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Optimal Planting Date of Kernza Intermediate Wheatgrass Intercropped with Red Clover

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Abstract: Intermediate wheatgrass (IWG) is a new perennial dual-use crop for grain and forage with growing interest among farmers. Intercropping IWG with red clover may increase yield and nutritive value through nitrogen transfer. IWG and red clover planting timing can affect grain and forage yield, and there has not been previous research on this management practice. At two locations (Arlington and Lancaster, WI, USA) a factorial experiment was established two years with two factors: (1) IWG planting date (August through October, and April) and (2) red clover planting season (in the fall with IWG or frost seeded in the next spring). Yield data were collected for two subsequent years. Grain yield was maximized at 515 kg ha^{-1} and 423 kg ha^{-1} at Arlington and Lancaster when planted by 26 August and 13 September, respectively. Planting date influenced grain yields in the first harvest year but not in the second. Seeding red clover in the spring increased IWG and red clover biomass compared to seeding it in the fall. In Wisconsin, planting IWG by early September at the latest and planting red clover in the spring is recommended to maximize grain yield.

Keywords: intermediate wheatgrass; planting date; intercrop; red clover; perennial grains; dual-use forages

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1. Introduction

Perennial crops offer many important opportunities to produce grains in a more environmentally, economically, and energetically sound manner than the current annual crops [1]. Perennial crops have extensive root systems that contribute to reducing soil erosion, nutrient, and pesticide use and losses [2,3], which lowers farmer expenses due to decreased annual inputs and costs. Recently, there have been increased research efforts to domesticate and breed perennial cereals for seed yield [4]. Among them, the cool-season grass intermediate wheatgrass (IWG, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) stands out because it provides grain, forage, and multiple ecosystem services [4–7]. This novel perennial dual-use crop has been developed through conventional breeding for increased seed production and registered under the tradename Kernza by The Land Institute; Salina, KS, USA [8]. As a dual-use crop, grain and forage of IWG can present multiple income streams to farmers [9]. However, there is minimal agronomic knowledge for the management of Kernza intermediate wheatgrass as a dual-use crop [10].

Many of the benefits of growing IWG may improve when it is intercropped with legumes. Growing more than one crop in the same area (i.e., intercropping) is advantageous because it can aid with minimizing weed competition, improving yields, increasing biodiversity, and fixing nitrogen (N) in the soil which helps decrease synthetic fertilizer use and runoff [11,12]. Furthermore, as IWG is a dual-use crop, intercropping with legumes allows a high-quality forage harvest. Particularly, IWG intercropped with red clover (*Trifolium pratense* L.) has shown a higher protein concentration, 161 g kg^{-1} dry matter (DM), than IWG monoculture, 119 g kg^{-1} DM, suggesting nitrogen transfer from legumes to the grass [13]. However, optimal intercropping management is crucial to achieving the maximization of the benefits, avoiding that the red clover competition reduces IWG yields.

One of the key management practices is planting time. Intermediate wheatgrass has a vernalization requirement, where the plant needs exposure to cold in its vegetative stage during winter to flower and produce seed in the next growing season [14]. This means that when IWG is planted in the spring, no grain can be harvested in the first summer. However, in the following years, it is not known whether spring planting could have any benefit over fall planting. The enormous progress in the domestication and breeding of IWG [4,15] must be accompanied by agronomic management recommendations because they strongly influence crop development and yield. Particularly, farmers are requesting more information about its establishment methods, such as optimal planting date and row spacing [16]. There has been no previous research on optimal planting dates for Kernza grain and forage in the North Central US, thus farmers are demanding information on the effect of planting date on yield [16].

Intermediate wheatgrass is typically planted early in the fall to ensure enough time for germination, shoot and root establishment [17,18]. The plant is exposed to a cold period over winter and resumes growth and development the following spring [14]. However, the planting date can be delayed due to farmers receiving seeds late (seed harvest is in late summer, seed availability is still low), or adverse weather conditions [16]. Planting early could potentially lead to high yields but may also increase the chances of pests or diseases interacting with the crop. On the other hand, planting too late in the fall may not allow enough time for the crop to emerge and grow, or increases weed pressure while planting in spring leads to missing one grain production year. Therefore, finding the optimal planting date is important.

Research on another cereal dual-use crop, winter wheat (*Triticum aestivum* L.) has shown that planting date affects yield. For instance, in Nebraska, grain yields were maximized with planting dates that accumulated about 400 growing degree days (GDD; with a base temperature of 4 °C) from planting until 31 December [19]. In Wisconsin, a 3 September planting date, with an approximated 790 GDD, produced the highest grain yield for winter wheat and there was no advantage planting at an earlier date [20]. Planting late reduces yield because the crop does not germinate or emerge effectively in the fall due to the lower air and soil temperature. Therefore, winter wheat should be planted between 20 September and 5 October, avoiding planting too late with low temperature and too early with potential active pests (i.e., Aphids) that transmit viruses such as barley yellow dwarf virus [21]. Intercropping with legumes may affect the optimal planting date because of competition during the establishment or facilitation due to minimizing competition with weeds and providing nitrogen [22].

The objective of this study was to determine the combined effects of (1) intermediate wheatgrass planting date and (2) red clover planting season on Kernza grain yield, intermediate wheatgrass biomass, red clover biomass and weed biomass in the first two years.

2. Materials and Methods

2.1. Site Characterization

The experiment was established at two locations from the University of Wisconsin-Madison Agricultural Research Stations: Arlington (43°18′9.47″ N, 89°20′43.32″ W) and Lancaster (42°49′52.56″ N, 90°48′1.78″ W). In each location, experiments were established in two consecutive years (2017 and 2018) and yield data were collected for two subsequent years after establishment. None of the experiments were irrigated. Soils were Plano silt loam (PnB) with 2 to 6% slope at Arlington and Fayette silt loam (FaC2) with 6 to 12% slope at Lancaster [23]. At Arlington, which was previously tall fescue (*Festuca arundinacea* L.), in both years, individual plots were rototilled with a Land Pride RTA2570 rototiller (Great Plains Manufacturing, Salina, KS, USA) to prepare a fresh seedbed before each planting. At Lancaster, which was previously corn (*Zea mays* L.), in both years the land was disked with a Ford 230 disk (Ford Manufacturing, Pittsburgh, PA, USA) and cultipacked with a Kewanee 85 cultimulcher (Kewaunee manufacturing, Kewaunee, IL, USA) due to the field being dry and to firm up the seedbed before the first scheduled planting. At Arlington, a

Carter small plot forage drill (Carter Manufacturing Co., Inc., Brookston, IN, USA) was used for planting and at Lancaster, a Great Plains 1006 no-till grain drill (Great Plains Manufacturing, Salina, KS, USA) was used for planting.

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

In 2018, the average temperature warmed up earlier after winter than 2019 or 2020 at both locations. Furthermore, in 2018 average temperature after planting started to cool earlier than the previous year. The average temperature did not vary a lot between locations with the majority of the months sharing similar temperatures (Figure 1). In all four years, Lancaster received more precipitation than Arlington, 2018 and 2019 being the wettest year for Lancaster and Arlington, respectively. September 2019 in Lancaster was recorded as the wettest month in all four years between locations with just over 470 mm of precipitation (Figure 2). Annual precipitation for Arlington from 2017 to 2020 was 856 mm, 1114 mm, 1180 mm, and 944 mm, respectively. Annual precipitation for Lancaster from 2017 to 2020 was 990 mm, 1490 mm, 1444 mm, and 1110 mm, respectively.

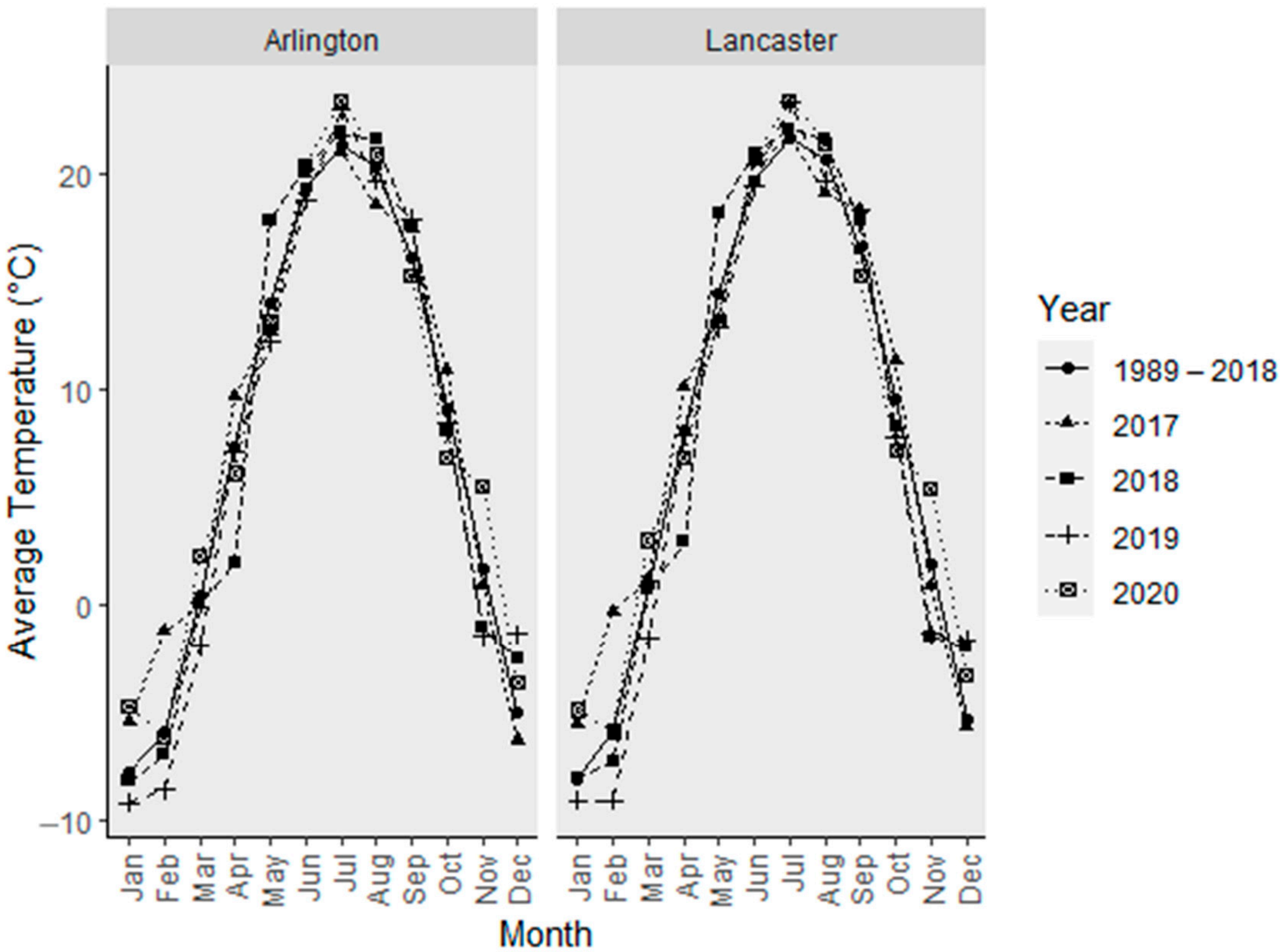

Figure 1. Average monthly temperature (°C) at Arlington and Lancaster, WI, USA for 30-year average (1989–2018) and experimental period (2017–2020) [24].

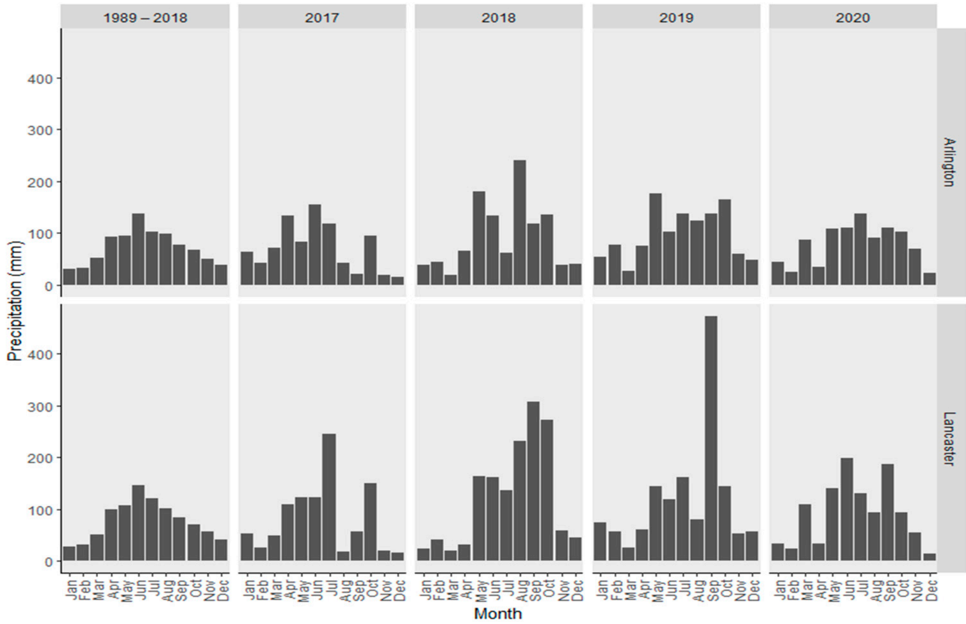

Figure 2. Monthly precipitation (mm) at Arlington and Lancaster for 30-year average (1989–2018) and experimental period (2017–2020) [24].

2.2. Experimental Design and Management

The treatment design was a full factorial with two factors: intermediate wheatgrass planting date and red clover planting season. Intermediate wheatgrass planting date factor had 6 levels: late August, mid-September, late September, mid-October, late October, and in April (spring) the following year (Table 1). Intermediate wheatgrass planting dates were not always the same across locations and years, due to weather conditions making it difficult to get into the field at times, so some plantings were delayed or missed entirely (Table 1). Red clover planting season factor had 2 levels: planting in the fall, at the same time as intermediate wheatgrass, or frost seeded in the spring.

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

	Arlington				Lancaster			
	2017		2018		2017		2018	
	Planting Date	GDD until Frost	Planting Date	GDD until Frost	Planting Date	GDD until Frost	Planting Date	GDD until Frost
Late August	30 Aug	972	-	-	-	-	30 Aug	865
Mid-September	15 Sep	728	-	-	13 Sep	798	13 Sep	597
Late September	27 Sep	468	21 Sep	420	28 Sep	473	27 Sep	335
Mid-October	20 Oct	162	14 Oct	138	10 Oct	291	12 Oct	156
Late October	-	-	30 Oct	54	26 Oct	103	-	-
Spring	30 Apr 2018	-	8 Apr 2019	-	15 Apr 2018	-	3 Apr 2019	-

The field plot design was complete randomized blocks with 4 replications for each location and planting year with each block having 10 plots. At Arlington, treatments were allocated as a full factorial, and at Lancaster, treatments were allocated as a split-plot with red clover planting season as the whole plot and intermediate wheatgrass planting date as split-plot (i.e., each block had 2 whole plots–red clover planting seasons–and each whole plot was split into 5 split-plots–Kernza planting dates). We chose a split-plot for Lancaster because it was a location further away from campus, so the Lancaster research station staff implemented the plantings, and a split-plot design was simpler than a full factorial design for planting. The Kernza seed for both locations was from The Land Institute (Salina, KS, USA) selection cycle 4, harvested in August 2017 at Arlington, WI, except for the Arlington 2018 planting, which used seed originating from The Land Institute selection cycle 5, harvested in August 2018 at Arlington, WI. Red clover was variety "FF 9615" from LaCrosse Seeds. Arlington and Lancaster received different fertilization because of different initial soil fertility in each location (Table 2).

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

	Arlington		**Lancaster**	
	2017	**2018**	**2017**	**2018**
Plot size	2.6×3.4 m^2	1.7×3 m^2	3×4.6 m^2	
IWG planting density	13.5 kg ha^{-1}		10.6 kg ha^{-1}	
Row spacing	30.5 cm		38.1 cm	
Fall red clover planting density	7.8 kg ha^{-1}		8.4 kg ha^{-1}	
Red clover frost seeding date	2 Mar 2018	8 Apr 2019	12 Mar 2018	2 Apr 2019
Red clover frost seeding density	11.2 kg ha^{-1}	10.1 kg ha^{-1}	10.1 kg ha^{-1}	
1st year Fertilization rate	44.8 kg ha^{-1} of N - -	- - -	56 kg ha^{-1} of N 45 kg ha^{-1} of P_2O_5 84 kg ha^{-1} of K_2O	102 kg ha^{-1} of N 34 kg ha^{-1} of P_2O_5 258 kg ha^{-1} of K_2O
1st year Fertilization time	Mid-May & Mid-June 2018	-	Late March 2018	May 2019
2nd year Fertilization rate	45 kg ha^{-1} of N - -	42 kg ha^{-1} of N - -	102 kg ha^{-1} of N 34 kg ha^{-1} of P_2O_5 258 kg ha^{-1} of K_2O	- - -
2nd year Fertilization time	Mid-June 2019	Mid-June 2019	May 2019	-
1st year Summer harvest date	7 Aug 2018	8 Aug 2019	3 Aug 2018	30 July 2019
1st year Fall harvest date	26 Oct 2018	22 Oct 2019	15 Oct 2018	25 Oct 2019
2nd year Summer harvest date	6 Aug 2019	30 July 2020	30 July 2019	4 Aug 2020

Temperature and precipitation records were obtained from the online database of the National Weather Service [24]. Daily average temperatures and the equation [24,25], were used to calculate growing degree days (GDD) in Celsius with a base temperature of 0 °C. The equation used was

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

T_{MAX} and T_{MIN} are daily maximum and minimum air temperatures, respectively, while T_{BASE} is the base temperature below which there is no accumulation of GDD (IWG T_{BASE} = 0 °C). GDD accumulation initiated at planting and ended when average daily temperatures remained below the base temperature for 5 consecutive days [13,17].

2.3. Forage and Grain Sampling

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

2.4. Statistical Analyses

Statistical analyses were conducted using PROC MIXED procedure in SAS 9.4 software [26]. Analysis of variance was conducted by location and age of stand, using a model for a randomized complete block design:

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

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

Using the least square means obtained previously, linear, quadratic, and cubic regression equations and p-values were obtained in PROC REG procedure in SAS 9.4 software [26], for Kernza grain yield, biomass yield, and weed biomass against accumulated fall growing degree days until the end of the season for each combination of location, age of stand, and clover planting season. Best fit equations for each variable were selected based on the Akaike information criterion (AIC) values. With the best fit model, optimal planting date in GDD was determined for each situation.

In order to convert GDD into calendar dates for the optimal planting dates, a 30-year mean was calculated for the end of GDD accumulation period. This was accomplished by obtaining daily weather records from the past 30 years [24]. For each year, the date for the end of GDD accumulation period, i.e., when average daily temperatures remained below 0 °C for 5 consecutive days, was identified first, and from that date, accumulation of GDD was calculated backward until the required GDD was reached. Later, each date that reached the required GDD was converted to a numerical day of the year (i.e., 1 Jan = 1 to 31 Dec = 365) and the mean for the 30 years was found. The mean value was then converted back to its corresponding date. The 90% probability of accumulating the required GDD was obtained by first ordering the numerical day of year values in ascending order for all 30 years. Then, a cumulative relative frequency was calculated for each day of year value.

3. Results

3.1. Grain Yield

In both locations, Kernza grain yield varied with intermediate wheatgrass planting date and red clover planting season (Figure 3, Supplemental Tables S1 and S2). Overall, planting later in the fall increased Kernza grain yield in the first year increases until it reached an optimum and then declined with an asymmetrical slope. The exception was a linear decline at Arlington when red clover was planted in the spring.

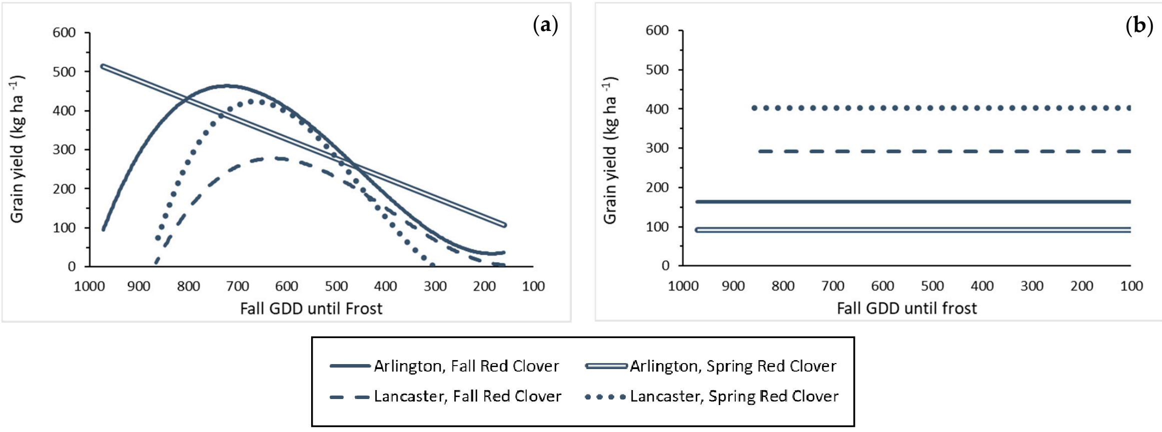

Figure 3. Lines of estimated values from best fit models of Kernza grain yield in kg ha^{-1} for Arlington and Lancaster's fall and spring planted red clover treatments in (**a**) the first harvest year and (**b**) the second harvest year. Y axis: Kernza grain yield; x axis: planting date represented by fall growing degree days until frost (earlier planting dates correspond to higher × values). The first year line equations and *p*-values are: Arlington, fall red clover: $Y = -0.000006x^3 + 0.01x^2 - 2.2x + 220.6$ ($p = 0.01$); Arlington, spring red clover: $Y = 0.5x + 27.8$ ($p = 0.01$); Lancaster, fall red clover: $Y = -0.000005x^3 + 0.01x^2 - 1.4x + 99.9$ ($p = 0.02$); Lancaster, spring red clover: $Y = -0.00001x^3 + 0.01x^2 - 4.5x + 390.1$ ($p < 0.05$).

At Arlington, the maximum Kernza grain yields were 463 kg ha^{-1}, when red clover was planted in the fall and IWG accumulated 721 GDD, and 515 kg ha^{-1}, when red clover was planted in the spring and IWG accumulated 972 GDD (Figure 3a). At Lancaster, the maximum Kernza grain yields were 278 kg ha^{-1}, when red clover was planted in the fall and IWG accumulated 630 GDD, and 423 kg ha^{-1}, when red clover was planted in the spring and IWG accumulated 664 GDD (Figure 3a). There was no effect of planting date on the grain yield in the second year, leading to a horizontal line as the best model (Figure 3b). In the second year, the mean Kernza yields at Arlington were 164 kg ha^{-1} when red clover was planted in the fall (a 65% decrease from the maximized yield from the previous year), and 91 kg ha^{-1} when red clover was planted in the spring (an 82% decrease from the maximized yield from the previous year). At Lancaster, they were 291 kg ha^{-1} when red clover was planted in the fall (a 5% increase from the maximized yield from the previous year), and 403 kg ha^{-1} when red clover was planted in the spring (a 5% decrease from the maximized yield from the previous year) (Figure 3b).

Kernza planted in the spring yielded 0 and 12 kg ha^{-1} in the first year at Arlington and Lancaster, respectively, and in the second year, yields were 64 and 205 kg ha^{-1} at Arlington and Lancaster, respectively.

3.2. Intermediate Wheatgrass Summer Biomass

At Arlington, there was no effect of planting date on intermediate wheatgrass summer biomass in the first year when red clover was planted in the fall, with a mean yield of 1584 kg ha^{-1} (Figure 4a, Supplemental Tables S1 and S2). When red clover was planted in the spring a linear decline was observed in the first year, from a maximum yield of 6811 kg ha^{-1} when intermediate wheatgrass accumulated 972 GDD (Figure 4b, Supplemental Tables S1 and S2). At Lancaster, there was also no effect of planting date on intermediate wheatgrass summer biomass in the first year when red clover was planted in the fall, with a mean yield of 1724 kg ha^{-1} (Figure 4c, Supplemental Tables S1 and S2). A linear decline in the first year from a maximum yield of 5158 kg ha^{-1} when intermediate wheatgrass accumulated 865 GDD was observed for plots with red clover planted in the spring at Lancaster (Figure 4d). There was no effect of planting date on the intermediate wheatgrass summer biomass in the second year on any of the treatments (Figure 4e–h, Supplemental Tables S1 and S2). The mean yields when red clover was planted in the fall and spring at Arlington were 1728 kg ha^{-1}, a 9% increase from the previous year, and 1147 kg ha^{-1} an 83% decrease from the maximized yield from the previous year, respectively. The mean yields when red clover was planted in the fall and spring at Lancaster were 5762 kg ha^{-1}, a 234% increase from the previous year, and 4154 kg ha^{-1} a 19% decrease from the maximized yield from the previous year, respectively.

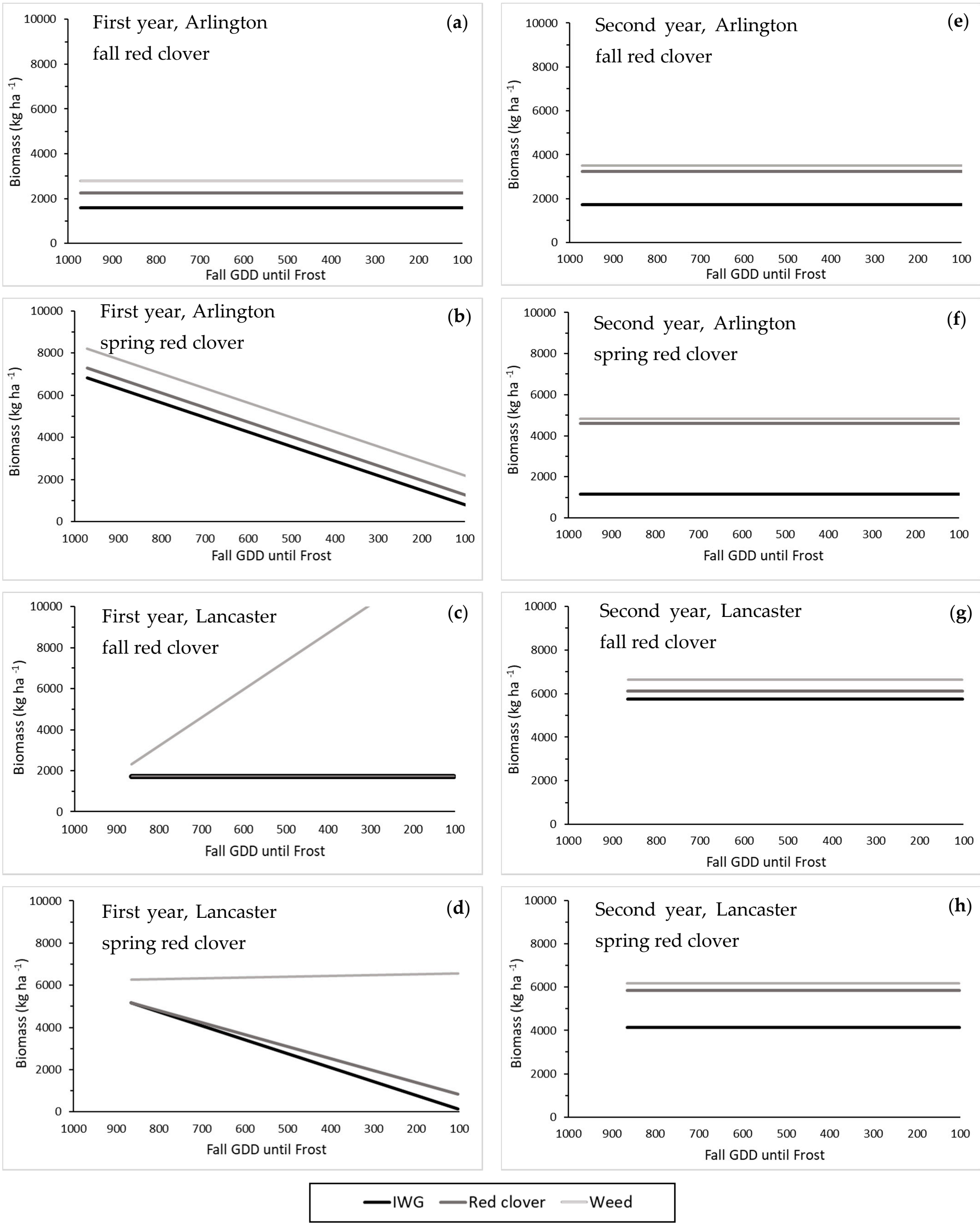

Figure 4. Stacked (i.e., cumulative) lines of estimated values from best fit models of summer Intermediate wheatgrass (IWG), red clover, and weed biomass in kg ha^{-1} in the first year for (**a**) Arlington fall red clover, (**b**) Arlington spring red clover, (**c**) Lancaster fall red clover, and (**d**) Lancaster spring red clover, and in the second year for (**e**) Arlington fall red clover, (**f**) Arlington spring red clover, (**g**) Lancaster fall red clover, and (**h**) Lancaster spring red clover treatments. The first year linear equations and *p*-values are Arlington, spring red clover: IWG = 6.9x + 104.2 ($p < 0.01$); Lancaster, fall red clover: Weed = −13.8x + 12511 ($p = 0.01$); and Lancaster, spring red clover: IWG = 6.6x − 550.9 ($p = 0.01$), Red clover = −0.9x + 798.1 ($p = 0.02$), Weed = −6.1x + 6366.5 ($p = 0.03$). In panel (**c**), the maximum stacked value of biomass is not shown for consistency of graph axis. Note that lines are stacked, so the biomass of each species is represented by the distance between the lines (Red clover line = IWG + Red clover biomass; Weed line = IWG + Red clover + Weed biomass).

Intermediate wheatgrass planted in the spring yielded 0 and 25 kg ha^{-1} in the first year at Arlington and Lancaster, respectively, and in the second year, yields were 526 and 2140 kg ha^{-1} at Arlington and Lancaster, respectively.

3.3. Fall and Total Intermediate Wheatgrass Biomass

At both locations, there was no effect of planting date on intermediate wheatgrass fall biomass in the first year (Supplemental Table S1). At Arlington, the mean yields were 256 kg ha^{-1} when red clover was planted in the fall, and 286 kg ha^{-1} when it was planted in the spring. At Lancaster, the mean yields were 744 kg ha^{-1} and 437 kg ha^{-1} when red clover was planted in the fall and spring, respectively. At Arlington, there was no effect of planting date on total intermediate wheatgrass biomass in the first year when red clover was planted in the fall, with a mean yield of 1999 kg ha^{-1}. When red clover was planted in the spring a linear decline was observed in the first year, from a maximum yield of 7819 kg ha^{-1} when intermediate wheatgrass accumulated 972 GDD. At Lancaster, there was no effect of planting date on total intermediate wheatgrass biomass in the first year when red clover was planted in the fall and spring, with mean yields of 2668 kg ha^{-1} and 2646 kg ha^{-1}, respectively.

3.4. Red Clover Summer Biomass

At Arlington, there was no effect of planting date on red clover biomass in the first year when red clover was planted in the fall and spring, with mean yields of 655 kg ha^{-1} and 488 kg ha^{-1}, respectively (Figure 4a,b, Supplemental Tables S1 and S2). At Lancaster, there was also no effect of planting date on red clover biomass in the first year when red clover was planted in the fall, with a mean yield of 8 kg ha^{-1} (Figure 4c). When red clover was planted in the spring a linear increase was observed in the first year as planting was delayed, to a maximum red clover yield of 705 kg ha^{-1} when intermediate wheatgrass accumulated 103 GDD (Figure 4d). There was no effect of planting date on red clover biomass in the second year on any of the treatments (Figure 4e–h, Supplemental Tables S1 and S2). The mean red clover yields when red clover was planted in the fall and spring at Arlington were 1513 kg ha^{-1} (131% increase from the previous year), and 3450 kg ha^{-1} (607% increase from the previous year), respectively. The mean red clover yields when red clover was planted in the fall and spring at Lancaster were 359 kg ha^{-1} (1950% increase from the previous year) and 1701 kg ha^{-1} (141% increase from the previous year), respectively.

When IWG was planted in the spring, red clover yielded in the first year 710 and 343 kg ha^{-1} at Arlington and Lancaster, respectively. In the second year, red clover yielded 2861 and 1756 kg ha^{-1} at Arlington and Lancaster, respectively.

3.5. Weed Summer Biomass

At Arlington, there was no effect of planting date on weed biomass in the first year when red clover was planted in the fall and spring, with means of 539 kg ha^{-1} and 908 kg ha^{-1}, respectively (Figure 4a,b; Supplemental Tables S1 and S2). At Lancaster, when red clover was planted in the fall and spring, a linear increase was observed in the first year as planting was delayed, with a minimum weed biomass of 574 kg ha^{-1} and 1091 kg ha^{-1}, respectively, when IWG accumulated 865 GDD (Figure 4c,d). There was no effect of planting date on weed biomass in the second year on any of the treatments (Figure 4e–h). The mean weed biomass amount when red clover was planted in the fall and spring at Arlington were 256 kg ha^{-1}, a 53% decrease from the previous year, and 242 kg ha^{-1}, a 73% decrease from the previous year, respectively. The mean weed biomass when red clover was planted in the fall and spring at Lancaster were 506 kg ha^{-1} (a 12% decrease from the minimum value of weed biomass in the first year) and 319 kg ha^{-1} (a 71% decrease from the minimum value of weed biomass in the first year), respectively.

When IWG was planted in the spring, weed biomass of the in the first year was 1481 and 2783 kg ha^{-1} at Arlington and Lancaster, respectively. In the second year, weed biomass was 355 and 466 kg ha^{-1} at Arlington and Lancaster, respectively.

3.6. Optimal Planting Dates

Based on 30-year weather records, end of GDD accumulation falls on 28 November on average and by 12 November with a 90% probability at Arlington and on 29 November on average, and by 13 November with a 90% probability at Lancaster (Table 3). At Arlington, the optimal planting date for Kernza grain, when red clover is planted in the fall is 8 September on average and on 1 September for a 90% probability of accumulating the required GDD. When red clover is planted in the spring, the optimal dates are 26 August on average and on 19 August for a 90% probability of accumulating the required GDD. At Lancaster, the optimal planting date of Kernza grain yield when red clover is planted in the fall is 15 September on average and on 8 September for a 90% probability of accumulating the required GDD. When red clover is planted in the spring, the optimal dates are 13 September on average and on 6 September for a 90% probability of accumulating the required GDD (Table 3).

Table 3. Growing degree days (GDD), 30-year mean optimal planting date, and 90% probability date (the planting date that will optimize yields 90% of the time) of Kernza grain yield, and the end of GDD accumulation for Arlington and Lancaster, WI, USA.

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

4. Discussion

4.1. Intermediate Wheatgrass Planting Date

In our study, the grain yield in the first year was consistent with results from a study on dual-use winter wheat in central Texas where the effect of planting date on grain yield had a third-order polynomial trend, with yields increasing up to a maximum and later decreasing [27]. Early planting dates in winter wheat can lower yields due to a higher susceptibility to insects and diseases [28,29]. It was reported that planting too early may lead to excessive fall growth that could potentially smother the crop [21]. Late planting dates in our study had a shorter growing season available to them, and they did not accumulate enough growing degree days, thus the crop did not establish well in the fall, leading to low yields in the first harvest or no yield at all. However, for IWG established as the sole crop in the fall (with red clover frost seeded in the next spring), there was a linear decline with planting date at Arlington for grain yield, suggesting that when there is no competition from another crop, earlier dates may be preferred. At Arlington, the optimal planting date was by 19 August for a 90% chance of accumulating the required GDD. We hypothesize this was not the case in Lancaster due to the vast presence of weeds that may have increased competition for resources. At Lancaster, the optimal planting date was by 6 September for a 90% chance of accumulating the required GDD. However, we may not have had early enough planting dates to entirely capture the first section of the curve. Further research is needed to confirm this with earlier planting dates.

It has been reported previously that Kernza grain yields tend to decline after the first year; grain yield components such as tiller number, spike number, spike weight, and others could be limiting the yield [9,30]. If the number of spikes is reduced, grain yield also decreases because grain yield is dependent on the number of spikes [30]. This was evident at Arlington where the second year grain yields decreased from their maximum by

65% when red clover was planted in the fall and by 82% when it was planted in spring. Interestingly, grain yields did not decline at Lancaster in the second year. One possible explanation could be the higher N fertilizer rate that the Lancaster plots received relative to what the Arlington plots received. This may suggest N fertilization could be related to yield stability, however previous research in Kernza monocultures has shown that this may not be the case [9]. Further research is needed on the effects of N fertilization of intermediate wheatgrass and its interaction with legume intercrops.

The IWG biomass yield in the first year of our experiment was higher when the planting was done in August through early September. Interestingly, a previous study at Arlington and Lancaster showed that cool-season grasses such as smooth bromegrass (*Bromus inermis* Leyss.), timothy (*Phleum pretense* L.), reed canarygrass (*Phalaris arundinacea* L.), and others produced similar results [31]. At both our locations, when red clover was planted in the spring, IWG biomass declined linearly with later planting dates, suggesting that if cool-season grasses grow with limited competition, either from an intercrop or weeds after planting, the later you plant the lower the summer biomass yield is the following year. A study in winter wheat has shown that the planting date effect on the first harvest of forage yield had a linear trend, the later you plant the lower the yield [27]. We observed similar results in our study, and at times there was no yield at all, possibly due to late planting. In our study, the latest planting date, 30 October, at the 2018 Arlington planting was not able to establish possibly due to the low amount of growing degree days accumulated. It is interesting to note that the earliest planting date of 30 August, one of the planting dates that produced some of our maximum yields, had precipitation events at most 2 days after planting with more than 7 mm of rainfall [24]. Most of the other planting dates either had their first precipitation event 3 or more days after planting or had it closer to the planting date but was around 2 mm of rainfall [24]. This variability in precipitation could have affected our results, and future studies should consider this. We think that having water available right after planting in sufficient amount led to a better establishment in the fall, which in turn helps maintain yields the following year. Furthermore, intermediate wheatgrass planting date affected establishment in the fall and growth in the subsequent (first) production year, but it did not affect the growth in the second production year. This suggests that intermediate wheatgrass can compensate from poor initial establishment in subsequent production years.

Regardless of planting date, the majority of weed biomass decreased from the first year to the second year. This is reinforced by previous research which has shown that weed biomass decreases significantly in IWG systems from 745 kg ha^{-1} in the first year stand to 87 kg ha^{-1} in the third-year stand [18]. It was also observed that when Kernza is planted, annual weed density decreases while perennial weed density increases [18]. This is potentially observed with our first and second year weed biomass accumulation. The large presence of the annual weeds in the first year at Lancaster may also have affected the grain yield and with the weed biomass reduced in the second year, it reduced the competition which may be one of the potential reasons for the higher grain yields in the second year at Lancaster. Our results support that IWG is effective at suppressing and controlling weeds in its cropping system, which is beneficial for farmers due to reducing the cost of herbicides [16,18].

Intermediate wheatgrass with red clover intercropping is an alternative to current agricultural systems that can provide grain, forage, and environmental benefits. Our study shows that much of the variability in the grain and forage harvest is due to the planting date. Between both locations, the optimal IWG planting date when red clover was planted in the spring with the highest yields for grain (515 kg ha^{-1}), IWG summer biomass (6811 kg ha^{-1}), and red clover biomass (705 kg ha^{-1}). Planting IWG late, in mid-October when red clover is planted in the next spring had a decrease in grain yield of 79% (109 kg ha^{-1}) and summer biomass of 82% (1222 kg ha^{-1}).

4.2. Red Clover Planting Season

As previously mentioned, IWG summer biomass yield followed a linear trend in the first year when red clover was planted in the spring. We speculate this happened due to the lack of competition between intermediate wheatgrass and red clover during the fall. Minimal competition allowed for better IWG establishment and development in the fall, resulting in a good stand the next year for those that were planted earlier. When red clover was planted in the fall, it led to more competition because red clover is well adapted to Wisconsin [32]. As its establishment is aggressive, it leads to strong competition with the IWG, explaining why the IWG planting date had little effect on its summer biomass yield with fall planted red clover treatments. Competition can reduce yields by affecting one or more yield components. For example, intercropping winter cereals with Kura clover (*Trifolium ambiguum* M. Bieb.) has shown reductions particularly in the number of tillers [33].

Red clover is well adapted to Wisconsin and has shade tolerance [34], allowing continued growth even with low light intensity that may be caused by IWG competition. At Lancaster, red clover biomass in the first year, for treatments with red clover planted in the spring, increased linearly with later IWG planting dates. This could be because planting IWG late in the fall caused poor IWG establishment and gave the red clover more opportunities to establish. With less competition, the red clover developed better. The fact that the highest red clover biomass came when the growing degree days accumulation was the lowest (103 GDD) supports this point.

When averaging across planting dates, the red clover planting season did not affect most of the variables, except grain yield at Arlington and the red clover summer biomass at both locations. At Arlington, when red clover was planted in the spring, it accumulated more red clover summer biomass (3450 kg ha^{-1}) than when red clover was planted in the fall (1513 kg ha^{-1}). At Lancaster, when red clover was planted in the spring (maximized at 705 kg ha^{-1}) it accumulated more red clover summer biomass than when red clover was planted in the fall (8 kg ha^{-1}). This could be because red clover planted in the spring competed less with IWG during establishment.

4.3. Planting Intermediate Wheatgrass in the Spring

When IWG was planted in the spring, red clover was only planted in the spring. In the first year, the IWG planted in spring essentially did not produce any grain due to not having a proper vernalization period to induce flowering [14]. In the second year, the grain yields of the IWG planted in spring at Arlington and Lancaster were 64 kg ha^{-1} and 205 kg ha^{-1}, respectively. Lancaster yielded more grain and forage than Arlington but, in both locations, the optimal fall plantings date yields were higher than the spring. Therefore, to maximize Kernza grain yield it would be beneficial to plant in the following fall and not in the spring if the original planting is delayed.

If farmers must delay fall Kernza planting, we recommend waiting till the following fall to maximize forage or grain yield, instead of planting in the spring. Looking forward, more research is still needed in this area. Understanding the effects of IWG planting date intercropped with other legumes such as alfalfa (*Medicago sativa* L.) or an annual clover such as crimson clover (*Trifolium incarnatum* L.) would be relevant. The intermediate wheatgrass-legume competition we observed could be different when another legume is utilized. Looking at the planting date, testing even earlier planting dates across more locations would also be important for farmers.

5. Conclusions

Delaying the fall planting date of Kernza reduced grain yields in the first harvest year but not the second harvest year. The optimal planting date for grain yield in the first harvest year would be by 19 August at Arlington and by 6 September at Lancaster, with 90% of probability of accumulating required GDD. We recommend planting before early September to optimize summer IWG biomass and increase weed suppression in the first year. To avoid low yields at the earlier planting dates, minimizing the potential competition with other species is recommended, either through delaying intercropping or controlling weeds. In some cases, red clover planted in the spring allowed higher IWG and red clover biomass yields, so planting red clover in the spring is recommended. Further research is needed to optimize perennial grain intercropping systems.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112227/s1, Table S1: *p*-values from the analysis of variance by location and age of stand for the effect of block, seeding year (2017 and 2018), red clover planting season (fall and spring), accumulated fall growing degree days from planting to end of GDD accumulation (nested within seeding year), and their respective two-way interactions for Kernza grain yield, intermediate wheatgrass summer biomass, fall biomass, total intermediate wheatgrass biomass (summer + fall), red clover summer biomass, and weed summer biomass. *p*-values with * significant at <0.05 and ** significant at <0.01, Table S2: Means by growing degree days (GDD) at Arlington and Lancaster when red clover is planted in the fall and spring of the first and second year intermediate wheatgrass managed for dual-use (grain and forage (IWG) harvest in the summer), red clover summer biomass (RC), and weed summer biomass (Weeds). Means with the same letter within each parameter are not different at alpha = 0.05.

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