superactive, mesic Typic Argiudolls) both with 2 to 6% slopes (NRCS-USDA, 2022).

For this study, four 3-4 year old Kernza IWG stands were used (Table 1). At each location experiments were established in two years (2021 and 2022). Three fields were evaluated for one year (referred as LA22-1, WM21-1, WM22-1) and one field was evaluated over two consecutive years (LA21-1, LA22-2). The Lancaster site was planted on April 23, 2018 with TLI-Cycle 4 IWG from grain harvested from the Lancaster ARS in August 2018. Seed was planted using a Great Plains 1006 no-till grain drill (Great Plains Manufacturing, Salina, KS, USA) at 10.6 kg ha^{-1} pure live seed in 38-cm row spacing. The previous crop was corn (*Zea mays* L.) harvested for silage. The site was fertilized with 56 kg N ha , 45kg P_20_5 ha^{-1}, and 84 kg ha^{-1} K_20 on April 26, 2018. To manage broadleaf weeds, the field was sprayed with 2.4-D amine at 1.07 kg ae ha^{-1} on July 6, 2018. From mid-July through October 2018 the field was mowed five times to reduce foxtail (*Setaria faberi L.*) densities. On May 5, 2019, the field was cut to a height of 10-cm for a spring forage harvest. Because of vernalization requirements (Locatelli et al., 2021), the first Kernza grain harvest occurred on July 30, 2019. A 0.5m^2 quadrat sample per plot was taken by hand by cutting and removing spikes and then cutting the remaining forage to a 10-cm height. Samples were dried at 47 °C. In fall 2020, soils were analyzed at the UW-Madison Soil and Forage Analysis lab where Bray-1 P and K were 18 and 127 ppm respectively and NO3-N was 5.1

ppm. Average soil pH of 6.4 and average organic matter (OM) of 2.4%. On March 22, 2021, 22 kg N ha^{-1}, 106 kg P_2O_5 ha^{-1}, and 213 kg ha^{-1} K_2O were applied. Rates were determined using the weight of the fertilizer, ratio of nutrients in the fertilizer, and area where the fertilizer was applied.

The WM21-1 study was planted on Sep 15, 2018 with cycle 5 IWG germplasm from The Land Institute (TLI, Salina, KS) from grain harvested from Arlington ARS in WI in August 2018. The previous winter wheat (*Triticum aestivum* L.) crop was harvested and glufosinate was applied with a Miller Pro 76cm boom sprayer at a rate of 0.70 kg ae ha^{-1} on September 6, 2018. The field was tilled with a field cultivator and was planted at 17.4 kg ha^{-1} with a 3-m wide International Harvester grain drill (International Harvester of Canada Limited, Hamilton, ON, CA) with 36-cm wide row spacing. In August 2019, the experiment was hand-harvested because of heavy infestation of quack grass (*Elymus repens* L.) which prevented harvest by combine. On July 28, 2020, the Kernza grain was harvested by combine and remaining straw was cut on July 30, 2020. Soil tests in fall 2020 showed an average soil pH of 6.9 and average organic matter (OM) of 3.0 %. Average P, K, and NO_3-N concentration was 20, 138, and 3.0 ppm, respectively.

The IWG stand in the WM22-1 study was planted on September 18, 2019. The previous crop was alfalfa. In early September, the field was prepared using a moldboard plow, Landoll vertical tiller, and a cultipacker. TLI-Cycle 5 seed was planted with a 3-m wide International Harvester grain drill in a 19-cm row spacing. On May 12, 2020, 2-4 D was applied at 1.07 kg ae ha^{-1} to manage broadleaf weeds. Grain was harvested by combine on July 28, 2020 and the remaining straw was baled on July 30, 2020. In fall 2020, soil samples 15cm deep were collected. Results of the analysis are shown on Table 2. On May 14, 2021, urea N fertilizer was applied at 185 kg ha^{-1}. Grain was harvested by combine on Aug 20, 2021. The Kernza IWG stands at sites LA21-1, WM21-1, and WM22-1 were 3 years old and LA22-1 was 4 years old when treatments were applied.

Table 1. Description of Kernza IWG establishment and dates for treatment applications in the nitrogen fertility and thinning study near Lancaster and West Madison, WI from 2018-2022.

	Lancaster		West Madison	
Factor	LA21	LA22	WM21	WM22
Planting date	April 23, 2018	April 23, 2018	September 15, 2018	September 20, 2019
Planting density (pure live seed)	10.6 kg ha^{-1}	10.6 kg ha^{-1}	17 kg ha^{-1}	11 kg ha^{-1}
IWG germplasm	TLI-C4	TLI-C4	TLI-C5	TLI-C5
N fertilization treatment date	March 29, 2021 April 11, 2022	April 11, 2022	March 29, 2021	April 4, 2022
Thinning treatment date	April 5, 2021	April 27, 2022	April 14, 2021	April 27, 2022
Harvest date	July 30, 2019 August 4, 2020 August 3, 2021 July 29, 2022	July 30, 2019 August, 2020 August, 2021 July 28, 2022	August, 2019 July 28, 2020 August 4, 2021	July 28, 2020 August 20, 2021 July 27, 2022

Table 2. Average pH, OM% (organic matter), P, K, and NO_3-N ppm in soil samples at each site. Soil was sampled to a 10 cm depth and was collected on October 14, 2020, the fall before 2021 N fertilization and thinning treatments were applied.

	Lancaster		West Madison	
Metric	LA21	LA22	WM21	WM22
pH	6.4	6.4	6.9	6.6
OM (%)	2.4	2.4	3.0	3.2
P (ppm)	18	18	20	27
K (ppm)	127	127	138	145
NO_3-N (mg/kg)	5.1	5.1	3.0	6.6

Climate records were obtained from the National Oceanic and Atmospheric Administration database (NOAA, 2022). Temperatures varied little among years and locations. At Lancaster, 30-year average monthly precipitation was 72mm. From 2018-2022, average monthly precipitation was 90, 95, 65, 79, and 72mm, respectively (Figure 4). At West Madison, 30-average monthly precipitation was 75mm. From 2018-2020, average monthly precipitation was 107, 98, 82, 48, 85mm (Figure 4). West Madison in 2021 had the driest growing season of the study with only 48mm per month of average precipitation. No sites were irrigated.

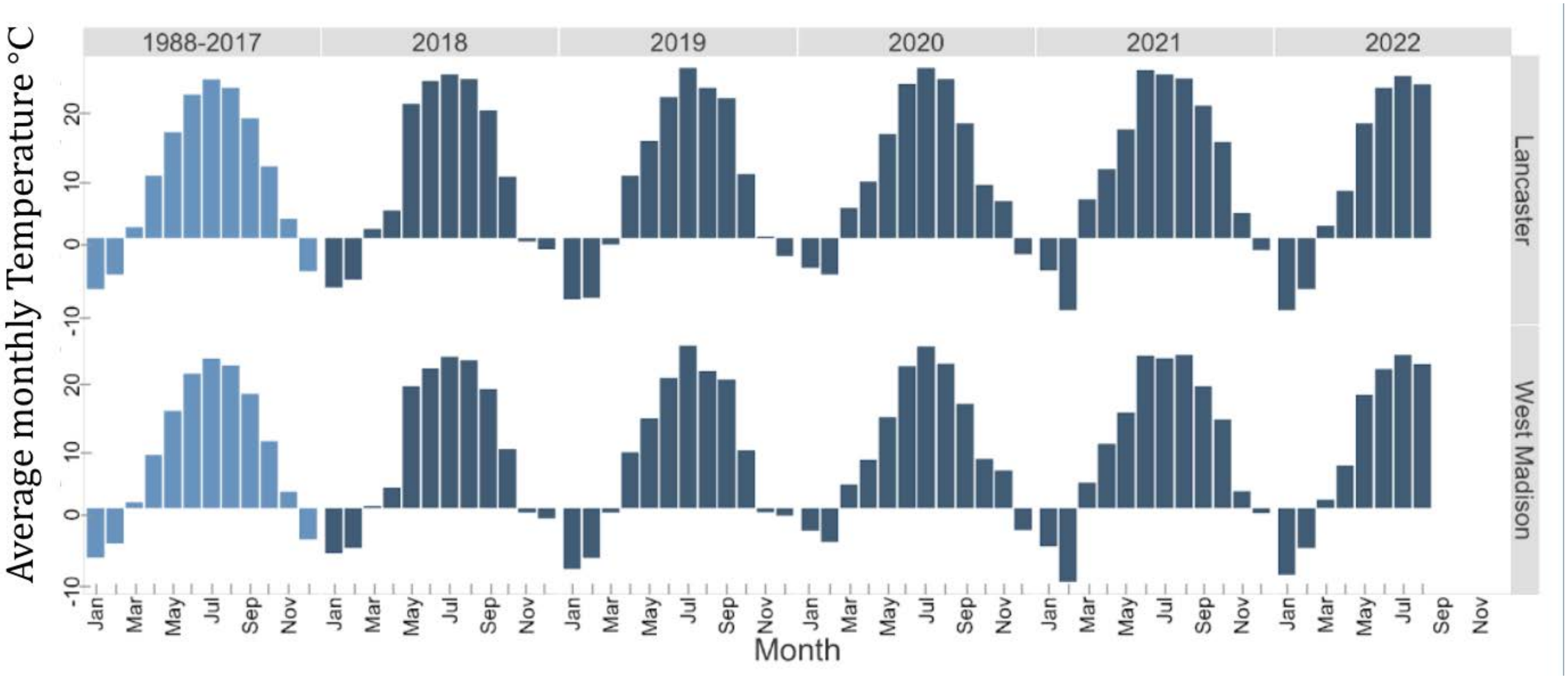

Figure 3. Average monthly temperature (°C) for 30-year average (1988–2017) and over the course of study at the 1) University of Wisconsin-Madison Lancaster Agricultural Research Station near Lancaster, WI and 2) University of Wisconsin-Madison West Madison Agricultural Research Station (WMARS) near West Madison, WI.

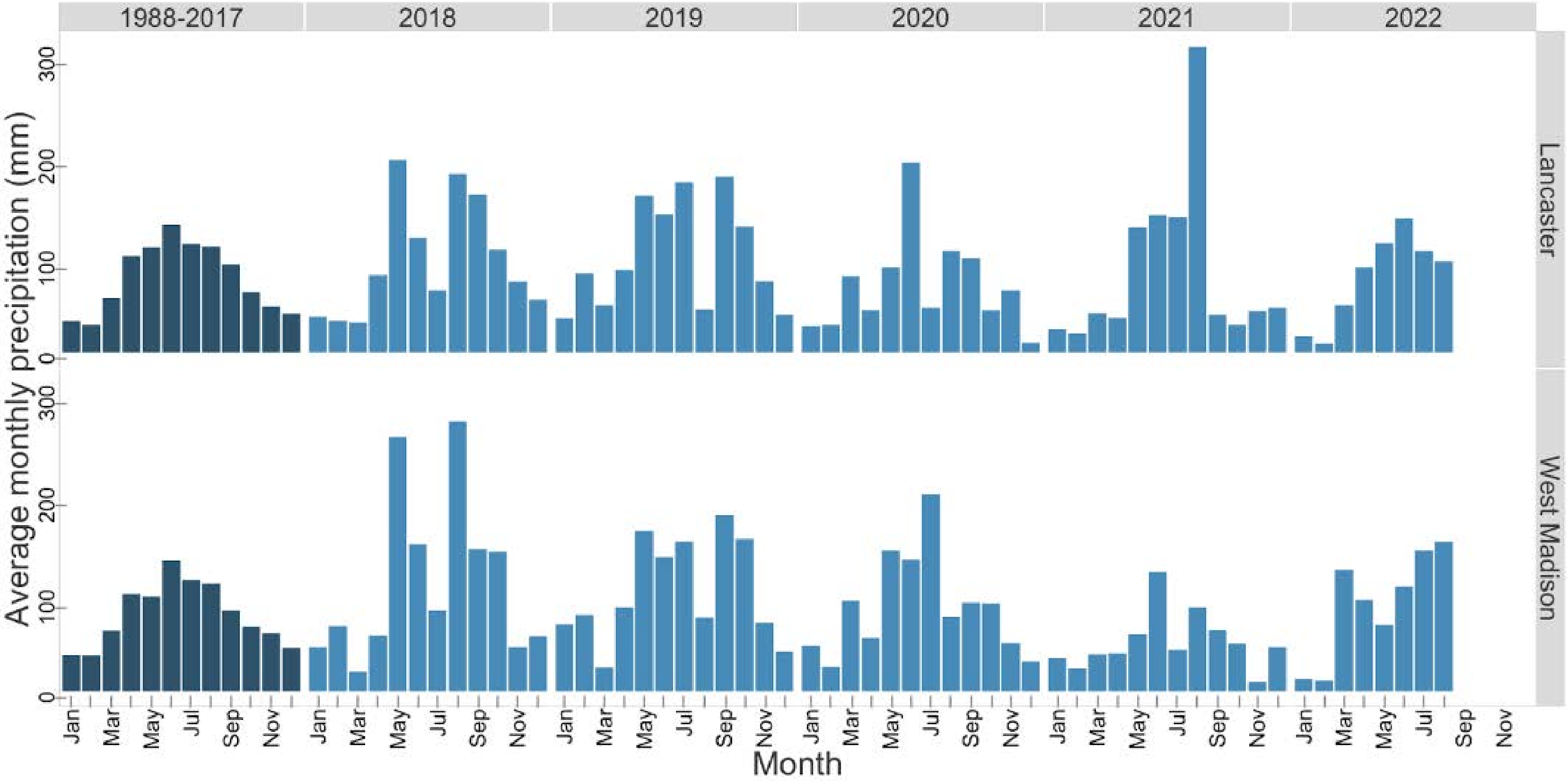

Figure 4. Average monthly precipitation (mm) accumulation for 30-year average (1988–2017) and over the course of study at the 1) University of Wisconsin-Madison Lancaster Agricultural Research Station near Lancaster, WI and 2) University of Wisconsin-Madison West Madison Agricultural Research Station (WMARS) near West Madison, WI.

2.2 | Study setup and design

Experimental design was a randomized complete block with four replications. Treatment design was a full factorial of two factors: N spring fertilization and Kernza IWG stand thinning. The N spring fertilization factor had three levels: 0 , 75 , and 150 kg N ha^{-1}. The thinning factor had four levels: 0, 2, 3, and 4 rows of IWG removed by herbicide per plot, reducing the stand by 0, 25, 38, and 50%, respectively. At Lancaster, new average row spacing was 38, 51, 57, and 76-cm, respectively and at West Madison, 36, 47, 53, and 71 cm, respectively. The nontreated controls were not fertilized or thinned. Plot size in each location was defined based on the initial row spacing of the Kernza planting to include 8 rows per plot. Therefore, plot size was 6.1 x 3.0 m ($l\ x\ w$) at Lancaster and 4.6 m x 2.8 m ($l\ x\ w$) at West Madison. Plots were separated by 1-m wide alleys maintained with mowing.

Nitrogen was applied as 44-0-0 ESN poly-coated urea. At Lancaster, a 3-m wide Gandy spreader was used to apply fertilizer at green-up (LA21-1 on March 29, 2021 and LA22-1 on April 11, 2022). At West Madison, a one pass of a 0.9-m wide Gandy push spreader (The Gandy Company, Owatonna, MN, USA) was used to apply the same fertilizer at spring-green up (WM21-1 on March 29, 20221 and WM22-1 on April 4, 2022).

After 15 cm growth occurred in the spring, thinning treatments were implemented by applying glyphosate mixed using a backpack sprayer. Per plot, 0, 2, 3, and 4 rows out of 8 rows of IWG were sprayed for a total of 0, 25, 38, and 50%, respectively, of the stand thinned (Figure 5). Intermediate wheatgrass mortality (100%) was visually assessed 2 weeks after glyphosate application. The LA22-2 site was the only one evaluated one year after thinning treatments were applied. N fertilization treatments were reapplied on April 11, 2022, as described above, while thinning treatments were not reapplied.

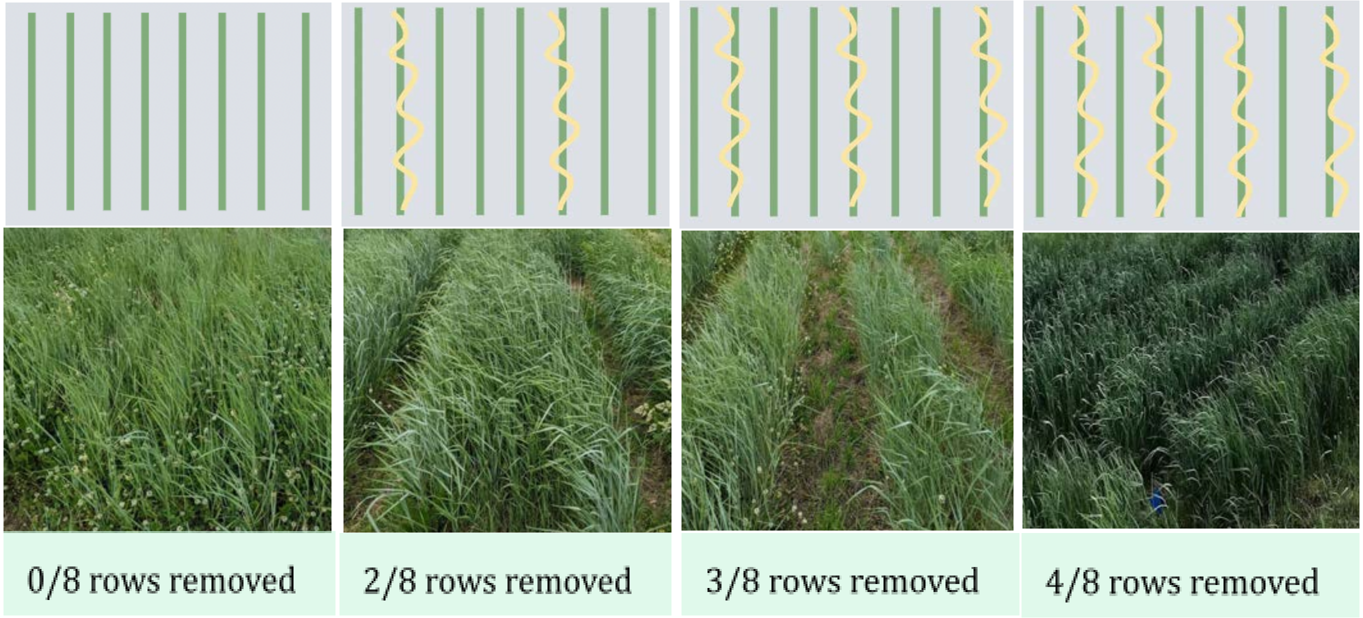

Figure 5. Illustration of the four levels of thinning used in this study. Yellow lines are the rows thinned using chemical thinning while green lines show rows remaining. Photos below the thinning treatments show an example of each thinning treatment from the West Madison Agricultural Research Station in Wisconsin in 2021. Images by Erica Shoenberger.

2.3 | Data collection

Grain and forage samples were collected by hand-harvesting Kernza spikes and biomass with a sickle at physiological maturity. Because of the difference in row spacing in each location, one 0.3 m x 1.5 m quadrat at Lancaster and one 0.3 m x 1.4 m quadrat at West Madison was harvested per plot to include 4 rows within the quadrat. In thinning treatments with three of eight rows removed or 38% of the stand thinned, two quadrats per plot was harvested. Kernza spikes from all tillers within the quadrat were cut. Spikes were dried at 47 °C for 10 days and threshed using a laboratory thresher to estimate grain yield. Forage and weeds were cut to 10 cm above the soil, separated, dried at 47 °C for 10 days, and weighed to calculate dry biomass of Kernza and weeds. After quadrat samples were taken, the remainder of the IWG stands were cut and biomass was removed from the plots. Dry matter yields ha^{-1} and m^{-1} of row were extrapolated from quadrat data. Seed mass was determined by counting and weighing 100 dry and de-hulled grains to determine average weight per grain. Harvest index was calculated by dividing the mass of the dry grain by the total mass of the dry grain and forage.

2.4 | Statistical analysis

Kernza IWG grain yield, seed mass, forage yield, weed biomass, and HI were tested for normal distribution and homogeneity of variance by assessing residual plots. All data were analyzed using one way ANOVA in RStudio, version 2022.07.1 (RStudio, PBC., Boston, MA). Grain yield ha^{-1}, weed biomass ha^{-1}, grain yield m^{-1} of row, and HI were square-root transformed while grain size was square-transformed to satisfy ANOVA assumptions and back-transformed for presentation.

The *lmer* function from the *lme4* package was used to analyze the linear mixed model with all treatments, location, harvest year, and study year (years since the application of thinning treatments), as fixed effects and block (nested within location) as a random effect. Interactions with all fixed effects were evaluated and analyzed separately if significant at $\alpha = 0.05$. Post-hoc mean comparison was conducted using Tukey honest significant difference (HSD) test at $\alpha = 0.05$.

The data for four sites in the year of thinning were pooled in the Model 1 where Y_{ijklm} = Kernza grain yield, forage yield, weed biomass, grain size, or HI; $\boldsymbol{\mu}$ = the overall mean; L_i = effect of location; N_j = effect of N fertilizer application; T_k = effect of thinning; H_l = effect of harvest year; B_m = block; E_{ijklm} = random residual.

$$Y_{ijklm} = \boldsymbol{\mu} + L_i + N_j + T_k + H_l + B_m + L_i{*}N_j + L_i{*}T_k + L_i{*}H_l + N_j{*}T_k + N_j{*}H_l + T_k{*}H_l$$
$$+ L_i{*}N_j{*}T_k + N_j{*}T_k{*}H_l + L_i{*}T_k{*}H_l + L_i{*}N_j{*}T_k{*}H_l + E_{ijklm} \qquad [1]$$

Because there was only one site to study the year after thinning, Lancaster sites LA22-1 and LA22-2 were analyzed together to account for study year effects on yield using Model 2: where: Y_{ijk} = Kernza grain yield, forage yield, weed biomass, grain size, or HI; $\boldsymbol{\mu}$ = the overall mean; S_i = effect of study year; N_j = effect of N fertilizer application; T_k = effect of thinning; B_m = block; E_{ijkm} = random residual.

$$Y_{ijk} = \boldsymbol{\mu} + S_i + N_j + T_k + B_m + S_i{*}N_j + S_i{*}T_k + N_j{*}T_k + S_i{*}N_j{*}T_k + E_{ijkm} \qquad [2]$$

3 | Results

3.1 | Effects of location and harvest year

The following analysis was evaluated using Model 1 which includes four sites harvested the summer after thinning treatments were applied. Grain and forage yield were affected by the location and by the harvest year (Table 3). Grain yields in Lancaster were higher than in West Madison, on average 549 and 146 kg ha^{-1}, respectively (Table 4). There was a location x harvest year interaction for grain yield ($p<0.01$; Table 3). Grain yields were higher in LA21-1 than LA22-1 which makes sense because these sites are the same fields aging (IWG stand age is 3 and then 4 years, respectively) (Table 4). Conversely, WM22-1 was higher than WM21-1 because WM21-1 was a poor stand and was harvested after physiological maturity when some of the grain had shattered and was not able to be collected for analysis. Forage yields were also generally higher in Lancaster than West Madison at 5070 and 3112 kg ha^{-1}, respectively (Table 4). Average weed biomass did not differ over the location or course of study. Further, HI was an average 0.09, except for West Madison in 2021 which was an average of 0.02.

Table 3. P-values for the main effects of location, N fertilizer, thinning, and harvest year, and their interactions for all grain and forage yield, weed biomass, grain size, and harvest index in the year of thinning and N fertilization treatment applications at four environments in two locations (Lancaster and West Madison, Wisconsin, USA) and two years (2021 and 2022).

Variables	Grain			Forage		Weed biomass	Harvest Index
	kg ha^{-1}	g m^{-1} of row	mg seed^{-1}	kg ha^{-1}	g m^{-1} of row	kg ha^{-1}	
Location (L)	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**0.02**	**<0.01**
N fertilizer (N)	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.60	**<0.01**
Thinning (T)	**0.02**	**<0.01**	0.54	**<0.01**	**<0.01**	**<0.01**	0.53
Harvest year (H)	**0.03**	**0.03**	0.93	**0.01**	**0.03**	0.44	**<0.01**
L x N	**0.01**	**<0.01**	0.78	0.51	0.33	0.68	0.25
L x T	0.91	**0.02**	0.45	0.84	**<0.01**	0.13	0.61
N x T	0.82	0.76	0.53	0.51	0.51	0.66	0.31
L x H	**<0.01**	**<0.01**	**<0.01**	0.47	0.42	0.84	**<0.01**
N x H	0.47	0.34	0.31	0.52	0.62	0.28	0.73
T x H	0.12	0.15	0.65	**0.03**	0.16	**<0.01**	0.68
L x N x T	0.81	0.76	0.86	0.88	0.90	0.86	0.42
L x N x H	0.53	0.58	0.93	0.99	0.97	0.63	0.62
L x T x H	0.67	0.40	**<0.01**	0.74	0.75	**<0.01**	0.67
N x T x H	0.25	0.23	0.99	0.83	0.94	0.19	0.15
L x N x T x H	0.93	0.93	0.45	0.94	0.87	0.91	0.50

Table 4. Average Kernza IWG grain and forage yield (ha^{-1} and m^{-1} of row), grain weight, weed biomass, and harvest index in the first year after application of thinning and N fertilization treatments by location and harvest year, across all nitrogen and thinning treatments. Means followed by the same lower case letter within a column do not differ according to Tukey's HSD at the α = 0.05.

Site	Stand age	Grain			Forage		Weed biomass	Harvest Index
	yr	kg ha^{-1}	g m^{-1} of row	mg seed^{-1}	kg ha^{-1}	g m^{-1} of row	kg ha^{-1}	
LA21-1	3	636 a	34.6 a	8.3 a	5520 a	296 a	1209 a	0.10a
LA22-1	4	461 b	25.4 b	6.8 b	4619 b	254 b	1641 a	0.08a
WM21-1	3	71 d	3.2 d	4.7 d	3379 c	163 c	2144 a	0.02b
WM22-1	3	220 c	11.2 c	6.4 c	2852 c	143 c	2102 a	0.08a

3.2 | Effects of nitrogen

No interaction was found for N fertilizer applications and thinning treatments (Table 3), therefore the effects of N fertilization and the effects of thinning are described separately. A location x N fertilization effect was found for grain yield ha^{-1} and m^{-1} of row, so the effects are described by location. In Lancaster, grain yield increased with each level of N fertilizer applied from 319 kg ha^{-1} to 597 kg ha^{-1} and to 730 kg ha^{-1}, an increase of 87% and 129% from 0 to 75 to 150 kg N ha^{-1}, respectively (Figure 6). In West Madison, grain yield also increased from 0 to 75 kg N ha^{-1} applied, an increase of 145% from 0 N fertilizer applied (Figure 6). No differences were observed between 150 kg N ha^{-1} and 75 kg ha^{-1}.

Average forage yields, HI, and seed size per level of N fertilization are reported because there was no location interaction (Table 3). Forage yield was 2,709 kg ha^{-1} with 0 N fertilizer applied. Yields with 75 and 150 kg N ha^{-1} do not differ, so average of both was 4,590 kg ha^{-1}, an increase of 70% (Figure 7A). HI was greater from 0.05 with 0 N fertilizer applied to an average of 0.08 with 75 and 150 kg N ha^{-1} applied (Figure 7B). Grain size followed the same trend with the smallest seed size of 5.9 mg seed^{-1} when 0 N fertilizer was applied and an average of 7.0 mg seed^{-1}, an increase of 19%, when 75 or 150 kg N ha^{-1} was applied (Figure 7C).

N fertilizer applications showed similar trends in the second year as the first year. Grain yield was 289 kg ha^{-1} with 0 N fertilizer applied and was 663 kg ha^{-1} with 75 or 150 kg N ha^{-1} applied, an increase of 129%. Similarly, forage yield ha^{-1} was 3,580 with 0 N fertilizer and 6,260 with 75 or 150 kg N ha^{-1}, an increase of 75%. HI was 0.07 with 0 N fertilizer and 0.10 with 75 or 150 kg N ha^{-1}, an increase of 43%. Weed biomass was not affected by N fertilizer. Grain size was 13% greater with 75 or 150 kg N ha^{-1} applied (6.4 to 7.2 mg seed^{-1}, respectively).

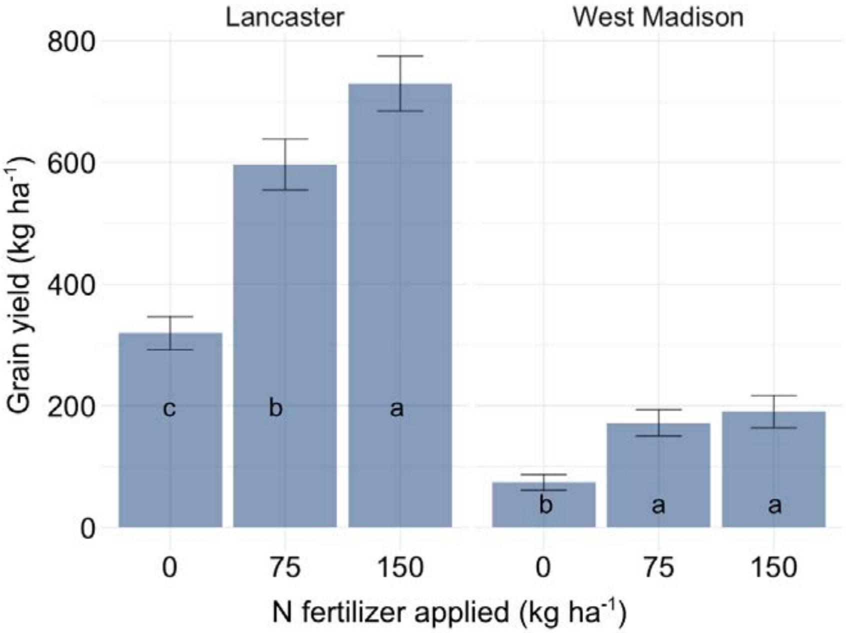

Figure 6. First year mean values of grain yield per rate of N fertilizer at near Lancaster and West Madison, WI, USA. Bars within a mean group with the same lower case letter do not differ according to Tukey's HSD at the α = .05.

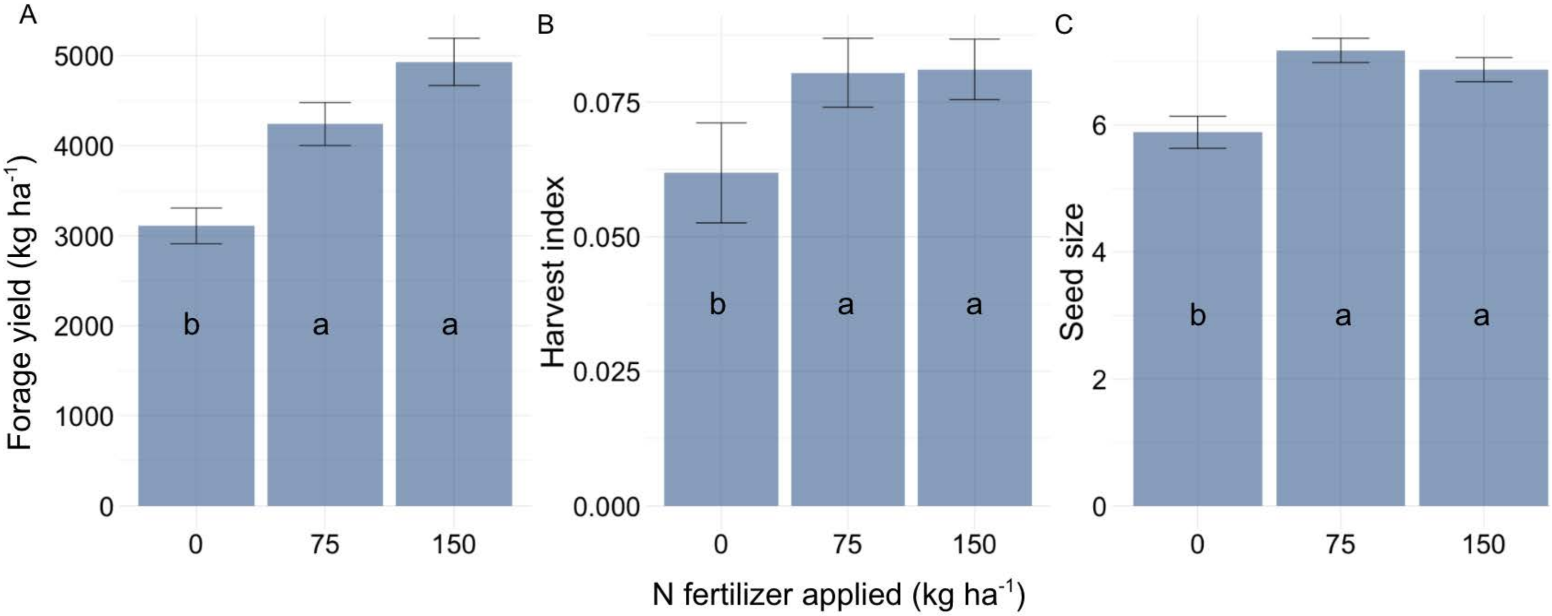

Figure 7. First year mean values of A) forage yield, B) harvest index, and C) seed size per rate of N fertilizer averaged across two locations in Wisconsin, USA. Bars within a mean group with the same lower case letter do not differ according to Tukey's HSD at the α = .05.

3.3 | Effects of thinning in the first study year

Thinning affected all variables except for seed size and HI (Table 3). No location interaction was found, so an average of both locations is reported. From the non-thinning control to maximum thinning (50% stand thinned), grain yield decreased by 21%, from 387 to 305 kg ha^{-1}, respectively (Figure 8). Yields for moderate thinning (25 and 38% stand thinned) did not differ from the non-thinning control or maximum thinning.

There was a location x thinning interaction for grain yield per row (Table 3), so they are described separately. In Lancaster, the non-thinned control yield was 22.8 g of grain per m of row. With 38% stand thinning, yield was 31.5 g m^{-1} of row, 38% greater than the non-thinned control. With 50% stand thinning, yield was 37.9 g m^{-1} of row, 66% greater than the non-thinned control. Stand thinning had no effect on grain yield per row at West Madison. As expected, maximum stand thinning had the highest grain yield per row compared to non-thinned control at Lancaster. There was a harvest year x thinning interaction for forage yield ha^{-1} (Table 3) where forage yield ha^{-1} differed in 2021 and did not differ in 2022. In 2021, when 25 and 50% of the stand was thinned compared to the non-thinned control, yield was 18 and 42% lower, respectively. Yield for 38% thinned did not differ from 25 or 50% thinned.

Though average weed biomass did not differ throughout the study, a three-way interaction, location x thinning x harvest year, was found (Table 3). In both years at Lancaster and at West Madison in 2022, no treatments affected the weed biomass. However, in West Madison 2021, weed biomass was 248 kg ha^{-1} with no thinning and 1,782 and 3,817 with 25 and 50% thinned, respectively. The 38% thinned treatment did not differ from 25 or 50% thinned.

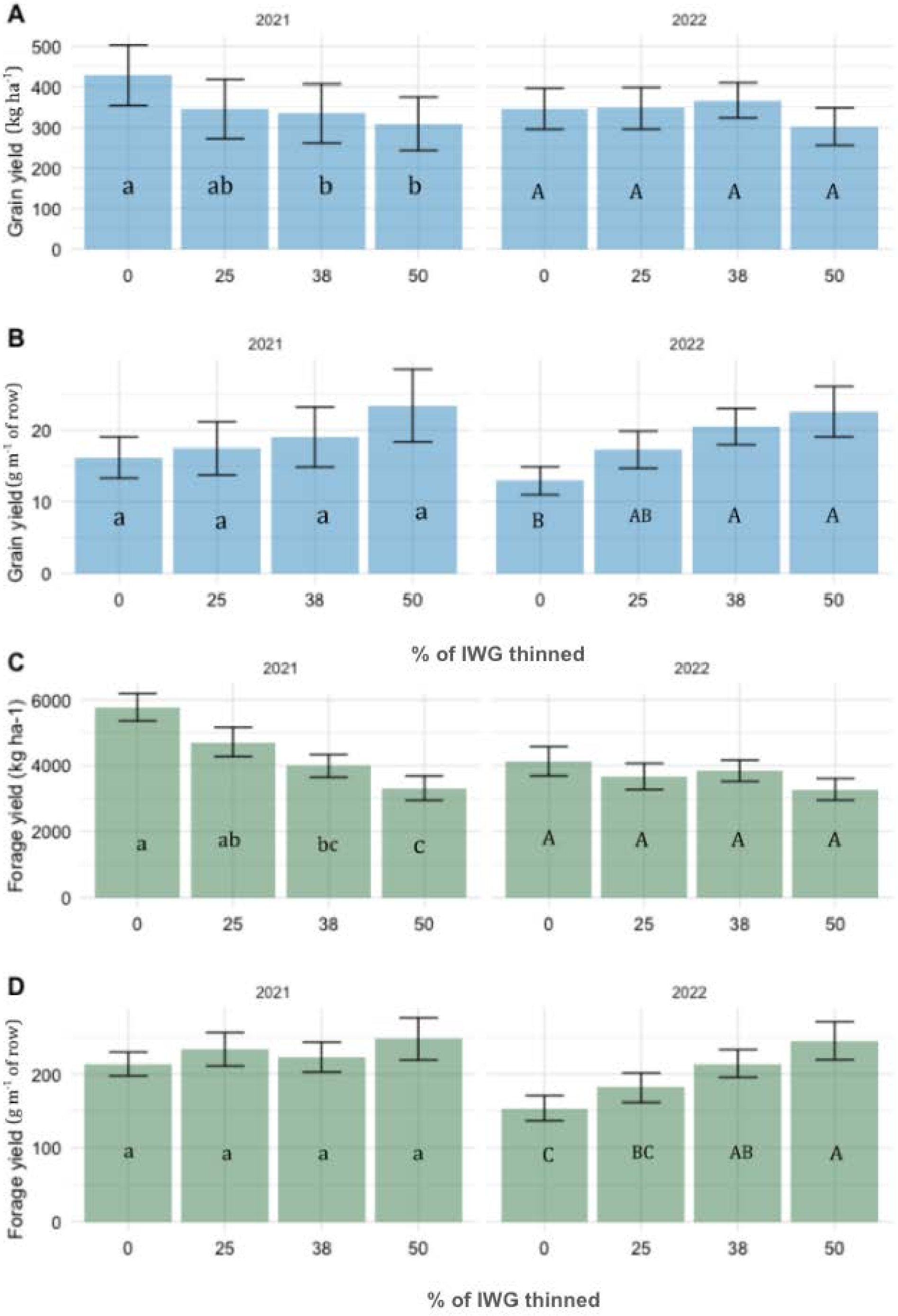

Figure 8. First year mean values for A) grain yield kg ha^{-1}, B) grain yield g m^{-1} of row, C) forage yield kg ha^{-1}, and D) forage yield g m^{-1} of row per level of thinning treatment at two locations in Wisconsin, USA. Bars within a mean group and harvest year with the same letter do not differ according to Tukey's HSD at the α = .05. Yield shown by harvest year (2021 and 2022) because of a two-way harvest year x thinning interaction.

3.4 | Effects of thinning in the second study year

In the first study year, there was no increase in yields with thinning. On the other hand, in the second study year, using only LA21-1 and LA22-2 environments, maximum thinning had 47% greater grain yield than the non-thinning control from 449 to 660 kg ha^{-1}, respectively (Figure 9A). In the first study year, grain yield m^{-1} of row was 78% greater with maximum thinning relative to non-thinned control. This effect was amplified in the second study year, where grain yield m^{-1} of row was 194% greater than the non-thinned control (Figure 9B).

There was no difference in forage yields ha^{-1}, though there was a difference in forage yield m^{-1} of row among study years (Figure 9C & D). In the first study year, forage yields m^{-1} of row were an average of 206 g m^{-1} with the non-thinning control and 335 g m^{-1} with maximum thinning , an increase of 63% (Figure 9D). In the second year, forage yields m^{-1} with maximum thinning increased from 204 to 402 g m^{-1} , an increase of 97% relative to non–thinning control (Figure 9D).

Weed biomass did not differ among all variables. There was a two-way interaction for HI between study year x thinning. HI did not differ among thinning treatments in the first study year. Conversely, HI was 33% greater in the second study year, from 0.08 with non-thinned control to 0.11 with maximum thinning.

Table 5: P-values for the main effects and interactions for all yield components, grain size, and harvest index in the year of and year after application of thinning treatments near Lancaster, Wisconsin, USA. P-values are significant at α = 0.05.

Variables	Grain			Forage		Weed biomass	Harvest Index
	kg ha^{-1}	g m^{-1} of row	mg $seed^{-1}$	kg ha^{-1}	g m^{-1} of row	kg ha^{-1}	
Study year (S)	0.05	0.02	0.21	0.10	0.05	0.84	0.70
N fertilizer (N)	<0.01	<0.01	<0.01	<0.01	<0.01	0.67	<0.01
Thinning (T)	0.40	<0.01	0.29	0.88	<0.01	0.54	0.08
S x N	0.44	0.38	0.55	0.24	0.24	0.57	0.23
S x T	0.13	0.05	0.64	0.63	0.49	0.69	0.04
N x T	0.71	0.64	0.56	0.70	0.57	0.58	0.68
S x N x T	0.63	0.66	0.15	0.86	0.93	0.75	0.67

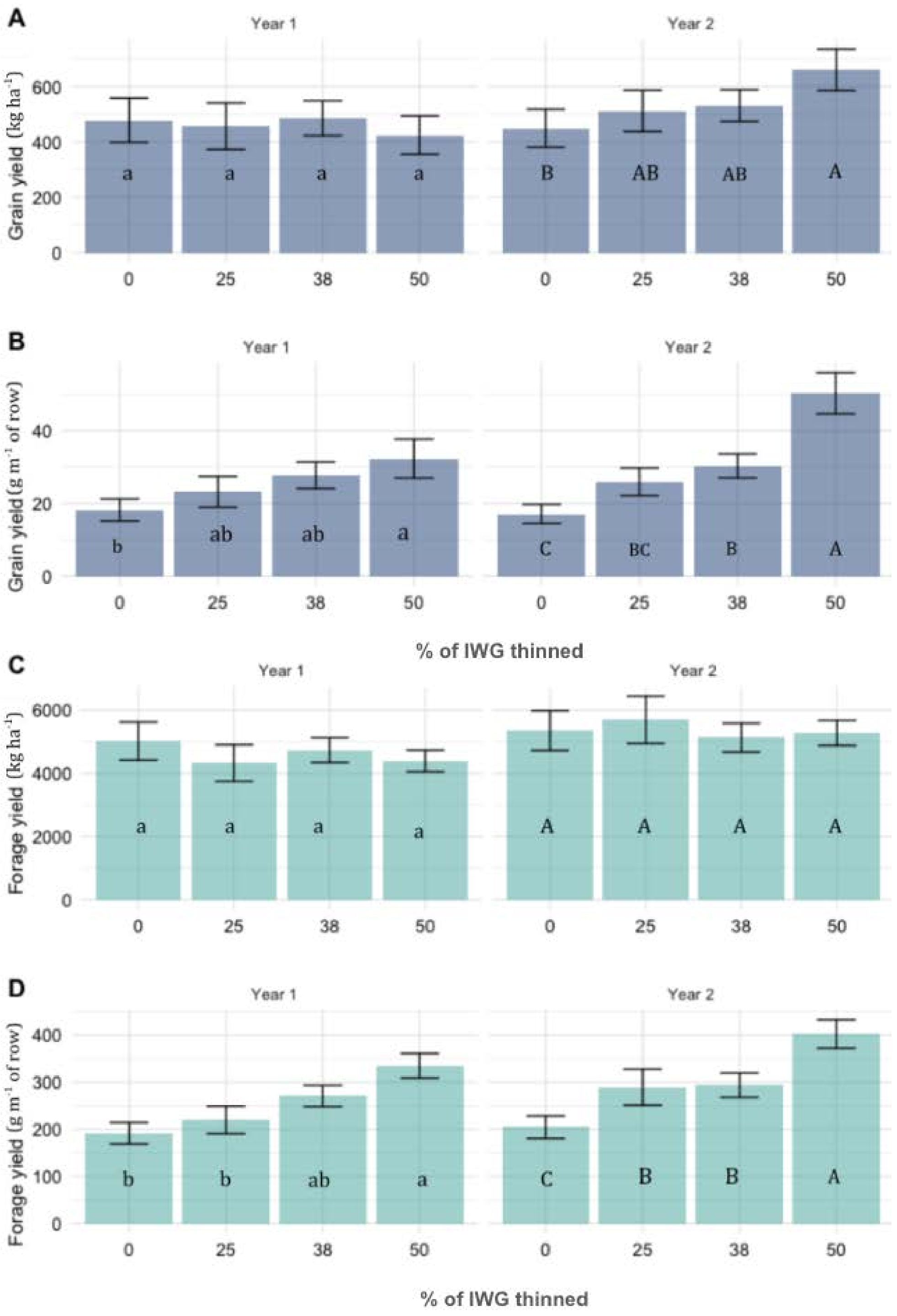

Figure 9. First and second study year comparison showing mean values per thinning treatment of: A) grain yield kg ha^{-1}, B) grain yield g m^{-1} of row, C) grain yield kg ha^{-1}, D) grain yield g m^{-1} of row near Lancaster, Wisconsin, USA. Bars within a mean group and study year with the same letter do not differ according to Tukey's HSD at the α = .05.

4 | Discussion

4.1 | Effects of nitrogen fertilization

Though the effects of N fertilization on grain yield differed slightly between sites, grain yield was consistently greater in N treated compared to no N treated plots. With 75 kg N ha^{-1} fertilizer application, grain yield was 87 and 145% greater in Lancaster and West Madison, respectively, compared to the non-treated control. It is likely that the West Madison site responded more dramatically to N fertilizer applications because only one West Madison site received an N fertilizer application previous to beginning of the study (WM21-1) while both Lancaster sites received two fertilizer applications. As root biomass increases and reduces available soil inorganic N in years after establishment, N applications are needed to recharge soil nutrient pools to maintain grain yields (Pugliese et al., 2019; Fernandez et al., 2020). Average nitrate-N concentration was 3.0 at WM21-1, 6.0 at WM22-1, and 5.1 at LA21-1 and LA22-2 in September 2020 before fertilizer applications. Even though these were shallow soil samples (15cm) compared to the deep root system of Kernza IWG, these samples are all quite low in plant-available N (10-50 mg kg^{-1} nitrate-N is considered a healthy range), so it is unsurprising that we saw a significant response to N application (Pattison et al., 2010). Grain yield responses to N noted above are less extreme than a 2020 study where grain yields were 217% greater in year 2 and 240% greater in year 3 of production when 80 kg N ha^{-1} was applied compared to no N treatments (Fernandez et al., 2020). This difference could be from differences among fertilizer application timing, Kernza IWG variety, site history, and climate.

The Lancaster site responded with a 22% grain yield increase from 75 to 150 kg N ha^{-1} while West Madison grain yield did not respond. This observation is consistent with another study in Wisconsin that demonstrated that grain yield was 16% greater with 135 kg N ha^{-1} compared to 90 kg N ha^{-1} (Zimbric et al., 2020). Researchers in Minnesota determined that the agronomically optimum range (AOR) of N fertilization for an older variety of Kernza IWG, TLI-C2, is between 61 and 96 kg N ha^{-1} (Jungers et al., 2017). Even though a high level of N fertilization occasionally increases grain yield more than application in the AOR, because of the relatively low grain yield increase proportional to the fertilizer applied, it may be more cost effective to apply the N fertilizers within the AOR as recommended by Jungers et al (2017).

A past study determined that the proportion of fertile tillers was the best predictor of grain yield and was not affected by N fertilizer application (Fernandez et al., 2020). We did not determine the number of fertile tillers in this study, so we cannot say whether or not our observations support this study. But in the first study year, we observed a 19% seed size increase with N fertilizer applications (Figure 7C). So, because we saw a general grain yield increase and seed size increase with N fertilizer, it is possible that even though the fertilizers may not have increased the number of fertile tillers, they may have increased the number of seeds per fertile tiller and size of the seed leading to a higher grain yield.

Further, forage yield increased by 70% with fertilizer applications, but there was no difference in forage yield between 75 or 150 kg N ha^{-1}. Summer forage harvests in a 2019 study showed a forage yield approximately 3,500-6,500 kg ha^{-1} in nontreated plots, a similar observation to the nontreated plots in our study (Pugliese et al., 2019). Another study found that summer above ground biomass in Kernza IWG did not differ with N fertilizer rates of 90 and 134 kg ha^{-1}, again mirroring what we observed in this study (Sakiroglu et al., 2020).

The HI also increased from 0.05 to 0.08 with N fertilizer applications compared to 0 fertilizer, but there was no difference between 75 or 150 kg N ha^{-1} applied. Harvest index is a measure of reproductive efficiency, so an increase in HI is indicative of an increase in grain yield as a percentage of the total weight of the crop. Past researchers have noted that HI decline in Kernza IWG overtime indicates that resources are not limited across the entire stand because the total biomass production tends to be steady or increase from season to season even as HI declines (Law, et al., 2020). Essentially, biomass tends to stay the same or increase while grain yield declines with stand age. Even with increased forage yield with a N fertilizer application (Figure 7A), we still saw an increase in HI indicating that regular N fertilizer applications in aging Kernza IWG stands can help ameliorate grain yield and HI decline overtime.

4.2 | Effects of thinning the first year after row thinning

Overall, spring thinning made no difference in HI and either made no difference or caused overall yield decline in the first study year post-thinning. In 2021, grain and forage yield ha^{-1} declined by 21 and 44%, respectively, with 4 rows thinned relative to the non-thinned control while grain and forage yield m^{-1} row did not differ. On the other hand, in 2022, grain and forage yield ha^{-1} did not differ while grain and forage yield m^{-1} row increased by 70 and 61% with 4 rows thinned relative to the non-thinned control.

Results from 2022 indicate that thinning reduced competition among the reproductive tillers resulting in higher grain yield per row (Figure 4A, B). These results are similar to a strip-tillage study which showed that spring thinning reduced stand density and competition among reproductive tillers with no difference in overall yield (Law et al, 2020). Though results from 2021 show that thinning decreased overall yield from non-thinned control to maximum thinning (50% thinned) 4 with no effect on yield m^{-1} row. This means that in 2021, stand density decreased and reduced competition did not result in higher grain yield per row. This could be related to the poor stand in the West Madison 2021 site that had extremely low grain yields at 71 kg ha^{-1} and HI of 0.02. This poor-performing stand also was the only site where weed biomass dramatically increased with thinning. This weed competition may have competed with the IWG post-thinning leading to an overall decrease in yield ha^{-1} and no change in yield

m^{-1}. Further, spring defoliation has been shown to stimulate vegetative tillers that directly compete with reproductive tillers (Aamlid et al., 1997; Clemence & Hebblethwaite, 1984). This competition appears to be more severe in 2021 than in 2022.

HI did not differ among thinning treatments in the first study year (Table 3). The total biomass of IWG decreased with thinning, but the harvest index remained the same which means that the proportion of grain to total biomass did not differ among thinning treatments.

4.3 | Effects of thinning the second year after row thinning

Counter to the first study year, grain yield ha^{-1} in the second study year increased by 47% while forage yield ha^{-1} did not differ with maximum thinning (50% thinned) relative to non-thinned control. Possible reasons for the highest grain yield in the most thinned treatment in the second study year is that the IWG had a fall and winter season post-thinning to produce more reproductive tillers, and consequently a greater grain yield the following summer.

One hypothesis for grain yield decline is that as a stand becomes more dense overtime, the red light that can penetrate the crown of the plant in the fall is reduced which decreases fertile tiller growth (Casal et al., 1985; Deregibus et al., 1985; Fernandez et al., 2020; Pinto et al., 2021). Essentially, thicker Kernza IWG canopies reduce the light needed for fertile tiller production which reduces tillering and consequently decreases grain yield (Fernandez et al, 2020). So, by thinning in the spring, the intraspecific competition in the stand was reduced in the fall, increasing the red light that could reach the crown and stimulate fertile tiller production. Further, primary induction in Kernza IWG occurs in the winter when reproductive tillers are produced, and secondary induction begins in the spring when reproductive tillers develop leading into flowering and seed development (Cooper & Calder, 1964; Heide, 1994; Duchene et al., 2021). When we thinned in the first study year, we did not see an overall grain yield increase because primary induction had already occurred. But by the second study year, grain yield increases in the most thinned treatment because intraspecific competition was reduced in the fall when fertile tiller production was stimulated and had a full winter season to undergro primary induction when the fertile tillers were produced.

4.4 | Weed dynamics

We were surprised to see that weed biomass did not differ across N treatments or in thinning treatments in three of four sites. The only site where weed biomass differed was already a poor Kernza IWG stand with low yields and competition from other perennial grasses. Several studies have cited generally low weed pressure in Kernza IWG systems with the greatest pressure being in establishment years and declining up to 91% in the years following (Dick et al., 2019; Olugbenle et al., 2021). Other studies have noted that weed biomass in Kernza IWG is generally low (Law et al., 2020; Sakiroglu et al., 2020). This study coupled with previous evidence that weed biomass in established Kernza IWG stands is low suggests that farmers need not be concerned with fertilization or thinning management practices leading to increased weed pressure unless the stand is already poorly established or in a site with pressure from other grass species.

5 | Conclusion

In this study, 75 kg N ha^{-1} was an adequate N fertilizer application for greatest grain and forage yield, HI, and seed size. It is beneficial for farmers to apply N in the spring to replenish soil nutrient pools and stimulate grain yields. Additionally, spring thinning treatments reduced or did not impact overall grain yield in the year of thinning, but treatments increased grain yield the year after thinning. Because of this, spring thinning treatments should be applied cautiously with farmers aware that grain yields may decline in the year of thinning but will increase in the second year. Future research should determine if fall thinning treatments increase grain yield the first year after they are applied. If this is the case, it may be most appropriate for farmers to thin the Kernza IWG stand in the fall so that they do not risk yield decline in the year of spring thinning and then apply N fertilizer in the spring to optimize summer grain yields.

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Chapter 2: Addressing the knowledge gap of synthetic auxin herbicide effects on Kernza intermediate wheatgrass *(Thinopyrum intermedium)* injury and grain yield

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Abstract

Kernza intermediate wheatgrass [IWG; *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is a cool-season perennial grass that has been bred as a dual-purpose grain and forage crop. One barrier to farmer adoption is that as of 2019, herbicides effects have not been studied nor have herbicides been labeled for use on IWG for Kernza grain production as of 2019. To evaluate herbicide effects, an experiment was conducted over two years (2019-2021) at sites in Wisconsin, Minnesota, New York, and North Dakota. The effects of broadleaf herbicides registered for use in wheat cropping systems 2,4-D amine, clopyralid, MCPA ester, and a mixture of MCPA + clopyralid were evaluated by measuring grain yield, crop injury, and weed control. Each herbicide was applied at 1X and 2X the labeled rate of application to newly planted and established (1-3 years old) IWG stands in the spring or in the fall. Applications were made before the boot stage in spring and at tillering in the fall. The application timing, herbicide type, and rate of application showed no effect on Kernza grain yield and or plant injury. Weed control ranged from 71 to 92% across herbicide treatments relative to the nontreated check at the WI site while weed control at the MN site was variable among treatments. Weed pressure was very low at NY and ND sites across all treatments. The results show that newly planted and established stands of Kernza IWG are tolerant to the synthetic auxin herbicides 2,4-D amine, clopyralid, and MCPA ester following application in the fall at tillering or in the spring before the boot stage. Synthetic auxins represent a potentially useful tool for weed suppression in Kernza IWG cropping systems, especially for problematic broadleaf weed species. However, herbicide use should be assessed in the context of tradeoffs with potential negative agroecosystem impacts.

1 | Introduction

Perennial crops can improve agricultural sustainability compared to annuals because their extensive root systems can sequester carbon, reduce soil erosion and nutrient leaching, and minimize pesticide requirements while simultaneously increasing farmer incomes due to decreased annual inputs and costs (Glover et al., 2010). The perennial cool-season forage grass, Kernza intermediate wheatgrass (IWG, *Thinopyrum intermedium L.*) has been bred for large seed size and grain yield and is the first perennial grain crop in the US (DeHaan et al., 2010). This grass has great potential as a human food and animal forage while providing environmental benefits. It has an extensive root system that has potential to limit nitrate leaching into groundwater, reduces soil erosion with year-round ground cover, and improves soil health (Culman et al., 2013; Jungers et al., 2019; Ryan et al., 2018; de Oliveira et al., 2020).

Commercial interest in IWG grain, i.e. Kernza® - has expanded in recent years (DeLage, 2015). Currently, the food industry demand for Kernza grain is greater than that produced by farmers. Used as a dual-purpose crop, farmers can harvest the forage as another revenue stream as a way to minimize risk solely from grain production. As a forage, IWG is productive, competitive with weeds, yields relatively high-quality forage, and is palatable to many types of livestock (Asay, 1996; Favrea et at., 2019; Hybner, 2012; Nelson et al., 1989; Zimbric et al., 2020). The forage can be harvested in early spring prior to elongation and/or after in the fall after the summer grain harvest. Having various potential uses improves Kernza IWG chances of increasing farmer income and farmer adoption.

Though many farmers are interested in growing this perennial grain for its ecological and economic benefits, weed management has been recognized as a considerable need and information gap in cultivating Kernza IWG (Lanker et al., 2020). Specifically, farmers are interested in herbicide options that can reduce weed impacts during critical Kernza IWG stand establishment and during early production years when needed (Lanker et al., 2020).

1.1 | Weed community dynamics in Kernza IWG systems

A recent study demonstrated that in the 3 years following Kernza IWG stand establishment, weed community composition transitioned from primarily winter annual species to perennial weed species when managed as a dual-use grain and forage system (Zimbric et al., 2020). This study also showed that from year 1 to 3 of the stand, above-ground weed biomass decreased by 88% regardless of the forage harvest schedule or N fertilization treatment. Another study focused on IWG and legume intercropping systems noted that Kernza IWG substantially suppressed annual broadleaf weeds and decreased above-ground biomass production by 91% from year 1 to year 2 (Dick et al., 2019). A later study on Kernza IWG intercropped with red clover (*Trifolium pratense* L.) showed that from year 1 to 2, weed biomass decreased by 76% at one site and by 83% at another (Olugbenle et al., 2021). This study also noted that, similar to Zimbric et al (2020), annual weed density decreased while perennial weed density increased over the life of the stand (Olugbenle et al., 2021). Other studies have noted that weed biomass in Kernza IWG is generally low (Law et al., 2020; Sakiroglu et al., 2020).

Farmers have specified that common weed species in their Kernza IWG fields are the perennial broadleaf species Canada thistle *(Cirsium arvense* L.*)*, red clover, the annual broadleaf species sweetclover *(Melilotus officinalis* L.*)*, and waterhemp *(Amaranthus tuberculatus* (Moquin-Tandon) Sauer) (Lanker et al., 2019). Though these species are prolific and competitive, the most concerning of these species are the aggressive and highly competitive species Canada thistle and red clover (Zimbric et al., 2020). The presence of these species has propelled interest in advancing herbicide options for weed management in Kernza IWG stands.

1.2 | Weed management options

Weed management in IWG systems is typically based on integration of cultural and mechanical methods. Recommendations are to plant in fields with low weed pressure, especially low pressure from perennial and highly competitive weeds that can become problematic throughout the life of the stand (DeHann et al. 2019). In cases with high winter annual weed density, timely mowing before Kernza IWG stem elongation occurs in the spring, is a recommended weed management tool (Zimbric et al., 2020). Otherwise, inter-row cultivation can be used to reduce weeds between rows if the row spacing is wide enough for the equipment. However, recommended planting practices encourage row spacing that is not wide enough for inter-row cultivation without risking damaged stands (DeHaan et al. 2019; Zimbric et al., 2019).

Herbicide efficacy on problematic weeds typically found in IWG cropping systems is well understood, but herbicide effects on IWG have not been studied. Registration of herbicides for use in Kernza IWG systems would have great potential to reduce weed competition in systems that do not allow for mechanical weed management. Specifically, this study assesses three synthetic growth regulator herbicides (synthetic auxins, Group 4) registered for use in wheat (*Triticum aestivum* L.) cropping systems to give farmers an alternative option for weed management: 2,4-D amine, clopyralid, and MCPA. Synthetic auxin herbicides are the most commonly used herbicides for broadleaf weed control since 1940's when the first type was introduced to the market (Busi et al., 2018). This group of herbicides are known as plant growth regulators that mimic the plant hormone, indole-3-acetic acid (Todd et al., 2022). Synthetic auxins are absorbed through the roots and foliage and disrupt cell formation resulting in altered growth, protein synthesis, cell division, and cell growth, the combination of which kills plants.

The synthetic auxin herbicides used in this study are registered for use in annual wheat systems, have good-excellent crop tolerance, and varying degrees of efficacy on winter and summer annual broadleaf weed species (Dewerff et al., 2019). Specifically, 2,4-D amine has good-excellent efficacy on several winter and summer annual broadleaf weed species, fair-good efficacy on dandelion, but only fair efficacy on Canada thistle and other perennial broadleaf species often found in small grain production systems. Conversely, clopyralid has good-excellent efficacy on Canada thistle, good efficacy on dandelion and red clover, fair efficacy on the winter annuals shepherd's-purse (*Capsella bursa-pastoris* L.) and field pennycress (*Thlaspi arvense* L.), and poor-no efficacy on pigweeds *(Amaranthus spp.)* and common lambsquarters *(Chenopodium album* L.*)*. MCPA has excellent efficacy on shepherd's-purse and pennycress, good efficacy on pigweeds and common lambsquarters, and only fair efficacy on Canada thistle. But, MCPA is not a broad spectrum herbicide and is not often recommended to use alone. Thus a premix of clopyralid + MCPA is commonly used to address individual herbicide deficiencies in broadleaf weed efficacy. This mix controls most broadleaf perennial, winter annual, and summer annual weed species found in small grains and grasses grown for seed. For this reason, this mix is the most promising herbicide for broadleaf weed management in Kernza IWG cropping systems.

1.3 | Objectives

The objective of this study was to determine the effects of 2,4-D, clopyralid, MCPA, and clopyralid + MCPA on injury and grain yield of newly seeded and established Kernza IWG stands under field conditions when applied during the tillering stage or before the boot growth stage as recommended for small grain crops. The second objective was to generate information needed to assess tradeoffs with herbicide use in Kernza IWG production systems. Our hypothesis was that these synthetic growth regulator herbicides will have little or no effect on IWG injury or grain yield.

2 | Material and Methods

2.1 | Site characterization

Experiments were conducted in the 2020 and 2021 growing seasons on field sites near 1) Arlington, Wisconsin (WI, 43.3380°N 89.3804°W) on Plano silt loam, 2) Rosemount, Minnesota (MN, 44.7394°N 93.1258°W) on Urban Land-Waukegan complex and eroded Timula-Bold silt loam, 3) Aurora, New York (NY, 42.7540°N 76.7024°W) on Lima silt loam (USDA-NRCS, 2022), and 4) Williston, North Dakota (ND, 48.1470° N 103.6180°W) on Williams-Bowbells loams.

Temperature and precipitation records were obtained from the online database of the National Weather Service (NWS, 2021). Daily average temperatures in ℃ and the equation from McMaster and Wilhelm (1997) was used to calculate growing degree days (GDD) where $GDD = [(T_{max} + T_{min}) / 2] - T_{base}$. T_{max} and T_{min} are daily maximum and minimum air temperature, respectively, and T_{base} is the base temperature (0 °C) (Frank, 1996). 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).

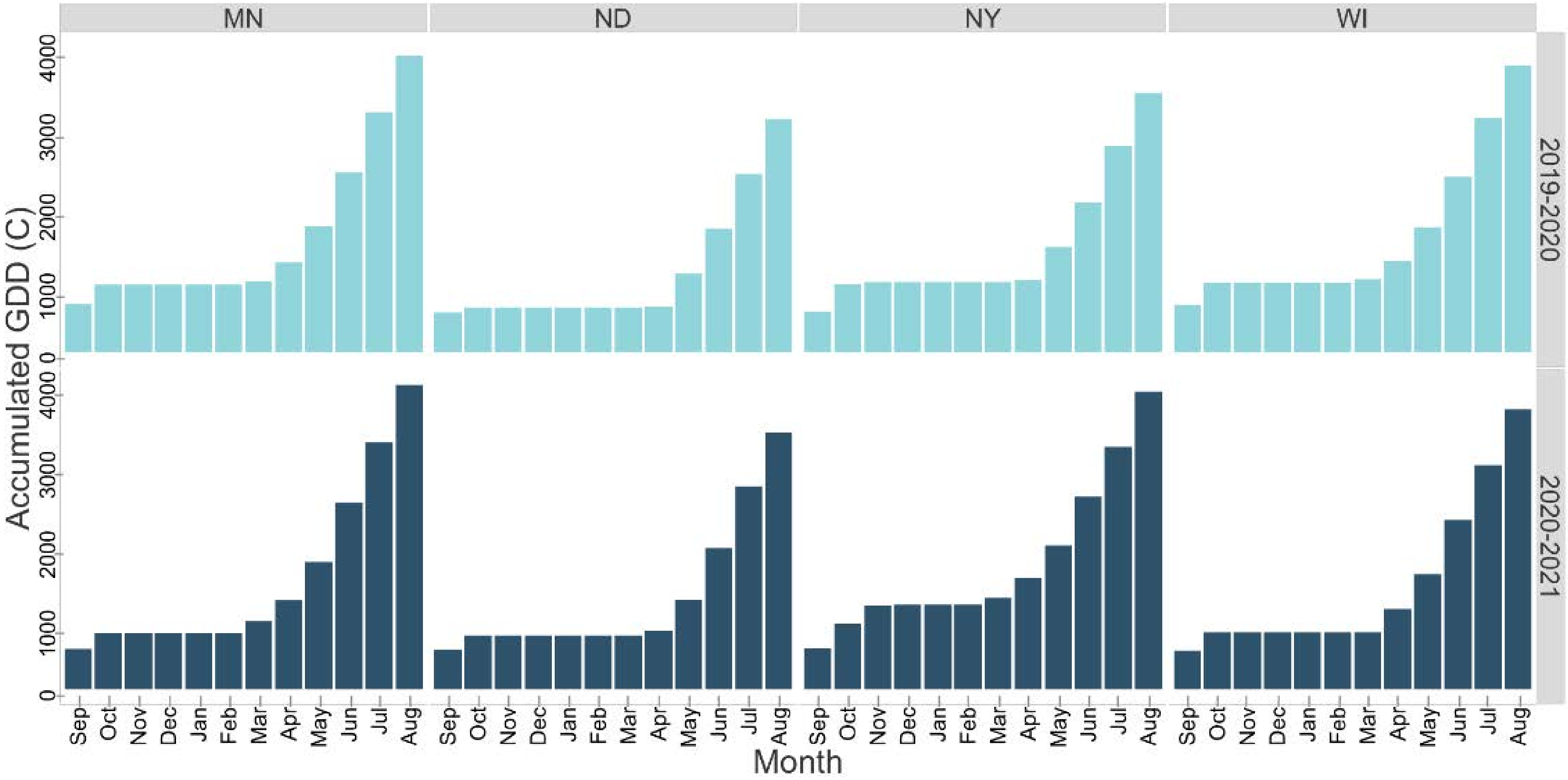

Figure 1. Total growing degree day (GDD) accumulation (base temperature = 0 ℃) from September 2019 to August 2020 and from September 2020 to August 2021 at 1) the University of MN-Rosemount Research and Outreach Center near Rosemount, MN, 2) the Williston Research Extension Center near Willison, ND, 3) the Musgrave Research farm near Aurora, NY, and 4) the University of Wisconsin-Madison Arlington Agricultural Research Station near Arlington, WI.

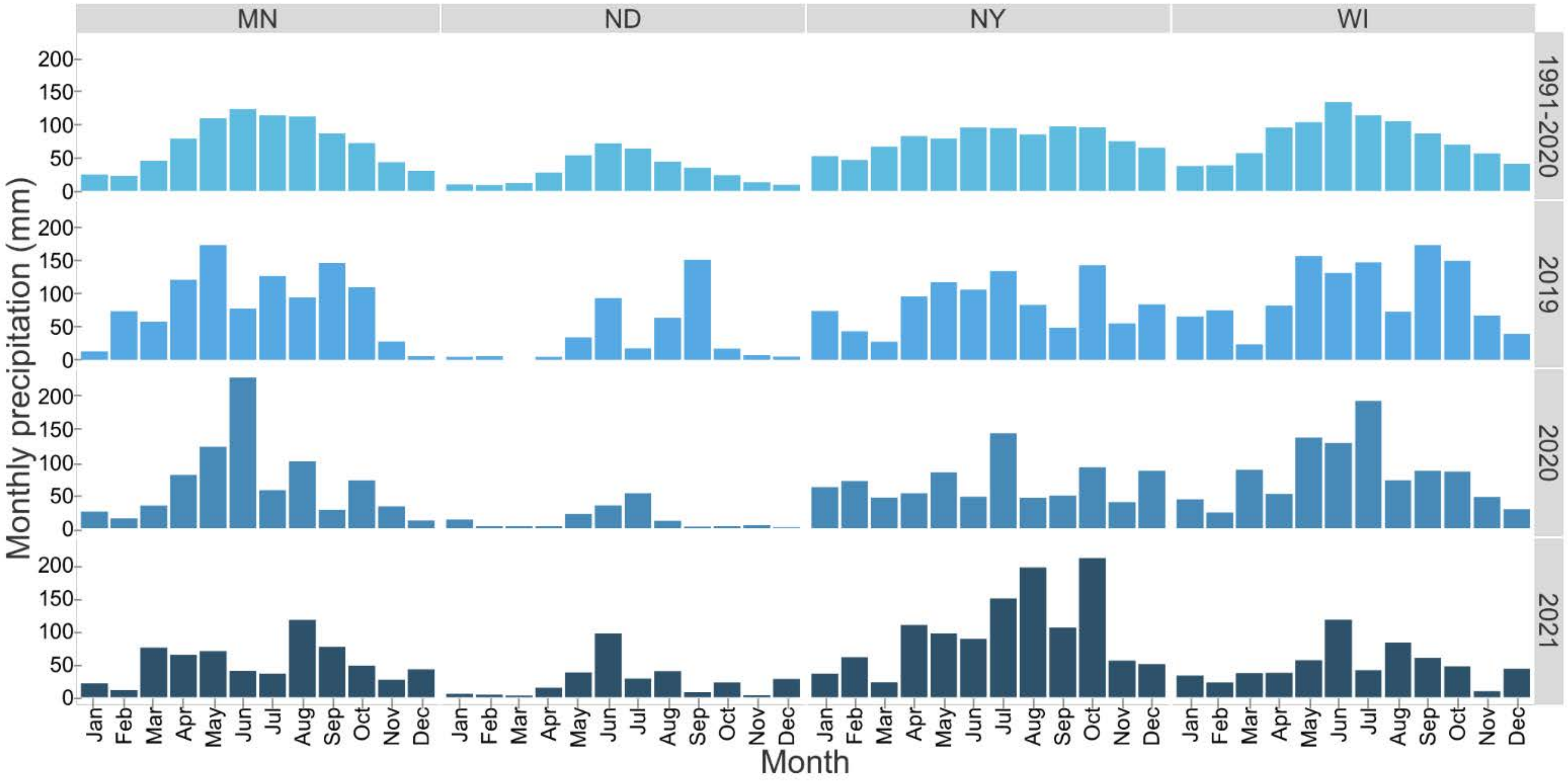

Figure 2. Average monthly precipitation (mm) at 1) the University of MN-Rosemount Research and Outreach Center near Rosemount, MN, 2) the Williston Research Extension Center near Willison, ND, 3) the Musgrave Research farm near Aurora, NY, and 4) the University of Wisconsin-Madison Arlington Agricultural Research Station near Arlington WI during 2019, 2020, and 2021. The 30- year monthly precipitation average is shown for 1991-2020.

2.2 | Study setup and design

Each experiment was arranged as a three-factor randomized complete block design replicated three times with herbicide type as Factor A, herbicide rate nested in herbicide type as Factor B, and herbicide application timing as Factor C. A nontreated control (NTC) was included within Factor A. The types of herbicides applied were: 1) 2,4-D amine, 2) clopyralid, 3) MCPA, and 4) MCPA + clopyralid. Each herbicide was applied at a 1X and 2X the labeled rate (Table 1). The two herbicide application times were in the fall and in the spring, and IWG stand age at herbicide application time varied from newly planted to established stands (Table 2). This study was established in the fall of 2019 and continued at some sites until the summer of 2021, after the Kernza grain harvest.

In WI, one experiment was conducted in an established IWG stand (TLI-C4 variety) planted in September 2015 and a second experiment was conducted in a new stand (MN-Clearwater variety) planted in September 2019. Field history for the established stand was conventionally-managed orchardgrass (*Dactylis glomerata L.*) in 2012–2014 and fallow during spring and summer 2015. Prior to IWG planting in September 2015, the site was tilled with a field cultivator followed by one pass with a cultipacker to improve soil-seed contact. The seeding rate was 18 kg ha^{-1} pure live seed in a 19-cm row spacing. Field history for the new stand was IWG intercropped with legumes (e.g., red clover, lupin). The site was planted in September 2019 using a grain drill at a seeding rate of 13.5 kg ha^{-1} pure live seed in a 19-cm row spacing. Seeding dates and fertilizer application rates are shown in Table 2. Plot size was 3 x 9 m^2.

In MN, three fields were planted in early September of 2018, 2019, and 2020. The seedbeds were prepared with multiple passes of a disk followed by a field cultivator prior to seeding with a grain drill. All fields were seeded with MN-Clearwater at 13 kg ha^{-1} pure live seed in a 30-cm row spacing. Previous crops in stands established in the fall of 2018, 2019, and 2020 were conventionally managed soybeans, alfalfa, and spring wheat, respectively. Fertilizers were applied according to Table 2. Plot size was 4 x 5 m^2.

In ND, IWG (TLI-C5 variety) was planted in August 2019 at a rate of 11 kg ha^{-1} pure live seed in a 19-cm row spacing using a no-till drill. The previous crop was conventionally-managed durum wheat. Glyphosate and 2,4-D amine were applied 1 day prior to planting as a burndown treatment . No fertilizers were applied. Plot size was 3 x 9 m^2.

In NY, the field was prepared with glyphosate burndown (1.7 kg ha^{-1}) followed by chisel plowing, discing, and cultipacking. Kernza seed was broadcast and rolled into the prepared seedbed using a Brillion grass seeder at 17 kg ha^{-1} on September 18, 2019. The previous crop was conventionally managed but had been used for hay production with minimal inputs for several years prior to the experiment. Nitrogen fertilizer was applied as 21-0-0 ammonium sulfate in late April 2020 and 2021 at 56 kg N ha^{-1}.

At each site, herbicide treatments were applied at the rates shown in Table 1 using a CO_2-pressurized backpack sprayer. Herbicides were applied during IWG tillering in the fall or at the jointing stage (1-2 nodes) before the boot stage in the spring (Table 3).

Table 1: Description of the type of synthetic auxin herbicide, herbicide rate, and application time in Kernza IWG experiments.

Herbicide type	Rate	Rate (kg ae ha^{-1})	Application time
2,4-D amine	1X	1.07	Fall
2,4-D amine	2X	2.14	Fall
2,4-D amine	1X	1.07	Spring
2,4-D amine	2X	2.14	Spring
Clopyralid	1X	0.1	Fall
Clopyralid	2X	0.2	Fall
Clopyralid	1X	0.1	Spring
Clopyralid	2X	0.2	Spring
Clopyralid + MCPA	1X	0.1 + 0.56	Fall
Clopyralid + MCPA	2X	0.2 + 1.12	Fall
Clopyralid + MCPA	1X	0.1 + 0.56	Spring
Clopyralid + MCPA	2X	0.2 + 1.12	Spring
MCPA	1X	0.56	Fall
MCPA	2X	1.12	Fall
MCPA	1X	0.56	Spring
MCPA	2X	1.12	Spring
Nontreated check (NTC)	- [a]	-	-

[a] Not applicable

Table 2. Description of the methods for Kernza IWG establishment and management for study sites near Arlington, WI, Rosemount, MN, Aurora, NY, and Williston, ND.

Site [a]	IWG stand age [b]	Soil type	Coordinates	IWG Planting				Fertilizer application	
				Variety	Date	Density	Row-spacing	Date	Type, rate
						kg ha^{-1}	cm		kg ha^{-1}
WI	0	Plano silt loam	43.3380° N 89.3804° W	MN-Clearwater	Fall, 2019	13.5	19	Spring, 2020 Spring, 2021	Urea, 56 N Urea, 79 N
WI	3+	Plano silt loam	43.3380° N 89.3804° W	TLI-C4 [c]	Fall, 2015	13.5	19	Spring 2020	Urea, 79 N
MN	0	Urban Land-Waukegan complex	44.7394° N 93.1258° W	MN-Clearwater	09/09/2019	13.0	30	04/27/2020	Urea, 90 N
MN	1	Urban Land-Waukegan complex	44.7394° N 93.1258° W	MN-Clearwater	Fall, 2018	13.0	30	04/27/2020	Urea, 90 N
MN	0	Timula-Bold silt loam	44.7394° N 93.1258° W	MN-Clearwater	09/03/2020	13.0	30	04/19/2021	Urea, 90 N
NY	0	Lima silt loam	42.7540° N 76.7024° W	MN-Clearwater	Fall, 2019	17.0	- [d]	04/2020 04/28/2021	Ammonium sulfate, 56 N
ND	0	Williams-Bowbells loam	48.1470° N 103.6180° W	TLI-C5	08/26/2019	11.0	19	-	-

[a] Sites within the continental United States of America. WI=Wisconsin, MN=Minnesota, NY=New York, ND=North Dakota

[b] Experiment stand age at the beginning of the study. 0=establishment year, 1=first production year, 2=second production year, 3+=third or more production year

[c] TLI - The Land Institute (Salina, KS)

[d] Not applicable

Table 3. Fall and spring herbicide application dates, IWG stage at herbicide application, rating date for weed and IWG injury, and abundant weed species at rating for study sites near Arlington, WI;, Rosemount, MN;, Aurora, NY;, and Williston, ND.

Site	IWG stand age [a]	Fall application				Spring application				IWG grain harvest date
		Date	IWG stage [b]	DAA [c]	Abundant weed species [d]	Date	IWG stage	DAA	Abundant weed species	
WI	0	-[e]	-	-	-	05/12/2019	Jointing, 1-2 nodes	14, 42	CAPBP, BROTE, LAMAM, ERIST, SINAR	07/28/2020
WI	3+	-	-	-	-	05/12/2020	Jointing, 1-2 nodes	14, 42	CAPBP, BROTE, LAMAM, ERIST, SINAR	07/28/2020
WI	1	09/14/2020	Jointing, 1-2 nodes	14, 42	TAROF, TRFPR, ERIAN, MEUOF, LUPPE	05/12/2020	Jointing, 1-2 nodes	14, 42	TAROF, TRFPR, ERIAN, MEUOF, LUPPE	08/04/2021
WI	3+	09/14/2020	Jointing, 1-2 nodes	14, 42	TAROF, CIRAR, PLAMA	-	-	-	-	-
MN	0	10/22/2019	Tillering	10	TAROF, BROTE, THLAR, CHEAL	05/04/2019	Jointing, 1-2 nodes	35	TAROF, BROTE, THLAR, CHEAL	08/07/2020
MN	1	10/22/2019	Tillering	10	-	05/04/2019	Jointing, 1-2 nodes	36	-	08/07/2020
MN	0	10/08/2020	Tillering	62	CAPBP, THLAR, CHEAL	05/15/2020	Jointing onset	41	CAPBP, THLAR, CHEAL	07/22/2021
MN	1	10/08/2020	Tillering	18	CAPBP, THLAR, CHEAL	05/15/2020	Jointing onset	41	CAPBP, THLAR, CHEAL	07/22/2021
NY	0	11/06/2019	Tillering	14	CAPBP, POANN, STEME, LAMPU, CERVU, VERAR	05/22/2019	Jointing, 2-4 nodes	14, 42	CAPBP, POANN, STEME, LAMPU, CERVU, VERAR	Summer, 2020
NY	1	10/14/2020	Tillering	14, 42	TAROF, TRFPR, POANN	05/13/2020	Jointing onset	25, 48	-	Summer 2021
ND	0	10/172019	Tillering	14	BROTE, ERICA, AGRCR, KCHSC, DESPI	05/29/2019	Jointing, 2-4 nodes	13, 42	BROTE, ERICA, AGRCR, KCHSC, DESPI	07/27/2020

[a] Experiment stand age at the beginning of the study. 0=establishment year, 1=first production year, 2=second production year, 3+=third or more production year

[b] IWG stage at herbicide application using the Feeks scale. Jointing, 1-2 nodes = Feeks scale 6-7; jointing, 2-4 nodes = Feeks scale 7-8, tillering=Feeks 3,4,5

[c] DAA=the number of days after application that IWG and weed injury ratings were conducted

[d] Abundant weed species at IWG injury and weed injury ratings. AGRCR=crested wheatgrass (*Agropyron cristatum*); BROTE=downy brome (*Bromus tectorum*); CAPBP=shepherd's purse (*Capsella bursa-pastoris*); CERVU= mouseear chickweed(*Cerastium vulgatum*); CHEAL= lambsquarters (*Chenopodium album*); CIRAR=canada thistle (*Cirsium arvense*); (ERIAN=annual fleabane (*Erigeron annuus*); ERIST=rough fleabane (*Erigeron strigosus*); ERICA=horseweed (*Conyza canadensis*); KCHSC= fireweed (*Kochia scoparia*); LACSE=prickly lettuce (*Lactuca serriola*); LAMAM=henbit (*Lamium amplexicaule*); LAMPU= purple deadnettle (*Lamium purpureum*); LUPPE=perennial lupine (*Lupinus perennis*); MALNE=common mallow (*Malva neglecta*); MEUOF=yellow sweetclover (*Melilotus officinalis*); PLAMA= broadleaf plantain (*Plantago major*); POANN=annual bluegrass (*Poa annua*); SINAR=wild mustard (*Sinapis arvensis*); STEME= common chickweed (*Stellaria media*); TAROF= dandelion (Taraxacum officinale); (THLAR =field pennycress (*Thlaspi arvense*); TRFPR=red clover (*Trifolium pratense*); VERAR=corn speedwell (*Veronica arvensis*)

[e] Data not collected and/or not applicable

2.3 | Data collection

In WI and MN, weed control was visually assessed 14 & 42 days after application (DAA) of herbicides on a scale of 0-100% (0%= no control, 100%= complete control) relative to the NTC. In ND and NY, weed cover was visually assessed 13 & 42 DAA and 25 & 48 DAA, respectively, on a scale of 0-100% (0 = no weed cover, 100% = complete weed cover). Intermediate wheatgrass injury was visually assessed at the same time of weed control (WI and MN) and weed cover (ND and NY) ratings. Injury ratings were based on a scale of 0-10 (0 = no crop injury, 10 = crop mortality) relative to the NTC. Grain yield was harvested by machine from the center 14.4-m^2 of each plot in WI and by two, 0.5-m^2 hand-harvested areas per plot at other sites. Seed heads (spikes) were cut from all tillers within the quadrats, dried at 60 ℃ until constant mass, threshed with a mechanical seed thresher, and weighed.

2.4 | Statistical analysis

All data were submitted to ANOVA in RStudio, version 2022.07.1 (RStudio, PBC., Boston, MA). Grain yield, weed control, and weed cover data were transformed when needed to meet normality and constant variance assumptions for ANOVA and back-transformed for presentation. Assumptions were assessed by evaluation of residual plots. The *lmer* function from the *lme4* package was used to analyze a linear mixed model. Location, herbicide type, herbicide rate nested within herbicide type, stand age, herbicide application timing, and all interactions were treated as fixed effects and block as a random effect. Interactions with all fixed effects were evaluated for significance and analyzed separately if significant at $\alpha = 0.05$. Post-hoc mean comparison was conducted using Tukey's honest significant difference test (HSD) at $\alpha = 0.05$.

The number and exact timing of injury ratings (Table 3) were not identical across sites, therefore the average rating was used for initial comparison across sites. If any variables affected the injury rating, then each site and timing was analyzed separately. Average IWG injury ratings were analyzed using logistic regression where 0 = no injury, 1 = injury present using the *glmer* function from the *lme4* package using the *logit* command. The MN dataset was removed from the IWG injury analysis because multiple frosts had occurred before the ratings and caused damage indistinguishable from herbicide injury.

The linear regression model used for grain yield analysis [Equation 1] is: Y_1 = grain yield, **μ** = the overall mean, *S* = effect of site, *A*=effect of stand age at grain harvest, *T*=effect of herbicide application time, *H/C*= effect of herbicide rate nested within herbicide type, *S x A x T x H/C* = effect of all interactions, *B*=block nested within location, and *E*=random residual. The model for logistic regression [Equation 2] is Y_1 = IWG injury; *I* = the intercept; *S* = effect of site; *A*=effect of stand age; *T*=effect of herbicide application time; *H*= effect of herbicide rate nested within herbicide type; *S x A x T x H/C* = effect of all interactions; *B*=block nested within location; *E*=random residual. Finally, each location was analyzed separately for weed control parameters. The linear regression model used for weed control [Equation 3] was *Y1* = weed control or weed cover; **μ** = the overall mean; *T*=effect of herbicide application time; *H*= effect herbicide type; *C*=effect of herbicide rate ; *T x H x C* = effect of all interactions; *B*=block nested within location; *E*=random residual.

$$Y_1 = \boldsymbol{\mu} + S\,x\,A\,x\,T\,x\,H/C + B + E \qquad [1]$$

$$Y_2 = 1/(1 + e^{-(I + S\,x\,A\,x\,T\,x\,H/C)}) + B + E \qquad [2]$$

$$Y_1 = \boldsymbol{\mu} + T\,x\,H\,x\,C + B + E \qquad [3]$$

3 | Results

3.1 | Temperature and precipitation over the course of study

There was no difference in accumulated GDD among sites (p=0.51) or study years (p=0.73). Accumulated GDD for MN, ND, NY, WI was 1427, 1084, 1449, and 1372, respectively (Figure 1). Spring GDD accumulation did not begin until May at the ND site while GDD accumulation began at the other three sites in March or April.

Per site, there was no difference in average precipitation across study years or compared to the 30- year average (p=0.08). Average monthly precipitation differed among sites (p<0.01). ND average precipitation was lower than all other sites with an average monthly precipitation of 25 mm. MN, NY, and WI sites average monthly precipitation did not differ with 69, 82, and 79 mm, respectively.

3.2 | Herbicide effects on grain yield

Grain yield was not affected by herbicide type or concentration (Table 4) with an average grain yield of 562 kg ha^{-1} across all treatments (data not shown). Grain yield did not differ between fall- and spring-applied herbicide treatments. Site and stand age were the only significant factors in explaining grain yield differences. Average grain yield in MN, WI, ND, and NY was 965, 319, 266, and 159 kg ha^{-1}, respectively (data not shown). In WI and MN, grain yield declined with stand age (p<0.01; Table 4, Figure 3). In NY, grain yields did not differ with stand age. Stand age effects were not determined for the ND site because grain was only harvested in the establishment year.

Table 4. P-values for effects and interactions of experimental variables on Kernza IWG grain yield. P-values are significant at α = 0.05. Three- and 4-way interactions were not significant (results not shown).

	IWG grain yield
Variable	P-value
Site (S)	< 0.01
Stand age (A)	<0.01
Application time (T)	0.66
Herbicide type/rate (H)	0.33
S x A	<0.01
S x T	0.18
S x H	0.80
A x T	0.18
A x H	0.39
T x H	0.77

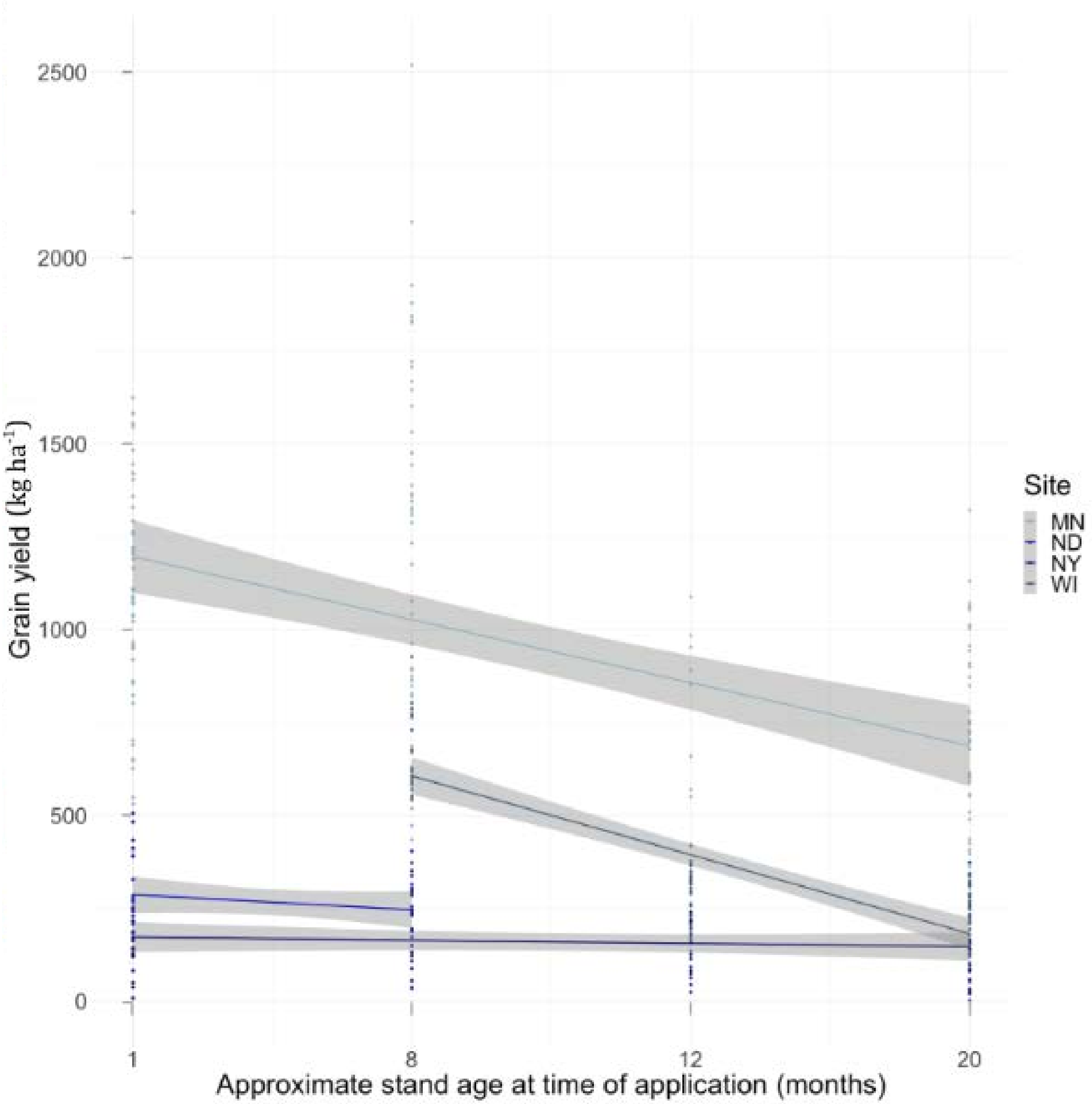

Figure 3. Kernza grain yield by approximate stand age at the time of herbicide application. Lines of best fit per location are as follows: MN, $y = -28.3x + 1196$, $R^2 = 0.20$; ND, $y = -6.8x + 287$, $R^2 = 0.10$; NY, $y = -1.4x + 173$, $R^2 = 0.01$; WI, $y = -107x + 630$, $R^2 = 0.44$. The slope of the best fit lines of best fit for MN and WI sites differ from zero with p-values <0.01 for both sites. The slope of best fit lines for ND and NY sites do not differ from zero with p-values of 0.12 and 0.54, respectively.

3.3| Herbicide effects on Kernza IWG injury

Herbicide treatment was not associated with IWG injury (Table 5). Neither herbicide type or concentration affected the probability of IWG injury. Similarly, herbicide application in the fall or spring showed no effect on the probability of IWG injury. No site or stand age effect was observed.

Table 5. P-values for effects and interactions of experiment variables on IWG injury. P-values are significant at α = 0.05. No interactions were significant (results not shown).

Variable	IWG injury P-value
Intercept †	1.00
Site (ND)	1.00
Site (NY)	1.00
Stand age (6 month)	1.00
Stand age (12 month)	1.00
Stand age (18 month)	1.00
Stand age (36+ month)	1.00
Herbicide application time (spring)	1.00
Herbicide type (2,4-D amine)	1.00
Herbicide type (Clopyralid)	1.00
Herbicide type (MCPA)	1.00
Herbicide type (Clopyralid + MCPA)	1.00
Rate (1X)	1.00
Rate (2X)	1.00

† The intercept is the WI site, stand age of 0 months, herbicide application time in fall, and the nontreated control (NTC) for herbicide type, and NTC for concentration.

3.4 | Herbicide effects on weed control

In WI, herbicide application time, herbicide type, and herbicide rate explained the level of weed control observed with an interaction between herbicide type and herbicide rate (Table 6). Weed control differed among herbicide type and rate with 2,4-D amine 2X, clopyralid 2X and clopyralid + MCPA 2X showing greater weed control than other treatments (Table 7). Weed control did not differ between 2,4-D amine 1X and clopyralid 1X, clopyralid + MCPA 1X, MCPA 1X, or MCPA 2X. However, weed control for clopyralid + MCPA 1X was greater than for clopyralid 1X or MCPA 1X. In ND and NY, neither herbicide application time, type, or rate affected weed cover (Tables 6 and 7).

Table 6. P-values for effects and interactions of herbicide application time, herbicide type, and concentration on weed control (WI) and weed cover (ND and NY). P-values are significant at α = 0.05.

	Weed control	Weed cover	
Sites	WI	ND	NY
	p-values		
Application time (T)	0.13	0.37	0.13
Herbicide type (H)	<0.01	0.20	0.56
Herbicide rate (R)	<0.01	0.70	0.39
T x H	0.99	0.43	0.52
T x C	0.06	0.66	0.12
H x C	0.03	0.89	0.31
A x H x C	0.67	0.76	0.50

Table 7. Weed control (WI) and weed cover (ND and NY) as affected by herbicide type and herbicide rate of application. Means followed by the same lower case letter within a site do not differ according to Tukey's HSD α = 0.05.

		Weed control [a]	Weed cover [b]	
Herbicide type	Concentration	WI	ND	NY
		%	——— % ———	
2,4-D amine	1X	79.6 bc	29.2 a	12.7 a
2,4-D amine	2X	91.7 a	26.7 a	14.2 a
Clopyralid	1X	72.6 c	44.7 a	15.0 a
Clopyralid	2X	87.1 a	39.4 a	12.1 a
Clopyralid + MCPA	1X	83.4 b	- [c]	14.7 a
Clopyralid + MCPA	2X	91.9 a	-	10.5 a
MCPA	1X	71.2 c	-	-
MCPA	2X	77.8 b	-	-
Nontreated	0	6.1 d	47.8 a	33.3 a

[a] Weed control was visually assessed in WI on a scale of 0-100 (0 = no control, 100 = total control).

[b] Weed cover was visually assessed in ND and NY on a scale of 0-100 (0= no cover, 100= total cover).

[c] Treatments were not applied, therefore data was not collected

4 | Discussion

4.1 | Herbicide effects on grain yield and crop injury

The synthetic auxin herbicides 2-4, D amine, clopyralid, MCPA, and mix of MCPA + clopyralid applied on Kernza IWG did not affect grain yield nor did they show crop injury, suggesting a high level of IWG tolerance to these herbicides applied in the spring before the boot stage or in the fall during the tillering stage.The lack of effect on IWG grain yield or crop injury associated with use of these herbicides is consistent with their use on small grains.

Overall, the only factors that influenced Kernza grain yield was the site and age of the stand at grain harvest. The MN site had the highest average yield, followed by WI, and ND, and NY. Though yields varied among sites, all sites fall within the range of reported Kernza IWG grain yields which vary based on planting dates, varieties, soil type, and climate.

The ND site may have had the lowest yields due to a lack of N fertilizer at establishment. Nitrogen fertilizer has been shown to increase Kernza IWG grain yields more than 3-fold relative to no N fertilization in the second and third year of production (Fernandez et al., 2020). However, Jungers et al. (2017) and Fernandez et al. (2020) both found that N fertilizer did not increase grain yield in the first production year which they attributed to high levels of residual soil-N. Low Kernza grain yield In ND may have been due to depletion of soil-N by the previous durum wheat crop.

Although N fertilizer was applied to IWG stands in WI and MN, lower yields in WI than MN may have been due to WI used MN-Clearwater and TLI-C4 seed for planting while MN only used MN-Clearwater, the first commercial food-grade variety of IWG (Bajgain et al., 2020). MN-Clearwater was selected for high grain yield, reduced seed shattering, high free grain threshing, reduced lodging, and uniform maturity, traits that would lead to higher grain yield over its counterpart, the older variety, TLI-C4 (Bajgain et al., 2020).

In MN and WI sites, grain yield differed with the age of the Kernaz IWG stand (Figure 3). Grain yield decline overtime in Kernza IWG is well-documented with many studies showing that yield is often highest in the establishment year and declines subsequently (Culman et al., 2013; Dick et al., 2019; Hunter et al., 2020a; Jungers et al., 2017, 2018; Lanker et al., 2021; Law et al., 2020; Pinto et al., 2021; Pugliese et al, 2019; Tautges et al., 2018; Zimbric et al., 2020). Yield decline overtime is thought to be related to intraspecific competition among the IWG that reduces reproductive tiller initiation in the fall thereby reducing grain yield the following summer (Pinto et al., 2021; Fernandez et al., 2020). It is also thought to be related to the increase in belowground competition for water, nutrients, and space throughout the life of the IWG stand (Fernandez et al, 2020; Hunter et al., 2020a; Tautges et al. 2018). Whatever the mechanism, observing yield decline overtime in this study was expected as it follows trends reported in previous bodies of literature.

4.2 | Herbicide efficacy on weed community

Wisconsin was the only site that showed differences among herbicide application time, herbicide type, and herbicide concentration on weed control. Weed control for clopyralid+MCPA 1X did not differ from that of 2,4-D amine 1X, but was greater than that for clopyralid 1X or MCPA 1X. Winter annual weeds found in WI Kernza IWG stands at the time of herbicide application were shepherd's purse, henbit (*Lamium amplexicaule* L.), wild mustard (*Sinapis arvensis* L.*).* Summer annuals found were annual fleabane [*Erigeron annuus* (L.) Persoon] and rough fleabane [*Erigeron strigosus* (Willdenow)]. The biennial, yellow sweetclover was found along with perennials dandelion

[*Taraxacum officinale* (Weber)], red clover, canada thistle , perennial lupine (*Lupinus perennis* L.), broadleaf plantain (*Plantago major* L.). The only grass weed found was downy brome (*Bromus tectorum* L.). Based on results from the WI site, clopyralid+MCPA and 2,4-D amine are promising for control of annual and perennial broadleaf weeds in Kernza IWG cropping systems.

In ND and NY, herbicides had no impact on the level of weed cover. In ND, the dominant weeds were downy brome, horseweed (*Conyza canadensis*), crested wheatgrass [*Agropyron cristatum* (L.) Gärtner], kochia [*Bassia scoparia* (L.) A. J. Scott], and tansymustard [*Descurainia pinna* (Walter) Britton]. The lack of herbicide effect on broadleaf weed species may have been due to the abundance of grass weeds which were not affected by the synthetic auxin herbicides. That is, the lack of weed control was likely due to a weed community that was dominated by species unaffected by the herbicides applied. In the future, it would be helpful to evaluate herbicides that control unwanted grasses to determine if there are options for grass control in Kernza IWG cropping systems that do not reduce grain yield.

Similarly, weed cover was not affected by herbicide treatment in NY. The dominant weeds were the perennials dandelion and red clover, winter annuals shepherd's purse, common chickweed [*Stellaria media* (L.) Villars], mouseear chickweed [*Cerastium vulgatum* (Hartmann) Greuter & Burdet], corn speedwell (*Veronica arvensis* L.), and annual bluegrass (*Poa annua* L.). The lack of herbicide effect on weed cover was unexpected, but was likely due to the abundance of annual bluegrass which was not affected by the synthetic auxin herbicides.

Weed control was variable over time at the MN site (data not shown). This was due in part to little or no weed pressure. Although the weed community consisted of dandelion, downy brome, field pennycress (*Thlaspi arvense* L.), and common lambsquarters (*Chenopodium album* L.), the IWG stand may have outcompeted these weeds, minimizing herbicide treatment effects.

5 | Conclusion

Kernza intermediate wheatgrass showed a high level of tolerance to the synthetic auxin herbicides, 2-4-D amine, clopyralid, MCPA, and the mixture of clopyralid+MCPA, with no impact on grain yield in the establishment year or in post-establishment years. Both 2,4-D amine 1X and clopyralid+MCPA 1X showed promise for broadleaf weed control in Kernza IWG production systems. As a result of this study, 2,4-D amine has recently been labeled for use on Kernza IWG. Herbicides in ND and NY sites did not affect the level of weed cover which could be because of significant grass weed populations. Therefore, future research in Kernza IWG systems could assess crop safety and efficacy of herbicides that target unwanted grass species.

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