The effect of nitrogen rates and plant density on grain yield components and persistence in intermediate wheatgrass (*Thinopyrum intermedium*) and mountain rye (*Secale strictum*)

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Abstract. Intermediate wheatgrass (IWG; *Thinopyrum intermedium*) and Mountain Rye (Mtn Rye; *Secale strictum*) have potential for release as dual-purpose (grazing and grain production) perennial grains in Australia due to their superior longevity compared to hybrid perennial wheats. Initially developed as perennial forage grasses, few management guidelines exist to inform agronomic practices to maximise grain yields and profitability in Australian environments. An experiment was established in 2020 to examine the effect of plant density and nitrogen rates on grain yield components. The experiment compared the two species (IWG, Mtn Rye) sown at three plant densities (50, 100 and 200 plants/m^2) with three nitrogen rates (0, 100, 200 kg/ha N). Overall, in the first year of production, Mtn Rye had higher grain yields than Kernza although yield decreased with increasing N rates. With further selection for floret fertility and seed size, Mtn Rye could prove a successful candidate for a perennial grain crop in Australia.

Introduction

Globally, annual grain crops provide 70% of human calorific requirements and occupy 70% of agricultural land (Glover and Reganold 2010). However, these grain production systems are associated with higher levels of soil disturbance, either through cultivation or the maintenance of chemical fallows between cropping sequences. Consequently, annual grain production has become associated with higher rates of soil loss, changed landscape hydrology, declining soil organic carbon and nutrient runoff. Changing the life cycle of grain crops from annual to perennial could offer a new production system which is more sustainable, resistant to climate mediated impacts, and with higher rates of carbon sequestration.

One method being pursued to develop perennial crops is domestication of perennial grasses to derive perennial cereals. Intermediate wheatgrass (IWG; *Thinopyrum intermedium*) , a perennial forage grass, has undergone selection for domestication traits and is one of the first perennial grain crops to be commercialised in the USA, under the name Kernza (DeHaan *et al.* 2020). Although there has been a significant increase in area sown to Kernza in North America, there is little experience with this species in Australian agriculture. Mountain Rye (Mtn Rye; *Secale strictum*) has a similar Eurasian origin to IWG and has been developed as a forage grass suited to acid duplex soils of south eastern Australia (Oram 1996). Improvements in seed harvestability led to a commercial cultivar of Mtn Rye being released in Australia, although was never widely adopted. Both IWG and Mtn Rye have the greatest near-term prospect for release as perennial grains in Australia, due to their superior persistence compared to hybrid wheats (Hayes *et al.* 2012). However, for both species, basic agronomic practices to maximise grain yields and profitability remain unclear in Australian environments. This study set out to understand the nitrogen (N) response and optimal planting density for these novel crops.

Methods

An experiment was established at the Orange Agricultural Institute (S33°19.565', E149°4.818') in 2020 to examine the effect of plant population and nitrogen rates on grain yield components of IWG and Mtn Rye. Soil type was a brown ferrosol (Isbell 1996) with $pH_{(CaCl2)}$ 5.5, 124 mg/kg and 36 mg/kg of mineral nitrogen and phosphorus (Colwell) in the top 10cm respectively. The experiment was sown on 21 April in a randomised design with three replicates. The area had been chemically fallowed since September 2019 and then cultivated after 3t/ha lime ($CaCO_3$) was applied on 17 February 2020. All treatments were sown using a cone seeder with 18cm row spacing, to produce plots which were 1.5m wide x 10m long. Seeding rates were adjusted according to seed size and germination percentage, to produce populations of each species that were 50, 100 and 200 plants/m^2. Nitrogen was applied as urea (46%N) in a split applications prior to rainfall, in June, July and September 2020. In total each plant population received one of three N rates, 0, 100, or 200 kg/ha.

Once each species had ripened, a representative sample of biomass was removed from each plot by cutting two rows at ground level, along a 50cm ruler (total area = 0.36 m^2) at either end of the plot in February 2021. This sample was used to determine the number of mature tillers/m^2. The grain was removed from this sample through a stationary thresher (Kimseed, Perth), from which the number of kernels were counted and weighed to determine relative seed size. A further sampling of five random inflorescences were selected from each plot. From these the number of spikelets where counted on each inflorescence and the seed removed and counted. This data was used to calculate the average number of seeds contained in each spikelet (floret occupancy).

Prior to harvesting plant height from ground level to the top of the tallest plant was measured in three random locations within each plot. At the same time, the level of lodging in each treatment was assessed using a visual (1-10) score, where 1= all plants standing and 10 = 100% plants laying horizontal to the soil surface. At harvest all grain was removed using a plot harvester (Kingaoy Engineering, Kingaroy) from which final grain yield was determined in kg/ha.

To determine plant survival over summer, assessments of basal frequency was taken in autumn following harvest for each treatment (Brown 1954). Fixed quadrats (1.0 x 1.0 m) divided into 100 cells (each 0.1 x 0.1 m) were located centrally in each plot. The number of cells which contained a live plant base was counted.

Data was analysed using a linear mixed model (Genstat, 21st edition) fitted with crop type, population and nitrogen rate and interactions as fixed effects, with replicate as the random term. All data was analysed at the 95% significance level (P =0.05).

Results and Discussion

There was no interaction between crop type, plant population and increasing rates of N fertilisation. Mountain Rye produced 71% more tillers than IWG (Table 1) while there was a 21% increase in the number of mature tillers from increasing the N rate from 0 to 200kg/ha for both species (P = 0.007). Overall Mtn Rye produced twice as much grain as IWG, however yields declined with increasing rates of N (P=0.064). In a similar experiment with IWG, Fernandez *et al.* (2020) found that increasing N application had no effect on grain yield in the first year of experimentation with positive responses in mature tillers to increasing plant population and nitrogen rate. Nitrogen responses in crops generally follow a sigmoid response curve in which production increases with increasing rates of N, before plateauing and sometimes declining (Russell 1963). The current study was located on a site with high soil N levels at sowing. Therefore, we maybe observing responses at the top end of the response curve showing little response to increasing rates of N. Unlike annual grain crops, response to N can be complicated by the perennial nature of these crops and variability in N demand as the perennial crop ages (Jungers *et al.* 2017). Further monitoring over the longer term will be important to gain insight into optimising N rates in both species.

There was no significant difference in seed occupancy of florets between plant populations and increasing N rates. However, the inflorescence of IWG contained more seeds per spikelet than Mtn Rye (Table 1) while Mtn Rye had a higher spikelet number/inflorescence. Small decreases in seed weight were observed with increasing N application (P = 0.009) at all densities (P < 0.001) of both species. On average, Mtn Rye had 22% larger seeds than IWG. Floret site utilisation and higher fecundity along with grain weight are major drivers of grain yield. The higher seed weight of Mtn Rye compared to IWG offers potential to improve grain yield in this species more quickly with an emphasis of selection

Table 1: Grain yield components of mature tillers at harvest, grain yield, seeds per spikelet seed size and lodging score, along with plant frequency (%) for IWG and Mtn Rye sown at three plant populations (50, 100 & 200 plants/m2) and three nitrogen treatments (0,100 & 200 kg/ha N).

	Crop		Plant Population (plants/m^2)			Nitrogen Rate (Kg/ha)			*P* value			lsd (5%)		
	IWG	Mtn Rye	50	100	200	0	100	200	Crop	Pop*	N#	Crop	Pop*	N#
Mature Tillers/m^2	455.2	783.7	600.1	582.1	676.3	588.9	576.0	713.5	<0.001	0.131	0.007	79.9	ns¥	97.8
Grain Yield (kg/ha)	553.1	1126.8	803.9	838.9	877.3	929.5	832.2	758.0	<0.001	0.585	0.064	116.8	ns	ns
Seed/ spiklet	2.3	1.3	1.9	1.7	1.7	1.8	1.6	1.8	<0.001	0.605	0.267	0.2	ns	ns
Spikelets/ inflorescence	21.4	37.5	28.3	30.3	89.7	29.4	29.7	29.2	<0.001	0.936	0.105	2.1	ns	ns
Seed weight (mg)	9.0	11.0	10.3	10.2	9.7	10.4	10.1	9.7	<0.001	0.009	<0.001	0.3	0.4	0.4
Plant Height (mm)	1603.0	1358.0	1511.0	1524.0	1407.0	1494.0	1484.0	1464.0	<0.001	0.007	0.722	62.7	76.8	ns
Lodging Score	0.3	5.3	2.8	2.3	3.8	2.6	2.6	3.2	<0.001	0.157	0.315	0.793	ns	ns
Plant Frequency (%)	50.2	29.8	34.1	41.8	43.7	39.7	40.9	39.1	<0.001	0.001	0.748	4.2	5.1	ns

*Plant population
#Nitrogen rate
¥ not significant

for improved floret fertility (Altendorf *et al.* 2021). Although, neither trait responded to increasing plant population or N rates in the first year of the current study.

The IWG grew taller than Mtn Rye, however there was a higher incidence of crop lodging in the Mtn Rye treatments, with no clear response between plant population and nitrogen treatment. In the study by Fernandez et al. (2020) there was limited lodging in IWG, however, high rates of N at high planting density increased the incidence of lodging. The higher rates of lodging in Mtn Rye suggest a weaker straw strength, compared to IWG and would indicate selection for improvement in this trait could lead to better harvestability and grain yield. Breeding programs have identified genetic markers for plant height and increased stem base dry weight in IWG, which lead to better seed yield (Fernandez *et al.* 2020).

Measurement of plant frequency demonstrated that both species had adequate survival through the first summer. IWG had 40% higher frequency scores by the start of the second growing season, which is indicative of its rhizomatous nature (Jungers *et al.* 2017) compared to Mtn Rye which is more caespitose. As expected, increasing plant population resulted in in an increase in plant frequency for both species, particularly when the higher plant densities (100 & 200 plants/m^2) where compared to the low plant density (50 plants/m^2, Table 1). Plant frequency will need to be monitored over the longer term to ascertain the optimal plant density to maintain persistence of each species and whether increased N rates are required to maintain plant numbers and grain yield.

Conclusions

In the first year of assessment crop type was the main driver of responses in grain yield components of both IWG and Mtn Rye, with no interaction between crop type, plant population and increasing rates of N fertilisation. The impact of plant density and N rate will need to be monitored over a longer period so that these factors can be optimised for production, while limiting any adverse effects from excess N. Overall, Mtn Rye had higher grain yields than IWG. As a forage grass Mtn Rye has had no selection for grain yield characteristics. With further emphasis for floret fertility and seed size, Mtn Rye could prove a successful candidate for a perennial grain crop via domestication in Australia.

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