

ARTICLE

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Effects of defoliation and row spacing on intermediate wheatgrass I: Grain production

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Abstract

Increasing intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] grain yield and maintaining yield over the life of a stand will be critical to the economic viability of Kernza (The Land Institute) grain production. Research on perennial grasses has shown that seed yield can be enhanced by (a) mechanically defoliating the stand for hay production and (b) increasing row spacing. We evaluated the interacting effects of row spacing and defoliation across the 4-yr life of an intermediate wheatgrass (IWG) stand in St. Paul, MN. We measured grain yield, harvest index, lodging, and yield components including grain mass and number of tillers, spikes, and grains. Data was analyzed with linear mixed models and partial least squares path analysis. Overall, grain yield declined substantially over time, from a mean of 880 kg ha⁻¹ in 2015 to 276 kg ha⁻¹ in 2018. Wider row spacings tended to increase grain yield. Defoliation increased grain yield in the first 2 yr, but may have decreased stand vigor in later years. Neither management practice fundamentally mitigated yield decline. The main cause of yield decline was the reduction in grain number per high-yielding spike, which dropped by roughly half after the first year. The proportion of spikes that were high yielding also declined over time. Increasing competition among reproductive units likely contributed to yield decline, but there is also evidence that resource allocation to reproduction declined over time. Future research in IWG breeding and management should focus on maintaining high grain number, reducing intra-stand competition, and increasing resource allocation to reproduction.

1 | INTRODUCTION

Perennial grains have the potential to contribute to the dual challenge of promoting human nutritional security while protecting environmental quality (Hunter, Smith, Schipanski, Atwood, & Mortensen, 2017) by simultaneously producing edible grain, building soil, and filtering water (Glover et al.,

2010; Jungers, DeHaan, Mulla, Sheaffer, & Wyse, 2019). Seeds from improved lines of the perennial cool-season grass intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] (IWG) have been marketed under the trade name Kernza (The Land Institute), making IWG the first commercially viable perennial grain crop in North America. However, the economic viability of this crop is limited by low initial grain yields that further decline over the life of the stand (Jungers, DeHaan, Betts, Sheaffer, & Wyse, 2017). Breeding and genomics efforts—aided by a recently released reference genome (*Thinopyrum intermedium* v2.1, U.S. Department of Energy Joint Genome Institute, 2019)—

Abbreviations: Ctrl, no defoliation; Fa, defoliation only in fall; HI, harvest index; IWG, intermediate wheatgrass; R/FR, red/far red; Sp, defoliation only in spring; SpFa, defoliation in spring and fall; TKW, thousand kernel weight; YC, yield component.

are underway to improve many agronomic traits of IWG, including yield and yield persistence (Bajgain, Zhang, & Anderson, 2019; DeHaan, Christians, Crain, & Poland, 2018; Zhang et al., 2016).

Improved agronomic management practices will also be needed to increase first-year grain yield and maintain yield over time. Research on perennial forage, turf, and native grasses indicates that both defoliation and increasing row spacing influence grain yield by modifying tiller density. Increasing the number of fertile tillers per unit area can improve perennial grass seed yield (Deleuran, Gislum, & Boelt, 2009 and 2010; Han et al., 2013), but at a certain tiller density this relationship saturates (Aamlid, Heide, Christie, & McGraw, 1997; Hampton & Fairey, 1997; Hare, 1993). Competition among tillers results in a cascade of compensatory responses among yield components (YCs), affecting first tiller fertility, then spikelets per fertile tiller, florets per spikelet, seeds per floret, and seed size (Hampton & Fairey, 1997).

Due to these compensatory responses, in many cases management interventions that strongly influence one yield component do not affect seed yield (Deleuran et al., 2009 and 2010; Hampton & Fairey, 1997; Han et al., 2013; Hare, 1993; Koeritz, Watkins, & Ehlke, 2015; Young, Youngberg, & Chilcote, 1996). Seed number per fertile tiller is typically the YC with the strongest effect on total yield (Hampton & Fairey, 1997; Hebblethwaite & Clemence, 1981; Hebblethwaite & Ivins, 1977). In contrast, seed size is relatively invariant and therefore is not commonly a main determinant of seed yield (Canode, 1964; Hampton & Fairey, 1997; Hebblethwaite & Ivins, 1977; Young et al., 1996; but see Hare, 1993 and Koeritz et al., 2015).

Given these feedbacks, management interventions are unlikely to improve yield by maximizing individual yield components. Instead, we hypothesize that management should aim to optimize the reproductive sink capacity by ensuring sufficient, but not excessive, tiller density for the resources available.

1.1 | Effects of defoliation

Defoliation can take many forms, including mechanical clipping, grazing, and burning, and the residual biomass can be either exported from the field or left in place. Here, our primary focus is on removal of stem and leaf material from the top of the canopy down to near the soil surface, with export from the field.

Defoliation stimulates tiller production in grasses by increasing both the intensity and the red/far red (R/FR) ratio of the light incident on the plant base (Aamlid et al., 1997; Deregibus, Sanchez, & Casal, 1983; Ugarte, Trupkin, Ghiglione, Slafer, & Casal, 2010; Youngner, 1972). It

Core Ideas

- Intermediate wheatgrass grain yield declined substantially over 4 yr, from 880 to 276 kg ha⁻¹.
- Both defoliation and wider row spacing tended to increase mean grain yield.
- Neither defoliation nor wider row spacing mitigated yield decline with stand age.
- There was excessive competition among culmed tillers in most years.
- Yield declined due to lower grain number and fewer highly productive spikes.

may also reduce lodging by reducing light competition. Grain yield of IWG is often limited by lodging (Frahm et al., 2018).

In species that require vernalization to induce reproductive growth, including IWG, fall defoliation should increase the proportion of reproductive tillers the following year. In contrast, spring defoliation stimulates vegetative tillers that directly compete with reproductive tillers (Aamlid et al., 1997; Clemence & Hebblethwaite, 1984). Excessive and repeated defoliation can negatively affect reproductive physiology by reducing overall photosynthetic capacity and depleting carbohydrate reserves (Alberda, 1957; Hampton & Fairey, 1997; Youngner, 1972). In most studies of perennial grass seed production, defoliating vigorous stands prior to reproductive development does not depress subsequent seed yield, and can be beneficial (Green & Evans, 1957; Hare, 1993; Hebblethwaite & Clemence, 1981). However, it should be noted that planting in wider row spacings may increase weed pressure due to lower competition from the crop.

Evidence of how defoliation affects IWG grain yield is very limited. Across eight sites and two years, Pugliese (2017) found inconsistent results: spring defoliation reduced seed yield in two of the sites in the first production year, but increased yield at one site in the second year. Newell and Hayes (2017) showed that harvesting forage between sowing and grain harvest did not affect first-year IWG grain yield. Dick, Cattani, and Entz (2018) found that grazing post-harvest regrowth shortly after chopping stubble increased grain yield relative to chopping or residue removal alone. However, spring grazing depressed grain yields of an IWG subspecies, pubescent wheatgrass [*Thinopyrum intermedium* subsp. *barbulatum* (Shur) Barkw. & D.R. Dewey], when grazing continued for 10 d following the onset of hollow stems (Hopkins et al., 2003). More information is needed on the effects of defoliation on IWG grain yield and yield components.

1.2 | Effects of row spacing

Planting in wider row spacings can also increase in-row tillering and reduce lodging by increasing both light intensity and R/FR ratio (Canode, 1964; Han et al., 2013). In addition, widening the row spacings gives each row greater access to edaphic resources (water, nutrients). However, due to the space left between the rows, tillers ha^{-1} is often lower in wide row spacings, compared to narrow row spacings (Deleuran, Gislum, & Boelt, 2010; Han et al., 2013; Koeritz et al., 2015). This can depress seed yield, especially in less productive environments where tillering is less aggressive (Han et al., 2013; Wang et al., 2017). However, in many cases the subsequent yield components fully compensate for lower tillers ha^{-1} , resulting in equivalent yield (Deleuran et al., 2009, 2010, 2013; Koeritz et al., 2015). Moreover, differences in tiller density among row spacings typically fade over the life of a stand (Deleuran, Kristensen, Gislum, & Boelt, 2013; Donald et al., 1954; Kays & Harper, 2009) due to proliferation of rhizomes and seedling recruitment from shattered seed, unless row spacings are maintained with cultivation or herbicide applications.

There is little published information on the effects of row spacing on IWG seed yield, and none with regard to recently improved germplasm. Crowle (1966) reported ~20% higher seed yield over 5 yr when IWG was planted in wider rows (91 vs. 30 cm). Black and Reitz (1969) found that seed yields were higher in wider row spacings in dry years and at narrow row spacings in wet years, with little overall effect across 5 yr. Canode (1964) found that thinning stands of IWG by removing every other 30 cm length of row reduced lodging and increased yields in the third, fourth, and fifth harvest years. These early reports indicate that IWG may benefit from planting at wide row spacings, especially in long-term stands. However, the germplasm used in these experiments was a forage type that had not been selected for higher seed yield, reduced lodging, and other domestication traits.

1.3 | Objectives

Understanding the effects of these management interventions on IWG reproductive physiology may help develop high-yielding Kernza production systems. Our objectives were to: (a) evaluate the interacting effects of defoliation and row spacing on grain yield, yield components, and lodging; (b) determine the mechanisms behind grain yield decline as the stand aged; and (c) elucidate how feedbacks among yield components ultimately affected yield.

We conducted this study across the 4-yr life of an IWG stand. Most studies of grain production from improved lines of IWG report only 2–3 yr of yield data (Culman, Snapp,

Ollenburger, Basso, & DeHaan, 2013; Jungers et al., 2017; Pugliese, 2017), so our results provide insight into a longer period of modern IWG stand development. This study also provides the first detailed report of management effects on intermediate wheatgrass grain yield components. The effects of defoliation and row spacing on forage production, forage quality, and economic returns are reported in a companion paper (Hunter et al., 2020).

2 | MATERIALS AND METHODS

The experiment was conducted under non-irrigated conditions at the University of Minnesota Agricultural Experiment Station in Saint Paul, MN (44.988291, -93.175625) on a Waukegan silt loam (fine-silty over sandy, mixed, superactive, mesic Typic Hapludoll). The previous alfalfa (*Medicago sativa* L.) crop was terminated prior to preparing a seedbed for IWG planting. An improved grain-type IWG from the fourth cycle of a breeding program at The Land Institute (Salina, KS) was seeded at a rate of 12 kg ha^{-1} pure live seed (1.9 million seeds ha^{-1}). The IWG was seeded in 15-cm rows with 20 rows per plot on 5 Sept. 2014. Total plot size was 3 by 4.5 m. The experiment encompassed four production years, from 2015 to 2018.

The experimental design was a split-plot, randomized complete block with defoliation as the main-plot treatment and row spacing as the split-plot treatment with four replications. Mechanical defoliation for hay production occurred in spring (Sp), fall (Fa), spring and fall (SpFa), or not at all (Ctrl). All vegetation was clipped to a height of 7.5 cm and removed immediately after clipping. Spring defoliation occurred prior to stem elongation on 8, 2, 5, and 21 May in 2015, 2016, 2017, and 2018, respectively. Fall defoliation occurred prior to senescence on 20, 5, and 26 October in 2015, 2016, and 2017, respectively. Fall defoliation did not occur in 2018 because the plots were terminated to enable transition to another experiment. Forage yields from these defoliation events are reported in the companion paper (Hunter et al., 2019).

Row spacing treatments of 15, 30, and 61 cm between rows were imposed within split plots by terminating rows of IWG about 14 d after emergence by hand hoeing. As a result, the initial seeding rate m^{-1} of row was the same across row spacings, but the seeding rate ha^{-1} was lower at wider row spacings (6 kg ha^{-1} in 30 cm rows and 3 kg ha^{-1} in 61-cm rows). After establishing row spacings, IWG recruitment between rows was not controlled, as this requires specialized equipment for interrow cultivation or herbicide banding that is not widely available on commercial farms.

The herbicides Dual Magnum (S-metolachlor 82.4%, Syngenta, Basel, Switzerland) and 2,4-D (2,4-dichlorophenoxyacetic acid) were applied to IWG at the

vegetative stage in early April annually at labeled rates for grass seed production. Nitrogen fertilizer was applied in early April as ammonium nitrate ($\text{NH}_4^+\text{-NO}_3^-$; 34–0–0) at a rate of 40 kg N ha⁻¹ 2015, and then as urea (46–0–0) at a rate of 56 kg N ha⁻¹ in 2016, 2017, and 2018. Baseline soil pH, P, and K levels were 6.8, 105 mg kg⁻¹, and 514 mg kg⁻¹, respectively, and no lime or P or K fertilizer was applied.

Daily temperature and precipitation data based on satellite observations were obtained from the NASA POWER Project (<https://power.larc.nasa.gov/>; Table 1). Growing degree days (GDD) were calculated with a base of 0°C and a maximum of 30°C, with accumulation beginning on the fifth consecutive day with non-zero GDD in spring.

2.1 | Yield data collection

Grain yield, straw yield, and lodging scores were collected annually at physiological maturity on about 4 August. In the 15-cm row spacing, yield was determined within a 46 by 91 cm quadrat placed to include 91 cm of three IWG rows. In the 30- and 61-cm row spacings, yield was determined in three separate 91 cm lengths of row. All spikes were clipped above the flag leaf and the remaining straw biomass was cut at 7.5 cm above the soil surface. Spikes and straw biomass were dried at 35 and 60°C, respectively, for 72 h or until constant mass, and weighed. Seeds were removed from spikes using a laboratory thresher (Wintersteiger LD 350) and then cleaned of chaff and other debris using hand sieves and a fractionating aspirator (Carter Day International, Inc.). Harvest index was calculated by dividing the mass of grain by the total mass of grain and straw. Lodging was scored prior to harvest on a scale from 0 to 10, with 0 representing stands with no lodging and 10 representing stands with all stems lying horizontal on the soil surface (Frahm et al., 2018).

By 2017, the space between planted IWG rows had become occupied by volunteer IWG plants arising from seed shatter and tillering and the rows were no long identifiable. Therefore, the length-of-row method used to estimate yield in the wide (30 and 61 cm) row spacings was underestimating yield. To address this, in 2018 “calibration” samples of grain yield were collected with a 61 by 69 cm quadrat in a subset of 30- and 61-cm row spacing split plots. Grain yield was estimated using the methods described above. Yields from the calibration quadrat samples were 1.67 and 2.93 times greater than yields from the length-of-row samples in the 30- and 61-cm split plots, respectively. Therefore, grain yields measured using the length-of-row method for the 30- and 61-cm split plots were multiplied by 1.67 and 2.93, respectively in 2017 and 2018. All results below are based on adjusted values.

2.2 | Yield components data collection

Yield components data were collected from a separate sample in each split plot at physiological maturity. Since the sampling locations and methods were not identical to those for the samples described above, the results are not always in alignment.

Yield component data included number of culmed tillers m⁻¹, number of spikes m⁻¹, ratio of spikes/tillers, number of grains spike⁻¹, grain mass spike⁻¹, and thousand kernel weight (TKW). Yield component samples were collected by cutting all culmed tillers 7.5 cm above-ground level from two separate 15-cm sections of row (30 cm total length) and counting culmed tillers and spikes.

The 10 best spikes, based on length and spikelet density, were subset and dried at 60°C for 72 h. When samples did not contain 10 spikes, all spikes were retained and the number of spikes was recorded. Seed was removed from spikes, cleaned using the methods described above, and weighed. Grain mass spike⁻¹ was determined by dividing the threshed grain weight by the number of spikes in the subsample (usually 10). To determine TKW, five subsets of 20 seeds were sampled from each 10-spike sample (with replacement) and weighed. Each subset mass was divided by 20, and the five subset averages were averaged to determine individual grain mass, then multiplied by 1000.

The number of culmed tillers and spikes ha⁻¹ was calculated by extrapolating from the 30-cm row samples. In the 15-cm row spacing split plot, this was done in the same way in each year, by assuming that the combined sample represented a 15 by 30 cm area. In the 30- and 61-cm row spacings, the extrapolation was done differently for 2015 and 2016, when the rows were still distinct, than for 2017 and 2018, when the interrow space was highly colonized. In 2015 and 2016, the sampling area was assumed to be the width of the row spacing (30 or 61 cm) by the length of the sample (30 cm). In 2017 and 2018, when the wider row spacings had filled in, we sampled a 15 by 30 cm area.

2.3 | Statistics

All statistical analyses were performed in R (R Core Team 2018). Split-plot mixed-effect linear models fit to a Gaussian distribution were specified with the lme function of the nlme package v. 3.1-137 (Pinheiro, Bates, DebRoy, & Sarkar D, 2018). Random intercepts were fitted to block, main plot, and year, when year was not included as a fixed effect. Initially, the interacting effects of year, row spacing, and defoliation were fit across all years. Then, to enable

TABLE 1 Monthly mean precipitation and temperature in St. Paul, MN, for August 2014–August 2018, with long-term means (normals) for 1981–2018

Weather variable	Year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Precipitation, mm	2014								120	45	38	29	23
	2015	11	10	20	62	113	120	157	116	100	59	97	69
	2016	12	21	79	58	78	116	154	190	185	79	42	51
	2017	33	30	29	119	145	116	94	139	52	139	12	19
	2018	27	34	30	47	102	147	90	140				
	Normal	22	22	48	77	105	129	104	110	81	63	40	31
Mean temperature, °C	2014								21.6	15.7	8.3	−4.4	−5.3
	2015	−9.0	−12.1	0.9	8.4	14.2	20.1	22.0	20.4	18.9	10.0	3.8	−3.2
	2016	−10.6	−5.8	3.6	7.6	14.8	20.3	22.5	21.7	17.0	9.8	4.6	−8.1
	2017	−9.3	−2.7	−0.2	8.2	13.0	19.6	22.0	18.7	17.8	8.9	−0.7	−8.2
	2018	−10.5	−10.2	−0.6	2.1	18.1	21.1	22.2	21.3				
	Normal	−10.6	−7.9	−0.3	7.0	14.1	19.7	22.3	21.1	16.4	8.5	0.0	−7.6

interpretation of overall effects across all four years, the effects of these predictors were modeled one-by-one, without covariates. Finally, the interacting effects of row spacing and defoliation were modeled within each year. Pairwise comparisons among treatment means were evaluated with the emmeans function of the emmeans package v. 1.3.0 (Lenth, 2018) with a Tukey adjustment for multiple comparisons. Models were evaluated to ensure they met the assumptions of independence and normality of residuals and, if necessary, response variables were transformed following the Box–Cox procedure. An alpha value of .05 was used to assess statistical significance.

Partial least squares path modeling, also known as path analysis, was performed with the plspm function of the plspm package v. 0.4.9 (Sanchez, Trinchera, & Russolillo, 2017). This approach enables simultaneous evaluation of the direct and total effects of key yield components on grain yield in a way that accounts for their physiological relationships. Prior to performing the path analysis, all variables were standardized across years to have a mean of zero and standard deviation of one (Youngerman, Ditommaso, Curran, Mirsky, & Ryan, 2018) using the scale function in R. This standardization approach allows for direct comparisons between path coefficients (Grace & Bollen, 2005). Models were fit for both the full dataset and subsets of the data by year, row spacing, and defoliation. All models were fit with bootstrap validation with 200 bootstrap resamples. Path coefficients were compared between submodels using the function plspm.groups of the plspm package. Path analysis has previously been used to evaluate the effects of management on yield formation in perennial grasses (e.g., Seker & Serin, 2004; Wang et al., 2017) and to evaluate the physiological effects of selection for grain yield in intermediate wheatgrass (Cattani & Asselin, 2018).

3 | RESULTS

3.1 | Weather and field conditions

Growing conditions were generally favorable throughout the experiment, with monthly mean precipitation and temperature typically near long-term values during the growing season (Table 1). Wet conditions in July of 2016, including a storm shortly before harvest, contributed to high lodging in that year. Growing degree day accumulation (base temperature = 0°C) prior to grain harvest was highest in 2016 (2240°C d), next highest in 2015 and 2017 (2180°C d), and lowest in 2018 (2090°C d).

3.2 | Grain yield

Overall, grain yield ha^{−1} declined substantially over time, from a mean of 880 kg ha^{−1} in 2015 to 276 kg ha^{−1} in 2018 (Figure 1 and Supplemental Figure S1A; Tables 2 and 3). Across all 4 yr, the SpFa defoliation led to higher grain yield than Fa defoliation and Ctrl (569, 472, and 468 kg ha^{−1}, respectively) and yield was higher in the 30- and 61-cm row spacings than in the 15-cm row spacing (566, 525, and 426 kg ha^{−1}, respectively). Differences in weed pressure either among defoliation treatments or among row spacings were not observed due to effective herbicidal control.

The effect of defoliation on grain yield changed over time (Supplemental Figure S1A; Tables 2 and 3). In 2015, there was an interaction between defoliation and row spacing in which spring defoliation increased yield in 30- and 61-cm rows but not in 15-cm rows. In 2016, SpFa defoliation yielded higher than Ctrl overall. However, the beneficial effect of defoliation was reversed in 2017, when Ctrl yielded higher

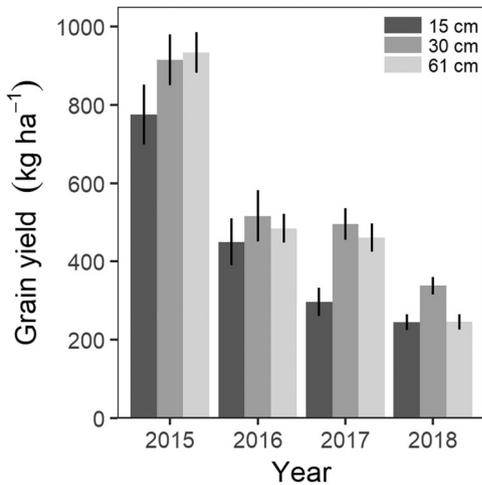


FIGURE 1 Intermediate wheatgrass grain yield (kg ha^{-1}) over time by row spacing (all defoliation treatments pooled). Interactions are displayed in Figure S1

than Sp defoliation, marginally higher than Fa defoliation, and was equivalent to SpFa. Defoliation did not affect grain yield in 2018.

Grain yield did not differ by row spacing in 2016 (Figure 1). However, in 2017, yield was higher in the 30- and 61-cm rows than in the 15-cm rows. In 2018, yield was higher in the 30-cm spacing than in the 15- and 61-cm spacings. Yield in the 30-cm row spacing was 67% higher than the 15-cm spacing in 2017 and 38% higher in 2018.

Total grain yield ha^{-1} was compared to grain yield ha^{-1} from the 10-spike YC sample to determine what proportion of overall yield the 10 best spikes contributed. The ratio of grain yield ha^{-1} from the main sample to grain yield ha^{-1} from the 10-spike YC subsample declined over time. This ratio was greater than 5 in 2015 and 2016, but averaged only 1.8 in 2017 and 2018 (Table 4).

3.3 | Harvest index

Harvest index (HI) declined every year (Figure 2 and Supplemental Figure S1B; Tables 2 and 3) despite relatively stable straw yield, which dropped by 24% after 2015 and then did not change (straw yield results are reported in the companion paper, Hunter et al., 2019). Across all 4 yr, SpFa and Sp defoliation resulted in the highest HI, Fa defoliation resulted in the lowest, and HI increased with each increase in row spacing. The effects of row spacing had begun to fade in 2016, when HI was equivalent in the 30- and 15-cm rows, though still lower than in the 61-cm rows. There were no differences among row spacings in 2017 or 2018. Defoliation resulted in contradictory effects on HI in different years. In 2015, Sp defoliation increased HI, but in 2017 Ctrl had among the highest HI. In

TABLE 2 Mean grain yield, harvest index, and lodging score by year, row spacing, and defoliation treatment. Within each category demarcated by horizontal lines, rows that share a letter are not significantly different at the $\alpha = .05$ level with a Tukey correction for multiple comparisons. Letters in bold indicate years in which there was a significant interaction between defoliation and row spacing. Interactions are displayed in Figure S1

Factor	Level	Grain yield	Harvest index	Lodging 0–10
		————— kg ha^{-1} —————		
Year	2015	876a	0.082a	6.1b
	2016	484b	0.063b	7.4a
	2017	418b	0.050c	6.3b
	2018	276c	0.037d	3.6c
Row spacing	15 cm	436b	0.049c	6.6a
	30 cm	566a	0.057b	5.6b
	61 cm	525a	0.068a	5.3b
Defoliation	Ctrl ^a	468b	0.056bc	6.4a
	Fa	472b	0.047c	5.6a
	Sp	530ab	0.061ab	5.9a
	SpFa	569a	0.068a	5.5a
2015 ^b	15 cm	775b	0.066c	7.9a
	30 cm	915ab	0.082b	5.9b
2016	61 cm	934a	0.098a	4.5c
	Ctrl + Fa	768b	0.068b	6.4a
	Sp + SpFa	983a	0.096a	5.8a
	15 cm	450a	0.047b	7.9a
	30 cm	516a	0.061b	7.2ab
	61 cm	485a	0.082a	7.1b
	Ctrl	327b	0.061a	7.5a
	Fa	502ab	0.054a	7.3a
	Sp	486ab	0.064a	7.7a
	SpFa	621a	0.075a	7.1a
2017	15 cm	297b	0.047a	6.6a
	30 cm	496a	0.048a	6.1a
	61 cm	461a	0.055a	6.2a
	Ctrl	514a	0.065a	5.4a
2018	Fa	381ab	0.039b	6.1a
	Sp	323b	0.037b	7.3a
	SpFa	453ab	0.061ab	6.5a
	15 cm	244b	0.035a	3.9a
2018	30 cm	338a	0.038a	3.2a
	61 cm	246b	0.037a	3.6a
	Ctrl	253a	0.027b	6.3a
	Fa	271a	0.029b	2.5b
2018	Sp	300a	0.047a	3.3b
	SpFa	280a	0.043a	2.2b

^aCtrl, no defoliation; Fa, defoliation in fall only; Sp, defoliation in spring only; SpFa, defoliation in spring and fall.

^bBecause the fall defoliation had not occurred yet in 2015, the Ctrl and Fa defoliation treatments were pooled and the Sp and SpFa defoliation treatments were pooled.

TABLE 3 Tests of fixed effects on key response variables with various specifications of split-plot mixed-effect linear models

Model	Fixed effect	Grain yield index	Harvest index	Lodging	Tillers m ⁻¹	Tillers ha ⁻¹	Spikes m ⁻¹	Spikes ha ⁻¹	Spikes/tillers	Grain number spike ⁻¹	TKW ^a	Grain yield spike ⁻¹
Full model	Year	***	***	***	***	***	***	***	***	***	***	***
	Row spacing (Row)	***	***	***	***	***	*	NS ^c	NS ^c	***	*	***
	Defoliation (Defol)	*	***	*	*	*	NS	NS	NS	NS	NS	NS
	Yr×Row	NS	***	***	*	***	***	NS	NS	NS	NS	*
	Yr×Defol	***	***	***	NS	NS	***	***	NS	NS	*	*
	Row×Defol	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Yr×Row×Defol	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*
Year only		***	***	***	***	***	***	***	***	***	***	***
Row spacing only		***	***	***	***	***	*	NS	NS	***	*	***
Defoliation only		*	***	NS	**	NS	NS	NS	NS	NS	NS	NS
2015 ^b	Row spacing	*	***	***	***	***	***	NS	NS	**	*	**
	Defoliation	**	***	NS	NS	NS	NS	NS	NS	NS	*	NS
	Row×Defol	*	*	NS	NS	NS	NS	NS	NS	*	NS	*
2016	Row spacing	NS	***	*	***	***	***	*	*	*	NS	**
	Defoliation	*	NS	NS	*	*	**	NS	NS	NS	NS	NS
	Row×Defol	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
2017	Row spacing	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Defoliation	*	*	NS	NS	NS	NS	NS	NS	NS	NS	*
	Row×Defol	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2018	Row spacing	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Defoliation	NS	**	***	NS	NS	NS	**	**	NS	NS	NS
	Row×Defol	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS

^aTKW, thousand kernel weight.

^bBecause the fall defoliation had not occurred yet in 2015, the Ctrl (no defoliation) and Fa defoliation treatments (defoliation in fall only) were pooled and the Sp (defoliation in spring only) and SpFa defoliation treatments (defoliation in spring and fall) were pooled.

^cNS, not significant.

*Significant at $\alpha < .05$.

**Significant at $\alpha < .01$.

***Significant at $\alpha < .001$.

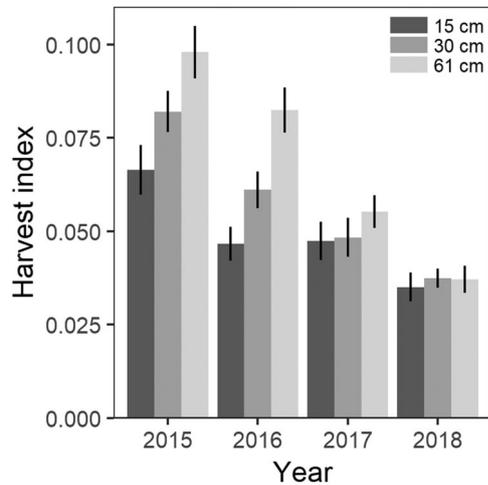


FIGURE 2 Intermediate wheatgrass harvest index over time by row spacing (all defoliation treatments pooled). Interactions are displayed in Figure S1

TABLE 4 Grain yield ha^{-1} calculated from the main sample, the 10-spike yield components subsample, and their ratio. All row spacing and defoliation treatments were pooled within each year

Year	Grain yield		Ratio of main sample to 10-spike subsample
	Main sample	10-Spike subsample	
	—kg ha^{-1} —		
2015	877	216	5.2
2016	485	104	5.6
2017	416	221	2.0
2018	276	206	1.6

2018, HI was low again in Ctrl, and was high following Sp and SpFa defoliation. Defoliation did not affect HI in 2016.

3.4 | Lodging

Lodging at grain harvest varied substantially among years and to some degree by management (Supplemental Figure S1C; Tables 2 and 3). Across all years, there was no overall effect of defoliation, but lodging was worse in 15-cm rows than in 30- or 61-cm rows (mean score of 6.6, 5.6, and 5.3, respectively). Lodging decreased with wider row spacings in 2015 and was worse in the 15 cm than in the 61-cm row spacing in 2016. Row spacing did not affect lodging in the final 2 yr. The effects of defoliation on lodging were inconsistent. In 2015, Sp defoliation reduced lodging in the 30-cm row spacing, but not in the others. Likewise, lodging was worse in Ctrl than all defoliation treatments in 2018.

Lodging was negatively associated with grain yield in each year ($r = -0.28, -0.096, -0.36,$ and -0.57 in 2015–2018, respectively), but the effects were only significant in 2015

and 2017. A one-unit increase in lodging score resulted in declines of 46.2 and 36.5 kg ha^{-1} of grain yield in 2015 and 2017, respectively ($p = .020$ and $.015$). In these years, lodging explained 9.2 and 13% of variability in grain yield, respectively. Lodging did not affect grain yield in 2016 ($p = .52$), likely due to very low variability in lodging scores. Surprisingly, lodging also did not affect grain yield in 2018 ($p = .59$), despite there being roughly twice as much lodging in Ctrl.

3.5 | Tillers

Tillers m^{-1} of row dropped sharply after the first year, by 54%, but then increased annually until the final year, when it was equivalent to the initial level (Supplemental Figure S2A; Tables 3 and 5). Across all years, tillers m^{-1} of row was greater with SpFa and Sp defoliation than in Ctrl. The effect of defoliation on tillers m^{-1} of row was highest in 2016, with more modest or inconsistent differences in later years. Planting in wider rows increased tillers m^{-1} of row in 2015 and 2016, even though the same number of seeds were planted m^{-1} of row. The effect of row spacing faded over time such that by 2017 and 2018 the wider row spacings no longer had more tillers m^{-1} of row. Tillers ha^{-1} also dropped sharply in 2016, but then recovered in each subsequent year, such that tillers ha^{-1} was almost twice as high in 2018 as it was in 2015 (Supplemental Figure S2B; Tables 3 and 5). In the 15-cm row spacing, this was because tillers m^{-1} of row was substantially greater in 2018 than in 2015. In contrast, in the wider row spacings, tillers m^{-1} of row was slightly lower than in 2015, but the interrow space was fully colonized. Across all 4 yr, there were more tillers ha^{-1} in the 15 cm than the 61-cm row spacing and Sp and SpFa defoliation resulted in more tillers ha^{-1} than Ctrl. Tillers ha^{-1} decreased with increasing row spacing in 2015 and 2016, but did not differ among row spacings in the final 2 yr. The effect of defoliation on tillers ha^{-1} was similar to that on tillers m^{-1} of row.

3.6 | Spikes

Spikes m^{-1} of row also fell dramatically following the first year, by 48%, remained stable in the third year, and then rebounded in the final year but not to the level of the first year (Figure 3 and Supplemental Figure S2C; Tables 3 and 5). Across all years, defoliation did not affect spikes m^{-1} of row, but row spacing did, with greater values in 30- and 61-cm rows than 15 cm. This effect was strongest in 2015, when spikes m^{-1} of row increased with each wider row spacing. By 2016, the number of spikes m^{-1} of row had converged in the 61- and 30-cm row spacings, though the 15-cm row spacing still had fewer spikes m^{-1} . By 2017 and 2018, all row

TABLE 5 Yield components by year, row spacing, and defoliation treatment (means). Within each category, rows that share a letter are not significantly different at the $\alpha = .05$ level with a Tukey correction for multiple comparisons. Letters in bold indicate years in which there was a significant interaction between defoliation and row spacing. Interactions are displayed in Figures S2 and S3

Factor	Level	Tillers m ⁻¹	Tillers ha ⁻¹	Spikes m ⁻¹	Spikes ha ⁻¹	Spikes/tillers	Grain num- ber spike ⁻¹	TKW ^a	Grain yield spike ⁻¹
			×10 ⁶		×10 ⁶			G	mg
Year	2015	407a	13.8b	225a	7.5b	0.56a	30.5a	6.0b	186a
	2016	188 c	6.2 c	117c	3.7c	0.59a	13.8b	6.9a	96b
	2017	232b	15.4b	116bc	7.7ab	0.53a	16.5b	6.1b	102b
	2018	409a	27.3a	144b	9.6a	0.37b	16.5b	5.9b	98b
Row spacing	15 cm	252c	16.8a	113b	7.5a	0.48a	17.3b	6.0a	103b
	30 cm	306b	15.3ab	158a	7.4a	0.54a	19.1ab	6.3a	120ab
	61 cm	368a	14.9b	180a	6.4b	0.51a	21.9a	6.3a	138a
Defoliation	Ctrl ^b	256b	12.8b	138a	7.0a	0.54a	20.0a	6.4a	129a
	Fa	302ab	15.4ab	137a	6.4a	0.48a	18.7a	6.4a	118a
	Sp	332a	16.6a	164a	7.5a	0.52a	20.2a	6.1a	124a
	SpFa	347a	18.0a	162a	7.5a	0.51a	18.6a	6.0a	111a
2015 ^c	15cm	287c	19.1a	148c	9.9a	0.53a	25.4b	5.5b	147b
	30 cm	406b	13.5b	231b	7.7b	0.57a	29.8ab	6.1a	184ab
	61 cm	530a	8.7c	297a	4.9c	0.58a	36.1a	6.2a	226a
	Ctrl + Fa	402a	13.8a	220a	7.6a	0.56a	30.6a	6.3a	194a
	Sp + SpFa	413a	13.8a	230a	7.4a	0.56a	30.3a	5.6b	178a
2016	15 cm	118b	7.8a	55b	3.7ab	0.49b	9.7b	6.7a	66b
	30 cm	205a	6.8a	144a	4.8a	0.68a	14.2ab	6.9a	99ab
	61 cm	241a	4.0b	152a	2.5b	0.60ab	17.2a	7.0a	122a
	Ctrl	131b	4.0b	60b	1.9b	0.47a	14.2a	6.8a	102a
	Fa	173ab	6.2ab	107ab	3.4ab	0.60a	12.9a	7.0a	86a
	Sp	247a	8.3a	168a	5.3a	0.64a	13.7a	6.8a	92a
	SpFa	200ab	6.4ab	132ab	4.0ab	0.65a	14.1a	6.9a	102a
2017	15 cm	214a	14.2a	101a	6.7a	0.51a	15.8a	6.0a	98a
	30 cm	238a	15.8a	124a	8.3a	0.56a	16.8a	6.2a	104a
	61 cm	244a	16.2a	121a	8.1a	0.51a	16.9a	6.0a	104a
	Ctrl	186a	12.4a	112a	7.5a	0.63a	19.5a	6.6a	128a
	Fa	219a	14.6a	95a	6.3a	0.44a	14.4a	5.9a	87b
	Sp	261a	17.4a	135a	9.0a	0.55a	15.8a	5.8a	92ab
	SpFa	261a	17.4a	120a	8.0a	0.49a	16.1a	6.0a	98ab
2018	15 cm	392a	26.1a	148a	9.9a	0.41a	17.1a	5.8a	100a
	30 cm	377a	25.1a	133a	8.9a	0.36a	15.3a	6.1a	94a
	61 cm	458a	30.6a	151a	10.0a	0.34a	17.1a	5.9a	99a
	Ctrl	320a	21.3a	161a	10.7a	0.51a	14.6a	5.8a	87a
	Fa	399a	26.6a	127a	8.5a	0.33b	16.3a	6.4a	102a
	Sp	401a	26.7a	124a	8.3a	0.31b	17.2a	5.8a	99a
	SpFa	517a	34.5a	164a	10.9a	0.33b	17.9a	5.7a	102a

^aTKW, thousand kernel weight.

^bCtrl, no defoliation; Fa, defoliation in fall only; Sp, defoliation in spring only; SpFa, defoliation in spring and fall.

^cBecause the fall defoliation had not occurred yet in 2015, the Ctrl and Fa defoliation treatments were pooled and the Sp and SpFa defoliation treatments were pooled.

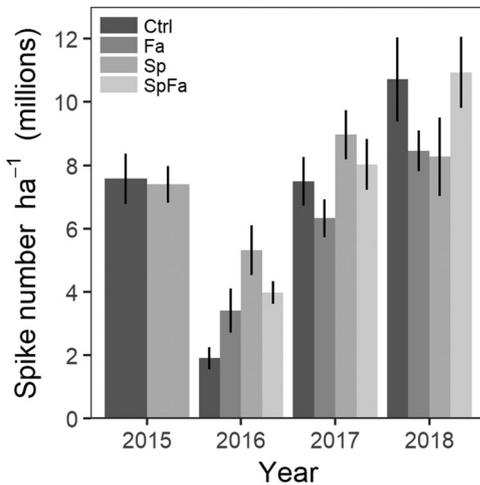


FIGURE 3 Intermediate wheatgrass spike number ha^{-1} (in millions) by defoliation treatments (all row spacings pooled). Because the fall defoliation had not occurred yet in 2015, Ctrl and Fa defoliation treatments were pooled (dark gray bar) and the Sp and SpFa defoliation treatments were pooled (light gray bar). Ctrl: no defoliation; Fa: defoliation in fall only; Sp: defoliation in spring only; SpFa: defoliation in spring and fall. Interactions are displayed in Supplemental Figure S2

spacings were equivalent. As with tillering, defoliation only affected spike number in 2016, when the Sp defoliation treatment resulted in greater spike numbers than Ctrl.

Due to the increase in stand density over time in the 30- and 61-cm rows, spikes ha^{-1} followed a different temporal pattern than spikes m^{-1} of row (Supplemental Figure S2D; Tables 3 and 5). Spikes ha^{-1} was highest in 2018, moderate in 2015, and lowest in 2016; 2017 numbers were equivalent to those in 2018 and 2015. As with spikes m^{-1} of row, defoliation did not affect spikes ha^{-1} across all years. However, the overall effect of row spacing on spikes ha^{-1} differed from spikes m^{-1} , with greater values in narrower rows (30 and 15 cm) than in the widest rows (61 cm). In 2015, spikes ha^{-1} consistently increased as rows narrowed, despite greater spikes m^{-1} in wider rows. By 2016 there was an equivalent number of spikes ha^{-1} in 15- and 30-cm rows, due to increased spikes m^{-1} of row in 30-cm rows, but there were fewer spikes ha^{-1} in 61-cm rows. Row spacing did not affect spikes ha^{-1} in 2017 or 2018.

3.7 | Ratio of spikes/tillers

The ratio spikes/tillers did not differ among the first 3 yr (mean = 0.56), but declined by 34% in 2018 (mean = 0.37) compared to the average of the previous 3 yr (Supplemental Figure S2E; Tables 3 and 5). There were no consistent effects of defoliation or row spacing on spikes/tillers across all years. However, row spacing had a limited effect on spikes/tillers in

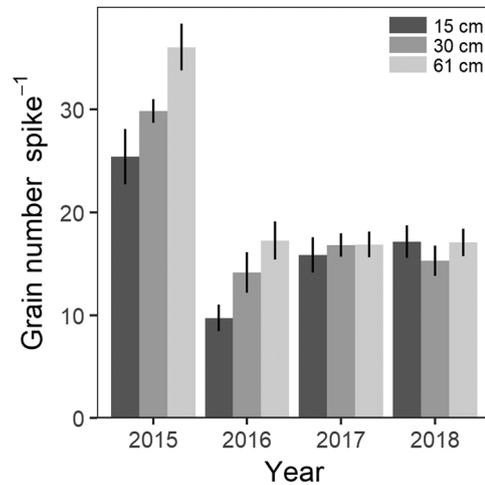


FIGURE 4 Intermediate wheatgrass grain number spike $^{-1}$ by row spacing (all defoliation treatments pooled). Interactions are displayed in Supplemental Figure S3

2016, with greater values in 30 cm compared to 15-cm row spacings.

The effect of defoliation changed as the stand aged. In 2016, defoliation seemed to increase spikes/tillers, though treatment differences were not statistically significant. However, this trend reversed in the later years, with the unclipped control producing greater spikes/tillers than all other treatments in 2018 (0.51 as compared to a mean of 0.32).

3.8 | Grain number per spike

Grain number spike $^{-1}$ differed strongly by stand age, but management only had moderate effects (Figures 4 and Supplemental Figure S3A; Tables 3 and 5). Grain number spike $^{-1}$ was highest in the first year (30.5) and decreased by half in the subsequent years (mean = 15.5). Grain number spike $^{-1}$ was numerically lower in 2016 (mean of 13.6) than in 2017 or 2018 (16.4 and 16.5, respectively), but the difference was not statistically significant. Across all 4 yr, grain number spike $^{-1}$ was greater in the 61-cm rows than in the 15-cm rows, while it did not differ between 30 cm and the other two spacings.

Defoliation did not have consistent effects on grain number spike $^{-1}$. In 2015, Sp defoliation tended to decrease grain number in the 15-cm row spacing and to increase it in the 61-cm row spacing, but both effects were only marginally significant ($p = .062$ and $.094$, respectively). In 2016, plants produced more grains spike $^{-1}$ in the 61 cm as compared to the 15-cm row spacings in the no-defoliation control treatment (22.8 vs. 6.8). There were no significant differences in grain number spike $^{-1}$ by defoliation or row spacing in 2017 or 2018.

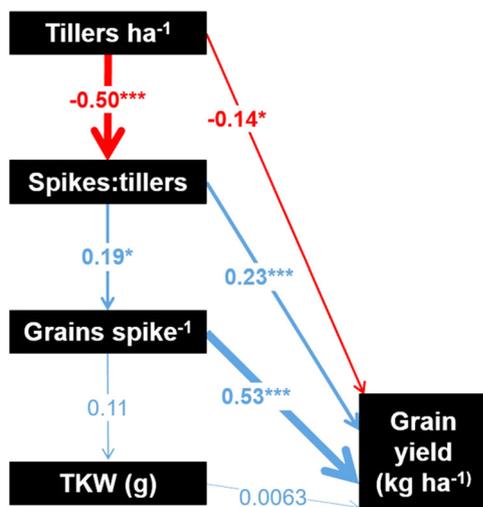


FIGURE 5 Path diagram of relationships among tillers ha⁻¹, ratio of spikes/tillers, grains spike⁻¹, thousand kernel weight (TKW) (g), and grain yield (kg ha⁻¹). Represents data from all years and treatments, standardized across years. Asterisks indicate statistical significance of each path (* $p < .05$, ** $p < .01$, *** $p < .001$). Significant path coefficients are bolded. Path analysis with the full dataset indicated that tillers ha⁻¹ was negatively correlated with both spikes/tillers and grain yield ha⁻¹ across all years and management treatments (Figure >5; Table 6). In other words, as tiller number increased, a lower proportion of culmed tillers produced spikes and grain yield decreased. Spikes/tillers and TKW had significant, positive, direct effects on grain yield ha⁻¹. So, as the proportion of reproductive tillers and TKW increased, grain yield was directly increased. Spikes/tillers also had a positive effect on grains spike⁻¹. The full model explained 43% of the variation in grain yield ha⁻¹

3.9 | Thousand kernel weight

Thousand kernel weight was relatively invariant among years, being statistically indistinguishable in 2015, 2017, and 2018 (mean = 6.0 g; Supplemental Figure S3B; Tables 3 and 5). However, TKW was 15% greater in 2016 (6.9 g) than the average of the other 3 yr (6.0 g). Across all 4 yr, there was no effect of defoliation on TKW and it was marginally greater in 30 cm than in 15-cm rows. In 2015, TKW was reduced with Sp defoliation in 15-cm row spacing. There were no differences in TKW in 2016 or 2017. However, Fa defoliation resulted in greater TKW than SpFa in 61-cm rows in 2018.

3.10 | Path analysis

To evaluate the interacting effects of yield components on grain yield, a path analysis was performed. In the path analysis diagram (Figure 5), arrows indicate causal relationships among variables. Negative relationships are shown in red and positive relationships are shown in blue. The strength of the relationships are indicated by the path coefficients, which are

in turn reflected by the width of the lines. Our model was constructed based on hypothesized relationships among IWG YC, as discussed above.

The relationships in the path analysis represent cascading limitations on grain yield (tillers ha⁻¹, then spikes/tillers, then grain number spike⁻¹, then TKW). Each yield component is hypothesized to affect grain yield both (a) directly and (b) indirectly through the yield components that come after it in the causal chain. For instance, tillers ha⁻¹ has a direct effect on grain yield (coefficient = -0.14). Tillers ha⁻¹ also affects grain yield through its effect on spikes/tillers. This indirect effect is equal to the product of the direct effect of tillers ha⁻¹ on spikes/tillers (-0.50) and the direct effect of spikes/tillers on grain yield (0.23), so this indirect effect is equal to -0.11. The total effect of tillers ha⁻¹ on grain yield is the sum of the direct effect and all indirect effects made up of the paths through each subsequent yield component.

Evaluating models for individual years of the study indicated that the direct effect of tillers ha⁻¹ on spikes/tillers was more negative in years with greater numbers of tillers ha⁻¹. The path coefficient was positive but nonsignificant in 2016 (0.17), the year with the lowest spikes ha⁻¹. The path coefficient was negative but nonsignificant in 2015 (-0.26). In both 2017 and 2018, the coefficient was both negative and significant (-0.45 and -0.36, respectively). This path was different between the model for 2016 and those for 2017 and 2018 ($p < .0001$ and $p = .0010$, respectively).

The direct effect of tillers ha⁻¹ on spikes/tillers was more negative with defoliation, across all years and row spacings. There was no relationship for the no-defoliation control (path coefficient = -0.0067). In contrast, the relationship was negative and significant for all the defoliation treatments, indicating that greater tillers ha⁻¹ led to lower spikes/tillers (Table 6). For all defoliation treatments, this path is significantly different from the same path in the Ctrl model ($p < .001$ in all cases).

The direct effect of tillers ha⁻¹ on spikes/tillers was also more negative in wider rows. In all row spacings, there was a significant, negative path coefficient between these two yield components, and the coefficient was greater in the wider row spacings (15 cm = -0.37, 30 cm = -0.58, 61 cm = -0.57). This path was marginally different between the 15- and 30-cm row spacings ($p = .061$) and significantly different between the 15- and 61-cm row spacings ($p = .049$).

4 | DISCUSSION

4.1 | Defoliation and row spacing effects on grain yield

Our results indicate that defoliation enhanced grain yield in a few different ways especially in the first 2 yr of the stand, but

TABLE 6 Direct path coefficients and total effects of paths between yield components and grain yield in the full path model (Figure 5) and submodels by year, row spacing, and defoliation. Bold values are significantly different from zero based on bootstrap validation ($\alpha < .05$)

Effect	Path	Full	Year				Row spacing			Defoliation			
			2015	2016	2017	2018	15 cm	30 cm	61 cm	Ctrl ^a	Fa	Sp	SpFa
Direct	Tillers ^b → Spikes	-0.50	-0.26	0.17	-0.45	-0.36	-0.37	-0.58	-0.57	0.01	-0.65	-0.55	-0.67
	Tillers → Yield	-0.14	0.16	0.07	0.03	-0.05	-0.07	0.00	-0.27	0.08	-0.35	-0.18	-0.29
	Spikes → Grains	0.19	0.14	0.23	0.40	0.06	0.24	0.12	0.21	0.26	0.08	0.26	0.09
	Spikes → Yield	0.23	0.20	0.51	0.32	-0.13	0.22	0.38	0.10	0.25	0.06	-0.01	0.47
	Grains → TKW	0.11	0.64	0.29	0.34	0.10	0.10	-0.10	0.24	0.16	0.13	0.11	0.03
	Grains → Yield	0.53	0.38	-0.09	-0.01	0.24	0.41	0.49	0.67	0.60	0.56	0.64	0.35
	TKW → Yield	0.01	0.01	0.19	0.31	0.42	0.02	-0.04	-0.02	0.15	0.16	0.16	-0.30
Total	Tillers → Yield	-0.30	0.10	0.15	-0.13	-0.01	-0.19	-0.26	-0.41	0.09	-0.42	-0.27	-0.62
	Spikes → Yield	0.33	0.26	0.50	0.36	-0.11	0.32	0.43	0.24	0.42	0.10	0.16	0.51
	Grains → Yield	0.53	0.39	-0.03	0.10	0.28	0.41	0.49	0.66	0.62	0.58	0.66	0.34
	TKW → Yield	0.01	0.01	0.19	0.31	0.42	0.02	-0.04	-0.02	0.15	0.16	0.16	-0.30

^aCtrl, no defoliation; Fa, defoliation in fall only; Sp, defoliation in spring only; SpFa, defoliation in spring and fall.

^bTillers = tillers ha⁻¹; Spikes = spikes/tillers; Grains = grain number spike⁻¹; TKW = thousand kernel weight (g); Yield = grain yield (kg ha⁻¹)

the mechanism was not consistent. For instance, in 2015, Sp defoliation reduced lodging in the 30-cm row spacing. In contrast, in 2016, all defoliation treatments increased tillers m⁻¹ and spikes/tillers, especially at 30- and 61-cm row spacings. This did not lead to excessive inter-tiller competition in 2016, since tiller numbers were lower, though the path analysis indicates that, across all 4 yr, defoliation did lead to increased competition among reproductive units.

Despite this inconsistency in mechanisms, it appears that defoliation may stimulate grain production in young, vigorous stands of IWG. However, the reduction in grain yield following defoliation in 2017, which likely resulted from a reduction in spikes/tillers and grains spike⁻¹, suggests that repeated defoliation may ultimately diminish stand vigor and grain yield. This may be due in part to depletion of N reserves (see Hunter et al., 2020).

The effects of row spacing on grain yield indicate that planting in wider rows may help increase grain yield and maintain it over time. Average annual grain yield was at least 100 kg ha⁻¹ higher in 30- and 61-cm rows than in 15-cm rows. Despite equivalent seeding rates m⁻¹ of row, there were more tillers m⁻¹ in the wider row spacings in the first 2 yr of the stand, indicating that tillering was more aggressive with lower between-row competition. However, due to the lower number of rows ha⁻¹, the number of tillers and spikes ha⁻¹ was initially lower than in narrower rows. Nevertheless, wider row spacings increased grain yield in 2015, likely by reducing lodging in 30-cm rows and increasing grain number spike⁻¹ in 61-cm rows.

Grain yield was also higher in wider rows in 2017 and in the 30-cm row spacing in 2018. However, the correction factor applied to the 2017 grain yield in 30- and 61-cm row spacings was calculated based on 2018 samples. If the stands were

denser in 2018 than in 2017, as would be expected, this correction factor would be too strong for 2017 data. This could account for the lack of yield decline in wider row spacings between 2016 and 2017. Moreover, harvest residue was not removed from the 15-cm rows, which may have depressed yields. More research is needed to determine the long-term effects of initial row spacing on intermediate wheatgrass grain yield.

4.2 | Reasons for yield decline over time

Despite the beneficial effects of defoliation and wider row spacing, grain yield still declined dramatically over time. This was primarily due to the decline in grain number spike⁻¹, which dropped by roughly half after the first year and then varied little. This pattern suggests a high degree of genetic control over grain number that is dependent on stand age. Specific physiological mechanisms could include lower floret initiation, lower floret fertility, increased seed abortion rates, or increased shattering. Grain number spike⁻¹ had the strongest direct effect on grain yield ha⁻¹ in the path analysis, with a 1 standard deviation change in grain number resulting in a 0.53 standard deviation change in grain yield. The importance of grain number in our study matches widespread findings in the perennial grass seed literature (e.g., Hampton & Fairey, 1997; Hebblethwaite & Clemence, 1981; Hebblethwaite & Ivins, 1977).

The other major factor driving yield decline over time was a reduction in the proportion of spikes that contributed substantially to total grain yield. This is shown by the decline in the ratio between the grain yield sample and the 10-spike subsample (from over 5 in 2015 and 2016 to ~1.8 in 2017 and 2018).

In the early years, the 10 best spikes only produced ~20–25% of the total yield. This indicates that there were many highly productive spikes beyond the 10 best. In the later years, by contrast, more than 50% of the yield was produced by the 10 best spikes, indicating that the remaining spikes in the sample had very low productivity. This explains why, even though the number of spikes ha^{-1} was maximized in 2018, grain yield was still very low.

Multiple lines of evidence point to increasing competition among reproductive units (Donald, 1954) as an important reason for the decline in the proportion of spikes that produced high yield. The path analysis revealed a strong compensatory tradeoff between tillers ha^{-1} and spikes/tillers, indicating that resources for reproductive growth were often limited. This tradeoff increased in severity in 2017 and 2018, the years with the highest number of tillers ha^{-1} . The path analysis also showed that defoliation treatments, which resulted in increased tillers ha^{-1} , also suffered a stronger compensatory reduction in spikes:tillers. Likewise, spikes/tillers was lowest in 2018, when tillers ha^{-1} peaked. Overall, this evidence confirms that stimulating production of additional tillers may not benefit grain yield in dense stands, as seen in previous work (Hampton & Fairey, 1997; Hare, 1993). It further indicates that the potential to enhance yield through defoliation may be limited by compensatory feedbacks among yield components.

However, there is evidence that resource allocation to reproduction declined over time, independently of increasing competition. The HI declined every year despite stable straw yield in 2016–2018, indicating that a smaller proportion of plant resources was allocated to grain production. Moreover, the path analysis provided stronger evidence for a tradeoff between tillers ha^{-1} and spikes/tillers in 2017 than in 2015, despite equivalent numbers of tillers ha^{-1} and spikes/tillers in these 2 yr. This indicates that competition among reproductive units was effectively greater, even though there were the same number of reproductive units, suggesting that fewer total resources were available for reproductive growth. The reason for this is unclear, since these well-established stands should have required lower investment in root growth than the first-year stand. This suggests that reproductive growth may be declining due to other factors, such as genetic control, nutrient limitation (see Hunter et al., 2020), or disease burden.

Results from 2016 confirm that grain yield was limited by more than inter-tiller competition. The proximal cause of the yield decline between 2015 and 2016 was a large decline in the number of tillers and spikes ha^{-1} and of grains spike $^{-1}$. However, the cause of these declines is unclear. The intervening winter was not particularly severe. Instead, it is possible that reproductive growth was genetically suppressed as the plants prioritized vegetative growth to colonize space. There may have still been intense competition among vegetative tillers,

but since we did not count unelongated tillers, we cannot assess this directly. Despite the very low spike numbers in 2016, and hence low inter-spike competition, total yield was still higher than in the subsequent years. This was because a greater proportion of tillers contributed to grain yield and TKW was greater, likely due to lower competition among reproductive units. The latter also may be due to the greater number of GDD accumulated in 2016 (2240 GDD, as compared to 2180 in 2015 and 2017 and 2090 in 2018).

The path analysis suggests that competition among culmed tillers in 2016 did not limit spikes/tillers, though it did in every other year. In this environment, the maximum number of culmed tillers ha^{-1} that did not cause a tradeoff in spikes/tillers is between 6.2 and 14 million culmed tillers ha^{-1} (the values in 2016 and the next lowest year, 2015, respectively). The corresponding values of spikes ha^{-1} for these 2 yr are 3.6 and 7.5 million. Seed yield has been shown to plateau at a similar number of spikes ha^{-1} in tall fescue (*Festuca arundinacea* Schreb.; Hare, 1992), but positive responses to tiller numbers greater than 8 million ha^{-1} have been reported in meadow fescue (*Festuca pratensis* Huds.) and perennial ryegrass (*Lolium perenne* L.) (Hampton, 1987; Hampton & Fairey, 1997). The optimal number of tillers ha^{-1} may shift with stand age if the overall investment in reproductive growth is declining.

4.3 | Lodging

Management effects on lodging followed expectations from the literature. Lodging decreased with wider row spacings in 2015, suggesting that narrow rows may increase lodging due to greater light competition or physical propagation of lodging from row to row. Defoliation reduced lodging in 30-cm rows in 2015, and across the board in 2018, likely due to decreased light competition and stand vigor. In high-fertility soils such as these and in regions prone to extreme weather, both defoliation and planting in wider row spacings may be critical for managing lodging. However, as the germplasm improves and lodging propensity decreases, this may become a lower priority.

4.4 | Defoliation and row spacing effects on yield components

Overall, defoliation had the expected effect on tiller production, with tillers m^{-1} frequently increased following defoliation in Sp or SpFa. In general, Fa defoliation stimulated tillering less than spring defoliation. This may be because vegetation was removed from the plots following grain harvest in August (except in 15-cm row spacing), so the light quality and

competitive environment were already conducive to tiller production in the fall.

However, timing of defoliation did not affect the proportion of tillers that were reproductive, which is contrary to expectations given the vernalization requirement of intermediate wheatgrass. Instead, spikes/tillers responded uniformly to defoliation regardless of timing, tending to increase in all defoliated treatments in 2016, and decreasing in all of them in 2018. This suggests that defoliation affected spikes/tillers by modifying stand vigor and intra-stand competition, rather than by stimulating tiller production at specific times. For instance, the decrease in spikes/tillers following defoliation in 2018 may be because defoliation resulted in greater tillers m^{-1} , diluting resources for spike production, likely nutrients. There is evidence that by 2017 this stand was experiencing N stress, especially in the defoliated treatments, and that P and K concentrations in the fall vegetative biomass were near the critical values for perennial grasses (Hunter et al., 2020).

Row spacing also affected tiller production as expected, with wider spacing increasing tillers m^{-1} of row when the stand was young, but still resulting in lower tillers ha^{-1} . Spike number responded similarly. These results indicate that within-row competition (in the wide rows) reduced tillering and spike production more than between-row competition (in the narrow rows). As the stands aged, however, the stand density converged among the row spacings due to colonization of the interrow space by tillers and rhizomes and to loss of in-row tillers due to competition. Therefore, row spacing did not affect tillers m^{-1} or spikes/tillers in the final 2 yr. This convergence of stand density may also help explain why interactions between row spacing and defoliation treatment were rare after the first year. Future research should explore the effects of maintaining the initial row spacing over time through interrow tillage or herbicide application.

Responses to defoliation and row spacing became less distinct in the subspike yield components: grain number spike $^{-1}$ and TKW. Grain number spike $^{-1}$ was greater in the 61 cm than the 15-cm row spacings overall, but the difference resulted from contradictory interactions (a positive interaction with Sp defoliation in 2015 and a positive interaction with Ctrl in 2016). Moreover, there was no effect of defoliation on grain number in the final 2 yr. This suggests that defoliation did not affect grain number directly, but instead that grain number shifted as a compensatory response to the resource environment.

As expected based on previous reports (Canode, 1964; Hampton & Fairey, 1997; Hebblethwaite & Ivins, 1977; Young et al., 1996), TKW was only minimally responsive to management. The decrease in TKW in the 15-cm row spacing following Sp defoliation in 2015 was surprising, given that all of the preceding yield components in that treatment combination also tended to be low. This suggests that Sp defolia-

tion was injurious to the stand in the narrow row spacing that year.

5 | CONCLUSIONS

Our results indicate that intermediate wheatgrass reproductive physiology is influenced by both competition among reproductive units and other, independent limitations to reproductive growth that increase with stand age. The management interventions we assessed—defoliation and row spacing—affected competition, but did not ameliorate yield decline over time. Therefore, our study has implications for both management and breeding of IWG.

Overall, both defoliation and planting in wider row spacings helped maximize IWG grain yield over a 4-yr stand life, with stronger evidence for the 30 cm than the 61-cm rows. The most direct beneficial effect of these management interventions was a reduction in lodging. In addition, both practices affected tillers ha^{-1} , which boosted grain yield in a number of year–treatment combinations by optimizing reproductive sink capacity. However, there was some evidence that repeated defoliation may risk depleting stand vigor over time.

Both excessive intra-stand competition and the steady decline in resource allocation to reproductive growth must be addressed in future research and germplasm improvement. First, ongoing breeding efforts (Bajgain et al., 2019; DeHaan et al., 2018; Zhang et al., 2016) should focus on developing IWG germplasm that maintains a high number of grains spike $^{-1}$ after the first production year, without over-producing culmed tillers. Reducing the rate of rhizomatous spread may also help avoid excessive intra-stand competition. In addition, the possibility that reproductive growth is being curtailed by declining nutrient availability and increasing disease burden must be addressed. A synergistic approach that combines improved genetics with optimized management tailored to specific environments will be required to realize the full potential of intermediate wheatgrass to produce abundant grain and robust ecosystem services.

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