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# Agronomic assessment of two populations of intermediate wheatgrass—Kernza<sup>®</sup> (*Thinopyrum intermedium*) in temperate South America

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## Abstract

**Background:** Kernza<sup>®</sup> intermediate wheatgrass is a perennial grain and forage crop that can provide several ecosystem services. Major research efforts focused on Kernza have taken place in high latitudes. The goal of this study was to evaluate, for the first time, the agronomic performance of Kernza in a low-latitude region with mild winters.

**Methods:** A KS-cycle 4 Kernza population (A) was planted in spring in Wisconsin, USA, and selected in one cycle for lower vernalization requirements, obtaining a new population (B). These two populations, at three nitrogen (N) fertilization rates, were evaluated in a full factorial, completely randomized field experiment in Uruguay over 2 years.

**Results:** The populations were similar in grain yields and flowering time in the 1st year, but population B had 63% lower grain yield in the 2nd year and 20% lower forage yield throughout the experiment. Increasing the N rate to 160 kg ha<sup>-1</sup> led to a 63% increase in grain yield and 28% increase in forage yield across populations. Forage yields and nutritive values were similar to those reported in the northern hemisphere. However, grain yields for both the 1st (316 kg ha<sup>-1</sup>) and 2nd year (41 kg ha<sup>-1</sup>) were lower due to reduced flowering and weed competition.

**Conclusions:** Expansion of Kernza to lower-latitude regions will require further breeding to improve reproductive performance.

## KEYWORDS

low-latitude regions, nitrogen fertilization rates, perennial grains, *Thinopyrum intermedium*, vernalization requirements

## INTRODUCTION

Annual grain crops widely dominate the world's arable land and are the main source of food on the planet (Pimentel et al., 2012). Due to shorter growing seasons and less developed root systems, annual crops provide less protection against soil erosion, have lower water and nutrient use

efficiency, store less carbon in the soil, and make the production system less resilient to both biotic and abiotic stresses than perennial plant communities (King & Blesh, 2018; Osterholz et al., 2019; Sanford, 2014; Sanford et al., 2021). Intermediate wheatgrass (*Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey, Kernza<sup>TM</sup>) is a cool season perennial grass with vernalization requirements

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to flowering that has been domesticated as a perennial grain crop in cold regions of North America based on its seed size, mechanical harvesting possibilities, and easy threshing (Wagoner & Schauer, 1990). At the beginning of the 21st century, scientists at the Land Institute, Salina, KS, started to work on the domestication of *T. intermedium* by selecting for improved yield per head, increased seed size, free threshing, shatter resistance, and reduced height, among other qualities (DeHaan et al., 2018; Zhang et al., 2016). As a result of this process of conventional breeding, *T. intermedium* became commercially available to farmers in the USA under the trade name Kernza™ (DeHaan et al., 2014). Later, “MN-Clearwater” was released by the University of Minnesota, and it is the first Kernza cultivar that produces grain approved for sale as Kernza perennial grain (Bajgain et al., 2020). On the other hand, its forage potential has always been remarkable, since *T. intermedium* was introduced from Eurasia to North America a century ago, and before it began to be improved as a grain crop, it was demonstrated to be a forage with good qualities and many forage cultivars were released (Jensen, Yan, et al., 2016). Although the size of Kernza grain has increased throughout its breeding, it is still four to five times smaller than wheat grain (Rahardjo et al., 2018). In comparison to wheat, Kernza has a higher content of protein, ash, fat, dietary fiber, and antioxidants, but a lower content of starch (Hayek, 2020; Mathiowetz, 2018; Rahardjo et al., 2018; Tyl & Ismail, 2019). Although its grain yield is currently lower than that of annual cereals (Franco et al., 2021), harvesting grain and forage can narrow the economic gap with annual grain systems (Bell et al., 2008), and may be satisfactory for farmers who have shown interest in the dual-use (grain-forage) potential of Kernza (Lanker et al., 2019). Improvements in grain and forage yield as well as additional forage harvests could improve the economic sustainability of Kernza (Law et al., 2022). Indeed, the harvest of forage and grain promoted nutrient availability and productivity of forage, root, and grain biomass, which highlights Kernza's dual-purpose possibilities (Pugliese et al., 2019; Sakiroglu et al., 2020).

Low-latitude regions of South America have contributed substantially to global grain production, after transforming native grasslands into annual crop systems, which led to the deterioration of natural resources and crop vulnerability to severe weather events (Modernel et al., 2016). In the low-latitude regions of South America, the main winter crops are wheat, barley, and oilseed rape, whose cultivars are mostly spring cultivars. The winters are predominantly mild and temperatures increase rapidly during the spring, so late anthesis leads to grain filling under unfavorably high temperatures, decreasing yield (Locatelli, Bhatta, et al., 2022). Kernza's geographic expansion to these regions could be seriously threatened as a dual-purpose crop due to its vernalization requirements, though phenotypic variability has been detected for these requirements (Locatelli, Gutierrez, et al., 2022). Like most temperate perennial grasses, Kernza could also require dual induction to flowering: primary induction by short day or low temperature, followed by secondary induction by long days with interchangeable effects of temperature and daylength (Heide, 1994; Ivancic et al., 2021). Primary induction requires some combination

of cool temperatures (vernalization) and/or short days, which enables the plant to initiate inflorescence primordia, while secondary induction involves long days and increasing temperatures, allowing culm elongation, inflorescence development, and anthesis (Chastain & Young, 1998; Heide, 1994). Low-latitude regions may not accumulate enough days of cold temperature to meet those requirements to initiate flowering, resulting in lower grain yields. The vernalization requirements of Kernza may not only be a constraint to its agronomic adaptation to low-latitude regions but are also being studied in high-latitude regions of the world with cold winters due to their negative impact on 1st-year grain yields (Cattani & Asselin, 2022; Jungers et al., 2022). Although the expansion of a perennial crop such as Kernza would be advantageous for every region, breeding advances have only taken place in cold areas of North America. Therefore, finding Kernza germplasm with adaptation to low-latitude regions testing the adaptation of Kernza populations with potentially contrasting vernalization requirements is needed. At nonvernalizing temperatures, research conducted in growth chambers with Kernza germplasm showed that about 15% to 25% of the plants were able to retain the ability to flower (Ivancic et al., 2021; Locatelli, Gutierrez, et al., 2022). Selecting plants with the ability to flower in low-stimulus environments to fulfill the vernalization requirements of Kernza should lead to germplasm with higher ability to produce grain and thus be used as a dual-purpose crop in low-latitude regions. Therefore, our first hypothesis is that a Kernza population selected in an environment without vernalization temperatures would produce higher yields in low latitudes.

Forage yields of Kernza intermediate wheatgrass have been reported from northern and central regions of North America, averaging 6450 and 7419 kg DM ha<sup>-1</sup> year<sup>-1</sup> for the 1st year and older stands (2nd, 3rd, or 4th year), respectively (Franco et al., 2021). Kernza can be managed as a dual-use crop, harvesting grain in the summer, and harvesting forage in the spring, summer, and fall. Spring and fall forage harvests contribute one-third to one-quarter of the biomass harvested in the summer, and they have higher forage nutritive value, contributing substantially to the profitability of Kernza (Favre et al., 2019; Hunter, Sheaffer, Culman, Lazarus, & Jungers, 2020; Pinto, Culman, et al., 2021). Kernza forage nutritive value in high-latitude regions was suitable for lactating beef cows, dairy cows, and growing heifers when harvested in the spring and fall, and for beef cows in summer (Favre et al., 2019; Pinto, Culman, et al., 2021). The forage quality can be strongly modified by factors of the production environment, such as climate, defoliation management, time of cutting, and fertilization (Hoveland & Monson, 1980; C. J. Nelson & Moser, 1994). Grain yield in pure stand has had widely varying values, from 112 kg ha<sup>-1</sup> (Culman et al., 2013) to 1089 kg ha<sup>-1</sup> (Favre et al., 2019) for the 1st year and from 3 kg ha<sup>-1</sup> (Pugliese, 2017) to 1662 kg ha<sup>-1</sup> (Culman et al., 2013) for the 2nd year (Franco et al., 2021). Although grain yield is highly variable, there is consensus in a decline in performance over time (Law et al., 2021; Pinto, De Haan, et al., 2021; Tautges et al., 2018; Zimbric et al., 2020). Grain yields are still low, although the response to selection for this trait has been high and

stable (DeHaan, 2022; DeHaan et al., 2014). The high variability in grain yields could be due to several combined factors such as differences between breeding cycles, pedoclimatic context, and field agronomic management operations. Therefore, our second hypothesis is that Kernza grown in low latitudes would have similar forage yield and nutritive value as Kernza grown in higher latitudes, but lower grain yields.

Nitrogen (N) availability can influence the timing and success of reproductive growth and plant fertility (Amanullah et al., 2009; Gungula et al., 2003; Wang & Tang, 2019). N fertilization in a dual-purpose crop is a key factor as it can affect both grain and forage production, for which knowing the response to the application of N is critical to the management of the crop (Koeritz et al., 2013; Vogel et al., 2002). Kernza's grain and forage yields response to N fertilization showed a quadratic behavior, with an optimum N rate of 61–96 kg ha<sup>-1</sup> and 81–121 kg ha<sup>-1</sup>, respectively (Jungers et al., 2017). Zimbric et al. (2020) found increases in both biomass and grain yield from 90 to 135 kg N ha<sup>-1</sup> rates. Therefore, our third hypothesis is that higher N rates will increase grain and forage yields.

Weed suppression is another key agronomic management factor in perennial crops. Depending on the timing, frequency, and intensity of forage harvesting, each cut may offer an opportunity for weeds to utilize resources and grow, damaging future forage and grain performance, or, on the contrary, to confer competitive advantages to the seeded species (Ghanizadeh & Harrington, 2019; Meiss et al., 2010). Severe defoliation (i.e., high intensity and high frequency) decreases the survival of perennial grasses in pastures, which is exacerbated by increased competition from invasive species (O'Connor, 1991). Indeed, farmers identified weed suppression as a major information gap in dual-use Kernza cropping systems (Lanker et al., 2019). During a 3-year experiment, weed biomass under dual-use Kernza systems decreased by an average of 88%, and weedy and weed-free treatments had similar grain and forage production, suggesting that weed competition may not have negative effects on Kernza (Zimbric et al., 2020). However, Weik et al. (2002) reported an increase in weed biomass from the 1st year to the 2nd year, although the magnitudes were too low to affect grain and forage

productivity. Therefore, our fourth hypothesis is that weed biomass in dual-use Kernza systems would decrease over time.

In this study, we report, for the first time, agronomic performance of Kernza managed for dual use in a temperate low-latitude region with mild winters. Specifically, we evaluated plant heading, heading time, grain and forage yield, harvest index, weed biomass, and forage nutritive value of Kernza intermediate wheatgrass in a replicated field experiment over 2 years.

## MATERIALS AND METHODS

### Plant material

Two Kernza populations were developed at the University of Wisconsin–Madison, using seeds from Kernza selection cycle 4 from The Land Institute (TLIC4): A and B (Figure 1). Population A consisted of seeds harvested in summer 2017 from plants seeded in the fall 2016 so they had fulfilled their vernalization requirements in the field in Arlington, WI, USA (43°18' N, 89°19' W). We expected that seeds from this population A would have diversity in vernalization requirements (Locatelli, Gutierrez, et al., 2022), and only 15%–25% of their plants would flower in an environment without vernalization according to the results of the greenhouse study of Locatelli, Gutierrez, et al. (2022). Population B consisted of seeds harvested on November 9, 2017 from plants of TLIC4 sown on April 19, 2017 in the field in Arlington, WI (AK3 Field 350B), so these plants completed their life cycle during one spring–summer–fall period, elongated in late July, flowered in August, and were harvested in November, experiencing little vernalizing stimuli. To obtain each population, seed from the same original population (TLIC4) was sown at two contrasting times in Wisconsin: fall 2016 and spring 2017. Therefore, plants from both trials were exposed to contrasting vernalizing stimuli before being harvested in the summer and fall of 2017 (Figure 1). We expected that seeds from population B would have a higher proportion of plants flowering in environments with low vernalization stimuli, because they were progeny from plants that flowered under restrictive vernalization conditions. We consider population B the

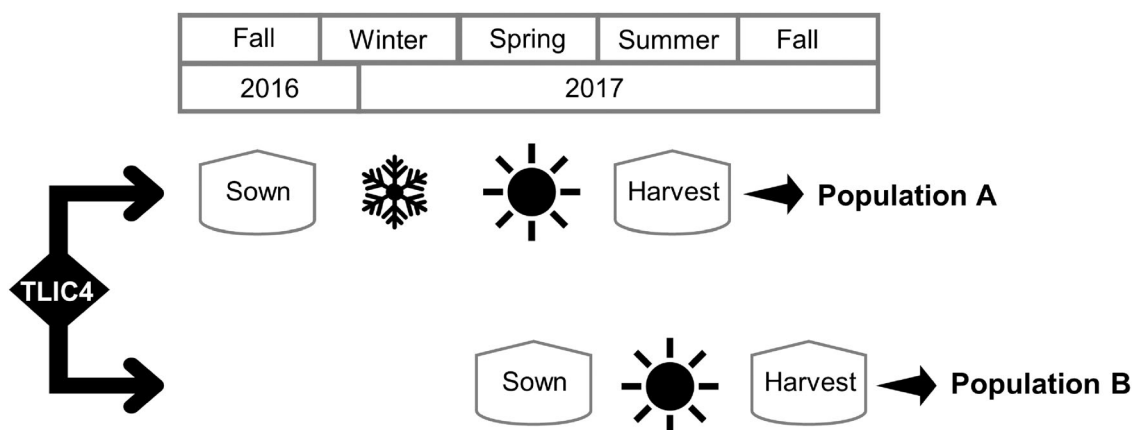


FIGURE 1 Explanatory scheme for the development of the populations in Arlington, WI, USA used in the study

product of one cycle of selection for reduced vernalization requirements. The plants that produced the seeds that comprised population B had fewer fertile stems ( $3.3 \pm 1.7$  spikes per plant) than those in A ( $14.0 \pm 3.6$  spikes per plant); they had smaller spikes (spike weight was  $0.4 \pm 0.1$  g for B and  $0.6 \pm 0.2$  g for A). The seeds from population B were also smaller (1000-seed weight was  $5.6 \pm 0.3$  g for B and  $7.7 \pm 0.4$  g for A). Seeds of both populations were imported to Uruguay subject to phytosanitary inspection and authorization (MGAP-AFIDI # 1318855, species code: THIIN21301034).

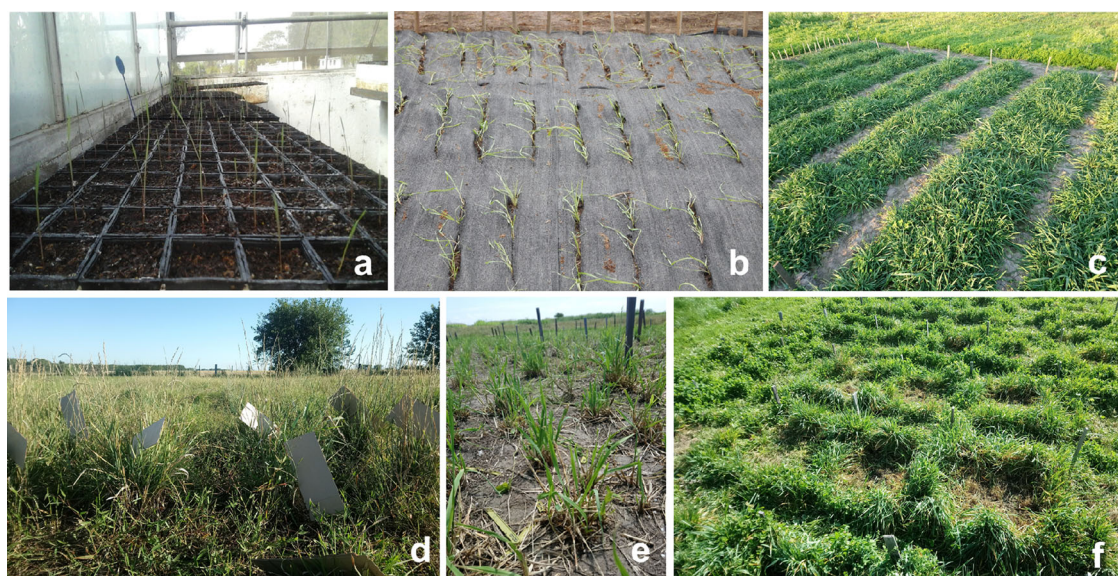
## Experimental design and management

The experiment was conducted in the fall (April) of 2018 at the Experimental Station “Dr Mario A. Cassinoni” in the University of Uruguay (UDELAR),  $32^{\circ}55' S$ ,  $58^{\circ}03' W$ , in Paysandu Uruguay. Soils were clay loamy fertile Typic Argiudoll with less than 1% slope that had previously been under perennial pasture. The treatment design was a complete factorial with two factors: Kernza population origin (two levels: A and B) and N fertilization rate (three levels: 0, 80, and  $160 \text{ kg ha}^{-1} \text{ year}^{-1}$ ). The experimental design was completely randomized, with six replications per treatment (Supporting Information: Figure S1). The experiment consisted of 36 plots, each one of  $1 \text{ m}^2$ , which consisted of 25 Kernza plants planted equidistantly to 20 cm, with 0.4 m alleys (Figure 2). Seeds were sown in containers (75 ml) and were grown in standard potting compost (composition for  $1 \text{ m}^3$ : 70% milled pine bark, 30% peat, 1 kg triple phosphate, 1 kg of a controlled-release fertilizer 19-6-10). Seedlings were grown in a greenhouse under natural light, until plants reached the three-leaf stage (from mid-April 2018 until mid-May 2018) and then transplanted to a field. From transplanting until the plants reached the tillering stage, a plastic shade net was used to control early weed infestation (Figure 2b). The previous perennial pasture, whose predominant species were white

clover (*Trifolium repens* L.), dallisgrass (*Paspalum dilatatum* Poir), and Westerworld (annual) ryegrass (*Lolium multiflorum* Lam. var. *westerwoldicum*), was destroyed with herbicide (glyphosate,  $5 \text{ L ha}^{-1}$ ) and then minimally tilled with a cultivator 2 months before transplant. The N fertilizer, urea (46-0-0), was applied in the fall and winter of the 2nd and 3rd years according to the schedule in Figure 3. Each N application was half the respective annual rate. Due to the impact of weeds on Kernza performance under field production conditions surveyed by Lanker et al. (2019) for northern hemisphere conditions, after establishment, the weeds were not controlled to evaluate weed biomass and its impact on Kernza production conditions in this new region.

## Grain, forage, and weed sampling

The grain samples were collected in the summer, while the forage and weed samples were collected three times a year in fall, winter, and summer, respectively (Figure 3). Grain harvests were done when the crop had reached physiological maturity (stage S4-5, Moore et al., 1991). Grain and forage samples were collected on January 21, 2019 (accumulated forage since sowing) and on March 3, 2020 (Figure 2d). A  $60 \text{ cm} \times 60 \text{ cm}$  quadrat per plot was used, located in the center of the plot. The seed heads from these samples (corresponding to the nine plants in the center of the plot) were cut 5 cm below the lowest seeds, dried, weighed, and threshed manually to estimate grain yield. The dry weight for spikes was added to the forage dry weight to estimate the total dry biomass for the harvest index calculations. Forage and weeds samples were collected by hand-harvesting quadrat samples at 2.5 cm above the soil. Both Kernza and weeds were separated in the field and placed in individual paper bags. Samples were then placed in a forced-air dryer at  $65^{\circ}\text{C}$  for 48 h. Biomass dry matter (DM) yields per hectare were then extrapolated from the quadrat data on an area basis and one sample per



**FIGURE 2** Pictures of Kernza in the Paysandu, Uruguay experiment at seedling (a), establishment in the field (b), beginning of stem elongation (c), grain and forage harvest in summer (d), fall regrowth (e), and winter forage harvest with quadrats (f)

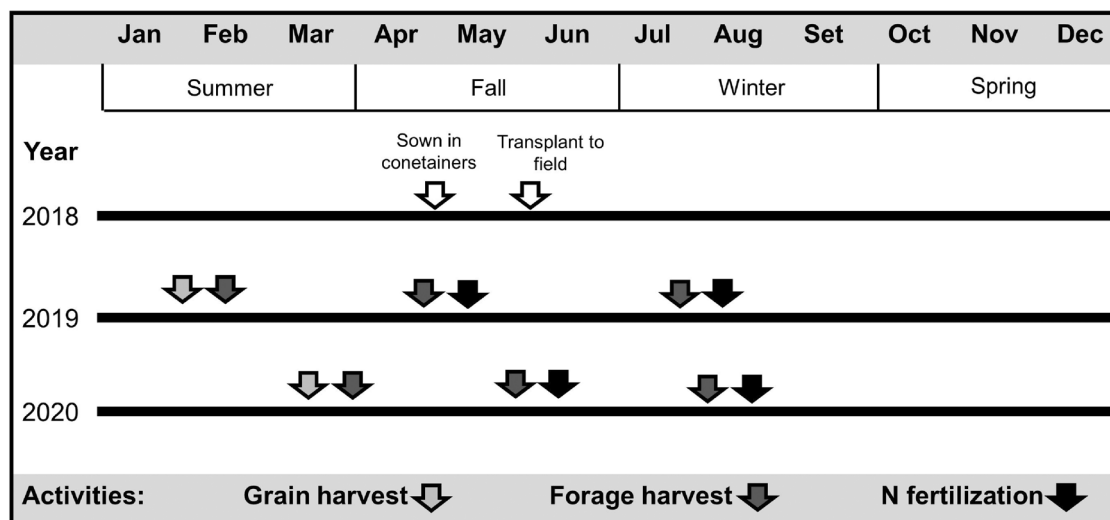


FIGURE 3 Schedule of management activities for each year of the experiment

plot was analyzed for nutritive value for each combination of treatments. The forage yield/weed biomass ratio was calculated as the result of dividing DM forage ( $\text{kg ha}^{-1}$ ) by DM weed biomass ( $\text{kg ha}^{-1}$ ). The forage fall 2019 and fall 2020 samples were collected on April 11, 2019 and May 18, 2020, respectively, and the winter ones were collected on July 15, 2019 and July 21, 2020, respectively (Figures 2e,f and 3). Forage and weeds outside the quadrants were also cut at 2.5 cm above the soil and removed after each harvest. The harvest index was calculated by dividing the mass of grain (grain yield) by the total mass of grain and straw (forage yield) in summer.

For all forage samples, a 30-g subsample was ground twice using a Wiley mill (Model 4 Thomas-Wiley Laboratory Mill; Thomas Scientific) to pass through a 1-mm screen for chemical composition analysis. Analyses of forage crude protein (CP, %) (Kjeldhal N percentage  $\times 6.25$ ) according to Horwitz and Latimer (2005), and neutral detergent fiber (NDF, %) as well as acid detergent fiber (ADF, %) (Fiber analyzer 200; Ankom Technology Corporation; Van Soest et al., 1991) were performed at the Nutrition Laboratory of the Faculty of Agronomy, University of Uruguay. CP, NDF, and ADF values for forage samples were reported as a proportion of the DM. The relative feed value (RFV) was calculated by multiplying digestible dry matter [DDM, %;  $\text{DDM} = 88.9 - (0.779 \times \text{ADF})$ ] by dry matter intake ( $120/\text{NDF}$ ) and then dividing by 1.29 (Linn & Martin, 1991).

## Weather data

Temperature and precipitation were recorded at an automatic weather station located within 100 m from the field experiment (Supporting Information: Table S1). The accumulation of heat units, expressed in growing degree-days (GDDs), was calculated using daily average temperatures and the equation from McMaster and Wilhelm (1997), using  $0^\circ\text{C}$  as the base temperature as described by Jungers et al. (2018). The accumulated Photo-Vernalo-Thermic Units (UPVTs) and vernalization units (VUs) between crop emergence and the first grain harvest and between the first and second grain harvests were calculated using the formalisms used by Duchene et al. (2021) from the STICS soil-crop model (Brisson et al., 1998, 2002, 2008).

From the STICS model, the impacts of temperature, photoperiod, and cold requirements were kept; therefore, the UPVTs were computed at the daily time step as follows:

$$UPVT(i) = GDD_t(i) \times SFP(i) \times SFV(i), \quad (1)$$

where  $i$  is the considered day,  $GDD_t$  are the growing degree-days limited by thresholds,  $SFP$  is a slow-down factor accounting for photoperiod, and  $SFV$  is a slow-down factor accounting for vernalization.

The impacts of temperature respond to a triangular function:

$$\begin{cases}
 GDD_t(i) = 0 & \text{if } T_{\text{mean}}(i) \leq DT_{\text{min}} \\
 & \text{or } T_{\text{mean}}(i) \geq DT_{\text{max}} \\
 GDD_t(i) = T_{\text{mean}}(i) - DT_{\text{min}} & \text{if } DT_{\text{min}} < T_{\text{mean}}(i) \leq DT_{\text{opt}} \\
 GDD_t(i) = \frac{DT_{\text{opt}} - DT_{\text{min}}}{DT_{\text{opt}} - DT_{\text{max}}} \times [T_{\text{mean}}(i) - DT_{\text{max}}] & \text{if } DT_{\text{opt}} < T_{\text{mean}}(i) < DT_{\text{max}}
 \end{cases} \quad (2)$$

where  $DT_{\min}$ ,  $DT_{\text{opt}}$ , and  $DT_{\max}$  are the minimal, optimal, and maximal temperatures to allow for crop development.  $DT_{\min}$  is equal to the base temperature, and the values for  $DT_{\text{opt}}$  and  $DT_{\max}$  used were 28°C and 35°C, respectively (Brisson et al., 2008).

The photoperiod [ $PhotoP(i)$ ] was calculated using astronomical input on the basis of the calendar day and the latitude using the NOAA solar calculator (<https://gml.noaa.gov/grad/solcalc/sunrise.html>).

The slow-down photoperiodic effect [ $SFP(i)$ ] could be calculated as follows:

$$\begin{cases} SFP(i) = \frac{[PhotoP(i) - PhotoP_{\text{sat}}]}{(PhotoP_{\text{sat}} - PhotoP_{\text{base}})} + 1, \\ SFP(i) = \max\{\min[SFP(i), 1], 0\}, \end{cases} \quad (3)$$

where  $PhotoP_{\text{base}}$  is the photoperiod below which there is no development and  $PhotoP_{\text{sat}}$  is the saturation photoperiod above which there is no limitation. The second term of this equation retains  $SFP(i)$  values between 0 and 1 in mathematical terms.

The estimation of the cold requirements consisted of two steps: the vernalizing value of a given day was first computed [ $VV(i)$ ], and then the slow-down factor [ $SFV(i)$ ] was calculated as the ratio of completion of the total vernalization requirements:

$$VV(i) = \max\left\{1 - \left[\frac{T_{\text{vern}} - T_{\text{mean}}(i)}{Ampli_{\text{vern}}}\right]^2, 0\right\}, \quad (4)$$

$$\begin{cases} SFV(i) = \frac{\left[\sum_{j=D_{\text{emer}} \text{ or } D_{\text{harv}}}^i VV(j)\right] - VV_{\min}}{VV_{\text{req}} - VV_{\min}}, \\ SFV(i) = \max\{\min[SFV(i), 1], 0\}, \end{cases} \quad (5)$$

where  $VV(i)$  is the vernalization unit on day  $i$ ,  $T_{\text{vern}}$  is the temperature to reach an optimal vernalization value,  $T_{\text{mean}}$  is the mean temperature on day  $i$ , and  $Ampli_{\text{vern}}$  is the temperature range with vernalizing effects. In this study, we used the best estimates calculated by Duchene et al. (2021) and also used by Jungers et al. (2022):

$T_{\text{vern}} = 4.5$  and  $Ampli_{\text{vern}} = 7.4$ . The accumulation of vernalization units was calculated by summing  $VV_i$  from the day of emergence to the first grain harvest and between this one and the second grain harvest (Table 1). Therefore, the accumulation of VU included all days that had vernalizing temperatures (within the  $Ampli_{\text{vern}}$  range) for both grain production years.

For each of the activities carried out throughout the experiment (grain harvests, forage harvests), days, thermal accumulation (GDD), and accumulated rainfall (mm) during the whole experiment were calculated (Table 1).

## Plant heading and heading time

Plant heading and heading time were measured for the 1st year of production only. Plant heading was monitored three times a week and was recorded when the first spike emerged from the flag leaf sheaf (stage R1, according to Moore et al., 1991). Plant heading was expressed as the percentage of plants that produced at least one head. Heading time was calculated in GDD from the emergence to heading date for each plant. During the 1st year, the number of spikes per plant was also assessed for the 90 plants with earliest flowering.

## Statistical analysis

Because the first N application was done after the first fall forage harvest (April 2019, Figure 3), the statistical models used for the 1st year did not include the N fertilization factor, whereas the models used for the 2nd year did. Therefore, for the 1st year, the treatment design consisted of only one factor (population) with two levels (A and B), and each treatment was replicated 18 times (i.e., 18 plots or experimental units) in a completely randomized design. The 2nd year was a complete factorial with the two populations and three N application rates (0, 80, and 160 kg N ha<sup>-1</sup>), and each treatment combination was replicated six times (i.e., six plots) in a completely randomized design.

Treatment effects on forage yield, grain yield, harvest index, weeds biomass, heading time, NDF, ADF, CP, and RFV were tested with analysis of variance conducted

**TABLE 1** Days, thermal accumulation (GDD), and accumulated rainfall (mm) during each experimental management activity (first grain and forage harvest, first fall forage harvest, first winter forage harvest, second grain and forage harvest, second fall forage harvest, and second winter forage harvest)

Harvest	Date	Days	GDD (°C day)	Accumulated rainfall (mm)	VU	UPVT
First grain and forage harvest	Jan 21, 2019	267	4320	1128	43	1210
First fall forage harvest	Apr 11, 2019	80	1756	282	-	-
First winter forage harvest	Jul 15, 2019	175	3148	512	-	-
Second grain and forage harvest	Mar 3, 2020	407	7551	1363	29	1033
Second fall forage harvest	Mar 3, 2020	76	1480	211	-	-
Second winter forage harvest	Jul 20, 2020	139	2263	355	-	-

*Note:* For the first grain and forage harvest, the thermal (GDD), days, UPVT, and VU accumulations are shown from emergence in containers (May 1, 2018), while rainfall accumulation began to be counted from transplanting to the field (May 15, 2018). With every grain harvest, the unit count resets to zero. UPVTs and VUs accumulations were calculated for the first and second grain and forage harvests.

Abbreviations: GDD, growing degree-days; UPVT, Photo-Vernalo-Thermic Unit; VU, vernalization unit.

using R version 4.0.3 (R Core Team, 2020) and considered significant at  $p < 0.05$ .

For the 1st-year analysis, for grain yield, harvest index, heading time, NDF, ADF, CP, and RFV, a randomized complete design was used and only the effect of population was included in the following linear model:

$$Y_{ij} = \mu + P_i + e_{ij}, \quad (6)$$

where  $Y_{ij}$  is the ( $ij$ )th observation of the response variable,  $\mu$  is the overall mean,  $P_i$  is the effect of the  $i$ th population (fixed effect), and  $e_{ij}$  is the residual error. An analysis of deviance using this same model was carried out on plant heading using the GLIMMIX procedure in SAS 9.4 (SAS Institute, 2004) with a binomial link because plant heading is a presence/absence trait.

For the 2nd year, the effects of population, N rate, and their two-way interaction on grain yield and harvest index were included in the following linear model:

$$Y_{ijk} = \mu + P_i + N_j + PN_{ij} + e_{ijk}, \quad (7)$$

where  $Y_{ijk}$  is the ( $ijk$ )th observation of the response variable,  $\mu$  is the overall mean,  $P_i$  is the effect of the  $i$ th population (fixed effect),  $N_j$  is the effect of the  $j$ th N rate (fixed effect),  $PN_{ij}$  is the interaction between population and harvest season (fixed effect), and  $e_{ijk}$  is the residual error.

During the 1st year, population, harvest season, and their two-way interaction effects on forage yield, weed biomass, and forage/weed biomass ratio were analyzed with a randomized complete design with population as the treatment and harvest season as the repeated measure. The following linear mixed model was used:

$$Y_{ijk} = \mu + P_i + S_j + PS_{ij} + e_{ijk}, \quad (8)$$

where  $Y_{ijk}$  is the ( $ijk$ )th observation of the response variable,  $\mu$  is the overall mean,  $P_i$  is the effect of the  $i$ th population (fixed effect),  $S_j$  is the effect of the  $j$ th harvest season (fixed effect),  $PS_{ij}$  is the interaction between population and harvest season (fixed effect), and  $e_{ijk}$  is the residual error.

For the 2nd year of the experiment, the effects of population, the N rate, and harvest season and their two- and three-way interactions on yield forage, weed biomass, and forage/weed biomass ratio were analyzed with a randomized complete design with population and N rate combinations as the treatment and harvest season as the repeated measure. The following linear mixed model was used:

$$Y_{ijkl} = \mu + P_i + N_j + S_k + PN_{ij} + PS_{ik} + NS_{jk} + PNS_{ijk} + e_{ijkl}, \quad (9)$$

where  $Y_{ijkl}$  is the ( $ijkl$ )th observation of the response variable,  $\mu$  is the overall mean,  $P_i$  is the effect of the  $i$ th population (fixed effect),  $N_j$  is the effect of the  $j$ th N rate (fixed effect),  $S_k$  is the effect of the  $k$ th harvest season (fixed effect),  $PN_{ij}$ ,  $PS_{ik}$ ,  $NS_{jk}$ , and  $PNS_{ijk}$  are the effects of the two- and three-way interactions, and  $e_{ijkl}$  is the residual error.

## RESULTS

### Weather and field conditions

From emergence in containers to the first grain harvest, 4320 GDD, 43 VU, and 1210 UPVT were accumulated. From the first grain harvest to the second one, 7551 GDD, and 29 VU, and 1033 UPVT were accumulated (Table 1). From the first grain harvest to the first fall forage harvest, more than 1700 GDD were accumulated; from the later to the first winter forage harvest, more than 1300 GDD were accumulated. The phenological delay observed in the 2nd year caused the grain to mature later (March), so thermal accumulation of the regrowth period was shorter. From transplanting to the field to the first grain harvest, rainfall was 1128 mm, and from the first to the second grain harvest, there was 1363 mm of rainfall. For each fall and winter forage regrowth period, the average rainfall was well over 200 mm (Table 1).

Kernza plant density did not change during the 1st year of the experiment; all Kernza plants survived. In the 2nd year, some plants were lost, mainly to weed competition.

### Plant heading, heading time, grain yield, and harvest index

Populations A and B showed no differences in plant heading or heading time in the 1st year (Table 2). Both populations had an average 47.5% plant heading percentage (A: 49.2%, B: 45.8%) and average 2892 GDD accumulation (A: 2874 GDD, B: 2909 GDD) at plant heading in the 1st year. The number of spikes per plant was very similar for both populations (A: 23.3, B: 22.5). Heading was not recorded in the 2nd year.

Kernza grain yield in the 1st year averaged  $316 \pm 29 \text{ kg ha}^{-1}$ , and it was not different between Kernza populations ( $p = 0.38$ ). In the 2nd year, an 87% reduction in grain yield occurred on average. No population  $\times$  N rate interaction ( $p = 0.53$ ) was detected; however, both effects population ( $p < 0.05$ ) and N rate ( $p = 0.09$ ) affected grain yield in the 2nd year (Table 2). Population A had three times the grain yield in the 2nd year than population B (Figure 4). Grain yield in the 2nd year were  $23 \text{ kg ha}^{-1}$  without N,  $37 \text{ kg ha}^{-1}$  with N80, and  $63 \text{ kg ha}^{-1}$  with N160.

As with grain yield, the harvest index in the 1st year was not affected by the Kernza population ( $p = 0.95$ ). In the 2nd year, the population had an effect on the harvest index ( $p < 0.05$ ), but not the N rate ( $p = 0.20$ ) or their interaction ( $p = 0.54$ , Table 2). Population A had a higher harvest index in the 2nd year (Figure 4).

### Forage yield and nutritive value

Population ( $p < 0.01$ ), harvest season ( $p < 0.01$ ), and population  $\times$  harvest season interaction ( $p < 0.05$ ) effects were detected on forage yield in the 1st year (Table 2). During the 1st year, population A accumulated more forage in

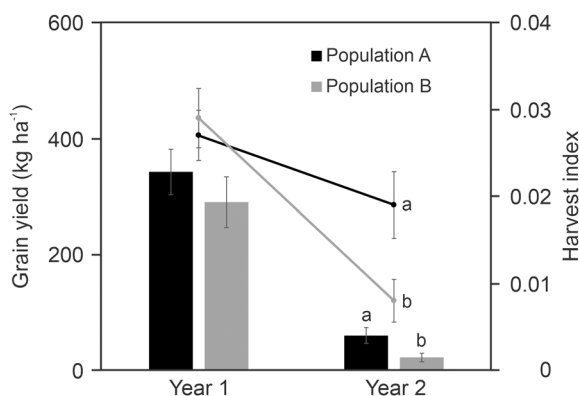


**TABLE 2** Percentage of the sum of squares of each effect over the total sum of the sources of variation from the analysis of variance of the effect of Kernza population (A vs. B), N rate (0, 80, 160 kg N ha<sup>-1</sup>), harvest season (summer, fall, and winter), and their respective interactions on grain yield, forage yield, weeds biomass, harvest index, and forage/weed biomass ratio, for each year (1st and 2nd) of the experiment, and plant heading, heading time, neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and relative feed value (RFV) for the 1st year of the experiment in Paysandu, Uruguay

Item	Year	Source of variance						
		Population (P)	Nitrogen (N)	Harvest season (HS)	P × N	P × HS	N × HS	P × N × HS
Grain yield	1	2.26	-	-	-	-	-	-
	2	15.57*	11.96	-	3.00	-	-	-
Forage yield	1	1.21**	-	85.46**	-	0.80*	-	-
	2	0.52	2.44*	74.77**	0.70	1.55*	1.90*	0.27
Weed biomass	1	3.90	-	32.2**	-	0.16	-	-
	2	0.91	0.88	40.78**	0.15	1.60	2.36	0.25
Harvest index	1	0.02	-	-	-	-	-	-
	2	11.8*	7.68	-	1.97	-	-	-
Forage/weed	1	5.70**	-	12.57**	-	11.34**	-	-
Biomass ratio	2	0.55	2.71	1.07	0.64	2.33	2.94	5.02
Plant heading	1	<sup>a</sup>	-	-	-	-	-	-
Heading time	1	3.46	-	-	-	-	-	-
NDF	1	3.49 × 10 <sup>-3</sup>	-	87.15**	-	0.31	-	-
ADF	1	2.95 × 10 <sup>-4</sup>	-	84.71**	-	0.34	-	-
CP	1	0.40	-	89.50**	-	0.09	-	-
RFV	1	0.01	-	86.56**	-	0.38	-	-

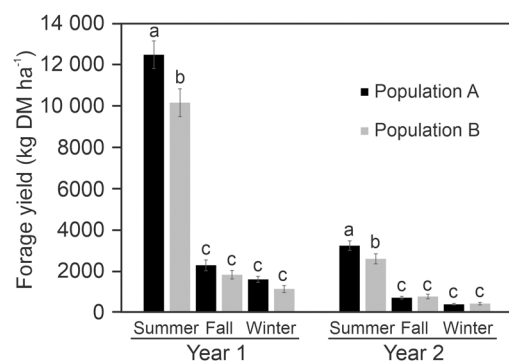
<sup>a</sup>Deviance analysis was carried out for plant heading ( $p = 0.40$ ) using the GLIMMIX procedure in SAS 9.4.

\* $p < 0.05$ , \*\* $p < 0.01$ .



**FIGURE 4** Grain yield (bars) and harvest index (lines) for the Kernza populations A (black) and B (gray) from the two 1st years of Kernza intermediate wheatgrass managed for dual use (forage harvest in the fall and winter, grain and forage harvest in the summer) in Paysandu, Uruguay. Means with the same letter within each parameter and for each year are not different at  $p = 0.05$ .

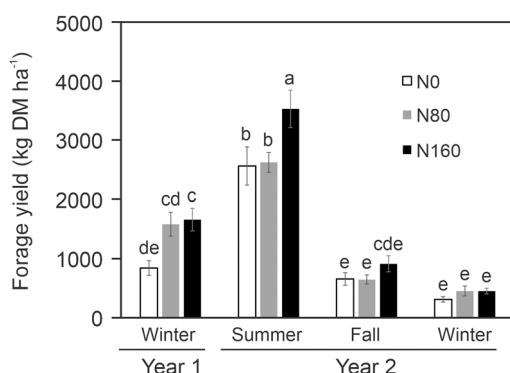
the summer than population B (Figure 5). In contrast, the average forage accumulation in both fall  $2043 \pm 167$  kg DM ha<sup>-1</sup> and winter  $1356 \pm 116$  kg DM ha<sup>-1</sup> was lower than that in summer and no differences were detected between populations (Figure 5). For the 2nd year, as in the 1st year, population A accumulated more forage than population B in the summer, but not in the following seasons (Figure 5).



**FIGURE 5** Forage yield for the Kernza populations A (black bars) and B (gray bars) in each of the harvests throughout the experiment in Paysandu, Uruguay. For the 2nd year, there was a nitrogen (N) rate × harvest season interaction, but means averaged over N rates are shown. Means with the same letters within each year are not different at  $p = 0.05$ .

In the 2nd year, N rate ( $p < 0.05$ ), harvest season ( $p < 0.01$ ), population × harvest season ( $p < 0.05$ ), and N rate × harvest season interaction ( $p < 0.05$ ) affected forage yield (Table 2). N160 produced more forage than the treatment with no N ( $1626$  vs.  $1173$  kg DM ha<sup>-1</sup>), whereas N80 ( $1237$  kg DM ha<sup>-1</sup>) did not differ from either N0 or N160 across populations and harvest seasons. The N160 treatment produced the most forage in the summer compared with the other N rates,

but no differences in forage production were observed among N rates during fall or winter (Figure 6). Forage production decreased from summer to fall and winter, and

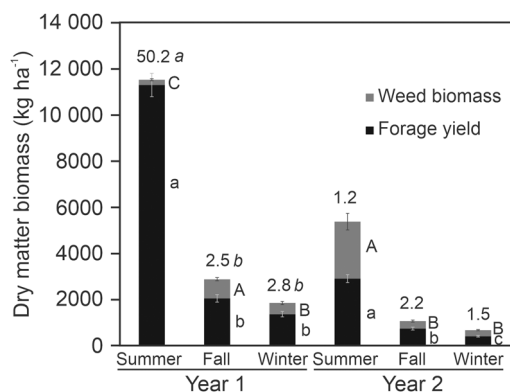


**FIGURE 6** Forage yield for summer, fall, and winter harvest across Kernza populations in response to the nitrogen (N) rate (N0: white bars, N80: gray bars, N160: black bars) in Paysandu, Uruguay. Analysis of variance was performed for four seasons (winter 2019 and summer, fall, and winter 2020). Different letters indicate significant differences among N rates and harvest seasons at  $p = 0.05$ .

**TABLE 3** Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), and relative feed value (RFV) of 1st-year Kernza intermediate wheatgrass managed for dual use (forage harvest in the fall and winter, grain and forage in the summer) in Paysandu, Uruguay

Parameter	Harvest season		
	Summer	Fall	Winter
NDF (g kg <sup>-1</sup> DM)	705.8 ± 0.4 <sup>a</sup>	662.6 ± 0.3 <sup>b</sup>	560.7 ± 0.4 <sup>c</sup>
ADF (g kg <sup>-1</sup> DM)	429.9 ± 0.5 <sup>a</sup>	352.2 ± 0.3 <sup>b</sup>	304.7 ± 0.5 <sup>c</sup>
CP (g kg <sup>-1</sup> DM)	82.0 ± 0.3 <sup>c</sup>	142.1 ± 0.2 <sup>b</sup>	197.3 ± 0.3 <sup>a</sup>
RFV	73.2 ± 0.9 <sup>c</sup>	86.4 ± 0.7 <sup>b</sup>	108.5 ± 1.2 <sup>a</sup>

Note: Values are presented as mean ± standard error. Means were calculated over two populations (A and B); means with the same letter within each parameter are not different at  $p = 0.05$ .



**FIGURE 7** Forage yield (black bars), weed biomass (gray bars), and forage/weed biomass ratio (values placed on top of each joint bar) for each harvest season throughout the experiment. Forage yield, weed biomass, and forage/weed biomass ratio with the same letter within each year are not different at  $p = 0.05$ . Within each year, capital and lower-case letters correspond to forage/weed biomass ratio, weed biomass, and forage yield, respectively.

no significant differences were detected between these in the last harvest season.

No population × harvest season interaction or population effects were detected on any forage quality parameters. Harvest season affected ( $p < 0.01$ ) all of them (Table 2). Forage quality increased from summer to winter, that is, NDF and ADF were greatest in the summer, intermediate in the fall, and lowest in the winter; on the contrary, CP and RFV were lowest in the summer, intermediate in the fall, and greatest in the winter (Table 3).

## Weed biomass

For the 1st year, weed biomass was very low in summer, high in fall, and intermediate in winter, and for the 2nd year, it was high in summer and lower in both fall and winter harvest seasons (Figure 7). Weed biomass accumulation doubled in the 2nd year in relation to the 1st year and this increase was mainly explained by the weed invasion that occurred by the second summer (Figure 7). Annual ryegrass and white clover from the previous pasture were the predominant weeds in both years. However, the ryegrass/white clover ratio increased sharply for the 2nd year as well as the presence of some perennial grasses such as dallisgrass. Since the first harvest (summer 2019), the forage/weed biomass ratio decreased and remained low during the successive cuts (Figure 7). Population ( $p < 0.01$ ), harvest season ( $p < 0.01$ ), and population × harvest season interaction ( $p < 0.01$ ) effects were detected on forage/weed biomass ratio during the 1st year (Table 2). Instead, no effect of the treatments evaluated was detected in the 2nd year. For the 1st year, population A had a much higher forage/weed biomass ratio than population B (1582 vs. 48) in summer only. Also, this ratio was lower in both fall and winter (Figure 7). In both years, only harvest season ( $p < 0.01$ ) affected weed production (Table 2).

## DISCUSSION

### Population effects on agronomic performance

Our first hypothesis was rejected: the population from seed harvested from plants that completed their life cycle during one spring–summer–fall period (population B) did not have superior agronomic performance for any of the traits evaluated. Several noncontradictory mechanisms can explain this surprising finding, and may serve as a hypothesis to test in future studies: (a) lack of response to selection due to genetic mechanisms, (b) experimental conditions providing enough vernalization stimuli, (c) seed quality (small seeds) masking genetic differences, and (d) photoperiod (short days) as a substitute for vernalization stimuli in Kernza. These potential mechanisms are discussed next.

Although the environmental conditions in Wisconsin where selection was performed to obtain population B were adequate for the purpose of obtaining germplasm with lower vernalization requirements (i.e., there was no

cold accumulation during spring–summer–fall to fulfill vernalization), population B differed from population A by only one selection cycle. Although the genetic basis for the response to vernalization is still unknown in *Kernza*, in annual wheat (genetically close to *Kernza*), there is allelic diversity and/or different number of gene copies in the main group of genes involved in vernalization requirements (*VRN1*, *VRN2*, *VRN3*, and *VRN4*), which strongly modifies both flowering ability and time to flowering (Díaz et al., 2012; Goncharov, 2004; Hyles et al., 2020; Kippes et al., 2018; Li et al., 2013). Therefore, the possible high genetic diversity associated with vernalization requirements in addition to the polyploidy present in *Kernza* could be key factors in explaining the lack of progress in agronomic adaptation expected for population B with a single selection cycle. However, population B was not grown in Wisconsin and further studies are needed to confirm this.

Additionally, the cold stimuli for vernalization where the seed of population B was produced were more restrictive than that offered in this experiment. In Wisconsin, the plants that formed population B were exposed to high and rising temperatures from emergence to plant heading (month temperature averages: May, 13°C; June, 20°C; July, 21°C; August, 19°C; September, 17°C). Although these plants were probably able to accumulate a few hours of vernalizing nocturnal temperatures without experiencing devernalization since the contrast with diurnal temperatures is not as strong under field conditions (Preston & Fjellheim, 2022), this accumulation was poor. In contrast, in Paysandu (Uruguay), the populations were exposed to a winter with lower temperatures (June, 10.2°C; July, 10.3°C; August, 11.1°C). Therefore, considering the variability in flowering and grain yield components shown in *Kernza* in response to different vernalizing stimuli in greenhouse studies (Ivancic et al., 2021; Locatelli, Gutierrez, et al., 2022), under Paysandu conditions, part of the germplasm of population A could have fulfilled vernalization requirements.

Contrary to our expectations, population A had higher forage yield accumulation in the summer of both years (Figure 5), possibly explained by its higher initial seed weight. We observed that the seedlings of population A had a noticeably larger size than population B at transplant (visual observation but no experimental measures). The effects of seed size on plant growth have been studied mostly in annual species but also in perennials, though no consensus has been reached yet. While some studies report that large seeds promote higher rates of seedling growth (Arnott, 1969; Bretagnolle et al., 1995; Glewen & Vogel, 1984; Kosiński, 2008; Leger et al., 2019; Peterson et al., 1989; Smith et al., 2003; Thomas, 1966; Trupp & Carlson, 1971), other studies highlight the limited effect of large seeds that is quickly lost (Beveridge & Wilsie, 1959; Lewis & Garcia, 1979; Naylor, 1980; Smart & Moser, 1999). As in other perennial crops, there is controversy about the effects of seed size on seedling growth in *Kernza* as well (Cattani & Asselin, 2022; Hunt & Miller, 1965), and there are no studies on the effects of seed size on the

growth and development of adult plants. However, for annual species, there is evidence that seed size had effects after the seedling stage, affecting grain yields (Baalbaki & Coopeland, 1997; Burriss et al., 1973; Gan & Stobbe, 1995; Kumar & Seth, 2004; Mian & Nafziger, 1992; Stobbe et al., 2008; Stougaard & Xue, 2005). Because most of the studies conducted on the issue compared different seed sizes between pots or plots but not intra-pot or intra-plot, and because weed control was strict, it was expected that early growth was related to seed size, but as intraspecific competition increased, average plant yield was determined by density within the stand rather than by seed size (Harper & Obeid, 1967; Stanton, 1984). In our work, similar to what has been reported in natural populations (Ehrlén & Lehtilä, 2002; Leishman & Westoby, 1994; Leishman et al., 1995; Rees, 1996), intra-plot competition occurred between *Kernza* plants and other species (no weed control), so the advantages of large seeds could be seen well beyond the seedling stage.

Although the population effect was not significant for the 2nd year on forage production, significant effect of the population  $\times$  harvest season interaction was detected (Table 2). Population A had higher forage production in the summer than population B, and no population differences were detected in subsequent fall and winter harvests (Figure 5). With the hypothesis that the larger seed size of population A resulted in more vigorous plants and greater ability to compete with weeds during the 1st year, it is logical to assume that for the 2nd year, some of these strengths already generated will be maintained at least in the periods of greater forage accumulation (summer). Although initial vigor was not measured, the higher forage/weed biomass ratio of the population with higher seed weight (A) during the 1st year could indicate the positive effect of seed size in the early stages of development on plant stand.

Our second hypothesis was not rejected: *Kernza* grown in low latitudes had similar forage yield and nutritive value as *Kernza* grown in higher latitudes, but lower grain yields. Grain yields were less than those usually observed in the higher latitudes of the northern hemisphere (Figure 4), which was expected. This experiment represents the first time this species was evaluated in a region with mild winters, which suggests the low degree of agronomic adaptation for the germplasm used. Indeed, due to the vernalization requirements for primary induction of the populations used, initially grown in northern regions (between 4°C and 5°C for 7 weeks as a putative optimal; Duchene et al., 2021; Ivancic et al., 2021; Locatelli, Gutierrez, et al., 2022), heading was strongly reduced, and only 47.5% of the plants showed flowering in the 1st year. However, considering that the grain yields in the 1st year were generated by less than half of the plants in the stand, it can be inferred that the yield per plant of the plants that produced grain was similar to that observed in the northern hemisphere. This observation highlights that the populations considered are highly diverse (Jensen, Yan, et al., 2016; Zhang et al., 2016) and could adapt quickly to a novel environment as was shown previously (Cattani & Asselin, 2018). In our situation, the fact that 47.5% flowering was observed in the 1st year could

suggest partial control of primary induction by short days, which has already been observed in other perennial grasses (Heide, 1994). A regular long day induction in spring can also be considered, but seems less likely as it is essentially found with annual species (Heide, 1994). The average daylength during the winter months is between 10.1 and 11 h (Supporting Information: Table S1) and can be considered as a “short day.” Such putative short day induction could be reinforced by further use of Kernza in low-latitudes regions or by specific breeding programs to obtain populations that do not rely on cold temperatures to induce fertile tillers primordia, like smooth brome (*Bromus inermis* Leyss) (Heide, 1984). On the other hand, another factor that could explain the low flowering percentage is the supply of long days needed for secondary induction. For cool season perennial grasses, the critical photoperiods for secondary induction can vary from 9 to 10 h in Mediterranean ecotypes to more than 16 h (Heide, 1994). Although the photoperiod in the inductive months (August, September, October, November) in Uruguay ranges from 11 to 14 h (Supporting Information: Table S1), part of the germplasm evaluated could have higher long-day requirements. In addition, the degree to which primary induction requirements have been completed may be modifying the demands of secondary induction requirements, that is, the more complete the primary induction process, the fewer long-day cycles may be required in secondary induction (L. T. Evans, 1964; Heide, 1994). This said, the interactions between vernalization, cold-temperature tolerance, photoperiod, flowering, and yields remain largely unclear (Fowler et al., 1996; Jokela et al., 2015; Limin & Fowler, 2006; Seppänen et al., 2013) and require continuous and progressive evaluation of new ecotypes in fields, rather than designing new breeding programs that are decontextualized and based on simplified factors.

## Weed competition and yield decline

Grain yields for the 2nd year decreased, consistent with the results reported by several authors (Hunter, Sheaffer, Culman, & Jungers, 2020; Law et al., 2021). In our experiment, the yield reduction was very steep, which might be explained by the weed competition (Figure 7). Annual ryegrass was the dominant weed, which has a short life cycle. The large seed bank in the soil (seeds produced before the 1st-year grain harvest) and the large interval between forage and grain harvests can account for this weed increase. The timing of forage harvests affects annual ryegrass seed production, which has a strong impact on subsequent weed invasion (Piltz et al., 2021). Furthermore, the abundance of this weed can also be increased by their short life cycle because it can complete the life cycle between two consecutive mowings or forage harvests (Meiss et al., 2010). Assuming a base temperature of 0°C, the total phenological cycle (emergence—physiological maturity) of annual ryegrass can be reached between 750 and 1000 GDD (Cousens, 1996). Therefore, from the first winter forage harvest (July 2019), all ryegrass seeds in the soil

accumulated before harvest of Kernza grain in the 1st year could complete their cycle by October or November, well ahead of the second Kernza grain harvest. In summary, by the 2nd-year grain harvest, the accumulation of annual ryegrass biomass was very noticeable (Figure 7). Additionally, in the 2nd year, an increase in the presence of perennial species was observed, which was led by dallisgrass, a warm-season grass native to temperate grasslands in southern South America (Speranza, 2009). This is consistent with what was reported by Zimbric et al. (2020). Thereby, our fourth hypothesis was rejected: weed biomass in the dual-purpose system carried out in this experiment increases over time.

Another factor that could explain the considerable decrease in grain yield in the 2nd year is the mean temperature during the winter months (June, July, and August), whose records for the 2nd year (14.7°C, 11.2°C, and 11.7°C) were well above the ones for the 1st year (10.2°C, 10.3°C, and 11.1°C) (Supporting Information: Table S1), which resulted in lower accumulation of UPVT from lower accumulation of VU for the second grain harvest (Table 1). The lower VU accumulated in the 2nd year could have led to both lower plant heading percentages and lower number of fertile stems, thus contributing to the decrease in the grain yield. In this study, the accumulation of VU for the 2nd year (29) was lower than the optimum reported by Locatelli, Gutierrez, et al. (2022) under controlled conditions (49) and 10 h, by Duchene et al. (2021) under field conditions in Europe (71), or the minimum VU needed reported by Jungers et al. (2022) to produce grain in field experiments in North America (78). This lower VU accumulation in the 2nd year may have resulted in increases in demand requirements during secondary induction and thus delayed harvest.

## Forage yield

Forage yield accumulation for the 1st year was high in the summer (Figure 5), which is explained by the long forage accumulation period between transplanting (May 2018) and the first harvest (January 2019). The interaction observed between population and harvest season is explained by this first summer forage accumulation, which was greater for population A, while no population differences were found in the fall or winter cuts (Figure 5).

Contrary to what has been mostly reported in previous literature (Culman et al., 2013; Fernandez et al., 2020; Hunter, Sheaffer, Culman, Lazarus, & Jungers, 2020; Law et al., 2020; Pugliese, 2017), forage yield in the 2nd year decreased strongly (Figure 5). As for grain yield, the increase in weeds by the second summer also negatively affected forage production. In addition to the timing, the intensity of forage harvesting can also have a strong effect on weed establishment. Although severe forage cuts can be a powerful strategy to remove flower heads from some weeds in spring, during summer and/or fall, this could provide an opportunity for new weeds to establish in pastures (Bourdôt et al., 2007; Ghanizadeh & Harrington, 2019). Indeed, the stubble height after forage cuts performed in this experiment (2.5 cm) could be excessively low, causing lower regrowth

rates and thus promoting the proliferation and competitiveness of weeds. Reducing the height of defoliation below 5 cm strongly increased the proportion of dead roots of perennial grass species, such as perennial ryegrass, orchardgrass, and timothy (*Phleum pratense* L.) (P. S. Evans, 1973). For perennial ryegrass, a stubble height from 4 to 8 cm, instead of more severe cuts, optimizes its persistence and productivity by allowing more rapid replenishment of water-soluble carbohydrate reserves, which will be allocated to maintain a more active root system and promote tillering (Donaghy & Fulkerson, 1998; Lee et al., 2008). Despite the negative impact that weeds may have had on this experiment, the cumulative forage production of Kernza in the first 2 years (19 320 kg DM ha<sup>-1</sup>) was similar to the average forage production of modern perennial cool-season grass cultivars evaluated in the same years with an annual N fertilization of about 100 kg ha<sup>-1</sup>; indeed, it was higher than that of orchardgrass (15 755 kg DM ha<sup>-1</sup>) and lower than that of Tall fescue (22 344 kg DM ha<sup>-1</sup>; INIA-INASE, 2022).

## Harvest index

Harvest index had a similar pattern as grain yield. For the 1st year, no effect of population was detected, but for the 2nd year, population A was higher (Figure 4). Values for this trait in the northern hemisphere have ranged from 0.04 to 0.11 for the 1st year and from 0.02 to 0.16 for the 2nd year with similar germplasm (Cattani & Asselin, 2018; Frahm et al., 2018; Olugbenle et al., 2021). Forage production declined in the 2nd year, which could suggest that the steep reduction in the harvest index was due to resource constraints. However, Kernza grain yield declines with stand age have not been previously attributed to resource limitation, because the total biomass production remains constant or increases with age (Law et al., 2021). In our experiment, both the lower supply of cold and the heavy weed invasion detected months before the 2nd-year grain harvest were the main factors responsible for the strong reduction in the harvest index in the 2nd year. In addition, the increase in weeds could further reduce light penetration, which is one of the main factors reported that could explain the reduction in grain yield and harvest index in Kernza as stand age increases (Hunter, Sheaffer, Culman, & Jungers, 2020).

## Nutritional parameters

The nutritional value of Kernza was similar to that reported in other latitudes for the same phenological stages. Since the geographical area in which the experiment was conducted has adequate climatic conditions for Kernza to maintain active growth during the winter (no soil freezing), forage harvesting is possible during winter. The highest nutritional values of forage in the northern hemisphere correspond to early spring harvests, when the plants have not yet reached stem elongation, while in low-latitude regions, the plants remain in tillering throughout the winter (June, July, August) and do not reach stem

elongation until spring (September, October). Therefore, the winter nutritional values observed closely resemble those in the northern hemisphere for spring harvest, with around 201–225 g kg<sup>-1</sup> CP, 456–504 g kg<sup>-1</sup> NDF, and 249–299 g kg<sup>-1</sup> ADF (Barriball, 2020; Favre et al., 2019; Jensen, Robins, et al., 2016; Pinto, Culman, et al., 2021). At grain harvest, a sharp decline in nutritional values was observed (Table 3). This drop has also been reported in the northern hemisphere, with values of around 49–100 g kg<sup>-1</sup> CP, 702–828 g kg<sup>-1</sup> NDF, and 427–501 g kg<sup>-1</sup> ADF (Barriball, 2020; Favre et al., 2019; Jensen, Robins, et al., 2016; M. L. Nelson et al., 1989; Pinto, Culman, et al., 2021). Nutritional values of the fall regrowth were intermediate, in agreement with those reported in the northern hemisphere: 100–119 g kg<sup>-1</sup> CP, 575–590 g kg<sup>-1</sup> NDF, and 316–337 g kg<sup>-1</sup> ADF (Coleman et al., 2010; Favre et al., 2019). With respect to RFV, our values are very similar to those in the northern hemisphere for summer harvest (57–75) and somewhat below those for fall (100–107) and spring (120–161) (Hunter, Sheaffer, Culman, & Jungers, 2020; Pinto, Culman, et al., 2021). The reason for these differences could be the cutting height: while we cut the forage at 2.5 cm from the ground, the other experiments made the cuts at approximately 7.5 cm, excluding the lower proportion of lignocellulosic tissue with lower nutritional value.

For most of the nutritional parameters, Kernza intermediate wheatgrass values were within the same range as those reported for other cool-season grasses. Tall fescue, orchardgrass, and canarygrass (*Phalaris* sp. L.) had mean nutritional values during fall and winter of around 128–170 g kg<sup>-1</sup>, 464–622 g kg<sup>-1</sup>, and 283–344 g kg<sup>-1</sup> and summer harvest residue values of around 84–102 g kg<sup>-1</sup>, 650–705 g kg<sup>-1</sup>, and 366–390 g kg<sup>-1</sup> for CP, NDF, and ADF, respectively (National Research Council, 2000). Orchardgrass and tall fescue evaluated in the same region as our experiment had higher nutritional values in summer, and similar fall and winter nutritional values as Kernza (Mieres et al., 2004). Kernza forage harvested in winter was more nutritious than Orchardgrass (157 g kg<sup>-1</sup> CP, 555 g kg<sup>-1</sup> NDF, 381 g kg<sup>-1</sup> ADF), while tall fescue had lower values for NDF (393 g kg<sup>-1</sup>) and ADF (254 g kg<sup>-1</sup>), but similar to Kernza for CP (219 g kg<sup>-1</sup>) in this season (Mieres et al., 2004).

## N fertilization

Our third hypothesis was not rejected: N fertilization increased grain and forage yields. Grain yield responded to the higher N rate (N160), which is above the values of 61–96 kg N ha<sup>-1</sup> observed by Jungers et al. (2017). The detection of response to higher rates of N could be due to the high weed invasion observed in the 2nd year, which competed for N with the Kernza plants. The N split applications (in fall and winter) could also explain the response in grain yields at a higher application rate than that observed in Jungers et al. (2017), where N was applied once in spring. Application of all N once at spikelet initiation resulted in higher seed yields than splitting N in the fall and spring in perennial ryegrass (*Lolium perenne* L.) (Hampton, 1986). Also, the split

application of N was not better than application of all N at double ridge or spikelet initiation in Chewing's fescue (*Festuca rubra* L. ssp. *Fallax* [Thuill] Nyman), tall fescue (*Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.), and orchardgrass (*Dactylis glomerata* L.) (Young et al., 1999).

Although in this study, yield responses to N rates were only measured for the winter of the 1st year and throughout the 2nd year (summer, fall, winter), forage yield response was observed with the highest N rate (160), which is consistent with the results of Fernandez et al. (2020) for a 2-year Kernza stand of a linear increase in forage production with the addition of N up to 80 kg ha<sup>-1</sup>. Jungers et al. (2017) found a quadratic relationship between biomass yield and N rates, with an agronomically optimum N rate ranging from 81 to 121 kg N ha<sup>-1</sup>. It is actually from the 2nd year onwards that greater effects of N on productive parameters are expected, because as stands age, root biomass increases and therefore its capacity to intake nutrients such as N increases (Pugliese et al., 2019). The detection of the N rate × harvest season interaction can be explained by the differential growth of the plants in each season: no differences in forage production were detected with N addition in fall or winter, when low biomass was observed overall due to vegetative stage, but the N addition rate of 160 kg ha<sup>-1</sup> increased forage production in summer, when biomass accumulation was higher overall due to reproductive stage (Figure 6). This experiment was done in only one location, so more research in other locations and years is needed to further understand the interaction between N fertilization, weather conditions, and weed invasion in terms of Kernza yields.

## CONCLUSIONS

Kernza low grain yields and the low plant heading percentage highlight the necessity to make further efforts to obtain germplasm with truly lower vernalization requirements to expand this promising grain to lower latitudes. Germplasm more adapted to low latitudes is currently being developed (A. Locatelli, unpublished). Kernza intermediate wheatgrass forage productivity and nutritional quality are comparable to other cool-season forage species adapted to low latitudes. N fertilization has been demonstrated to be a key management factor to achieve better results in both forage and grain production, but the precise timing and number of applications deserve more research attention. The development of weed management strategies has also emerged as a major issue for the agronomic development of this crop in this region. Unlike in high latitudes, in low latitudes, Kernza maintains active growth throughout the winter, increasing the possibilities of forage harvest; thus, the management of defoliation and its interaction with N fertilization and weeds management are key research topics for the development of this crop under a dual-purpose system.

## AUTHOR CONTRIBUTIONS

**Andres Locatelli:** Data curation; formal analysis; funding acquisition; project administration; resources; software; visualization; writing – original draft. **Lucia Gutierrez:** Formal analysis; methodology; supervision. **Olivier Duchene:** Conceptualization; writing – review and editing. **Pablo R. Speranza:** Conceptualization. **Valentin D. Picasso Riso:** Conceptualization; formal analysis; investigation; methodology; supervision; writing – review and editing.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

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