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Maize yield in response to alternating low- and high-density rows of diverse hybrids

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ABSTRACT

Production of rainfed summer crops like maize (Zea mays L.) must adapt to a changing climate and retain high productivity. Using diverse hybrids in the same stand is a simple way to adapt to unpredictable stresses during the growing season. However, some hybrids are available at a price premium and therefore their use needs to be cleverly incorporated in the system to balance cost and economic return. In a two-year study in Pennsylvania, we evaluated maize yield and economic return in response to combinations of two planting densities (4.6 and 8.9 plants m⁻²), two planting arrangements (rows with the same density or alternating rows of low and high density with medium overall density), and two hybrids (drought tolerant Aquamax and a non-drought tolerant Seedway hybrids), including mixtures of both density and hybrid. Both experimental years received adequate rainfall for production. Regression showed that increasing plant density by 1 plant m⁻² increased biomass and grain yield by 2% in the density range tested. Using the Aquamax hybrid increased grain yield by 6% compared with Seedway in one of two years. However, economic analysis indicated lower returns when using the higher planting density. Using low density, or a medium density by alternating low and high-density rows, optimized economic output and yield. In 2019, mixing hybrids suppressed yield by reducing the number kernels per ear by 9% compared to pure hybrid stands, mostly for the Aquamax hybrid, which suggests that combining compatible hybrids is of primary importance in mixtures. In rotations highly dependent on maize yield, a combination of defensive agronomic tactics that keeps costs low and yield slightly below the attainable yield as proposed here may result in a resilient and profitable agricultural system adapted to a variable climate.

1. Introduction

Managing rainfed summer crops like maize (*Zea mays* L.) requires utilizing management practices that take advantage of seasons with favorable growing conditions and prevent losses under unfavorable conditions. The ongoing climate change poses an additional challenge in some areas, as management must adapt to a climate with more intense and less predictable rain events during critical growth periods (Wolfe et al., 2018). These climate conditions can cause more frequent drought and flooding stress, sometimes alternating in the same season. Adaptation must therefore focus not so much on reaching maximum yield potentials but maintaining stable and positive economic returns. Systems that take advantage of the interaction of crop genetics (G), environment

(E), and management practices (M) will be pivotal in stabilizing yield and profit year-to-year. Yield gains may partially rely on genetic improvements tailored to specific environmental stresses (Tollenaar and Wu, 1999), but including innovative management techniques may help stabilize overall production and help producers avoid large, unexpected losses. In this research, we explore tactics based on combining plant genetics, plant densities, and plant spatial arrangements to maintain profitable and stable maize yields under varying yearly environmental conditions like water stress.

Crops experience drought stress when there is a mismatch between the atmospheric evaporative demand and the capacity of the soil-plant system to match that demand. It is often expressed as the ratio of actual to potential transpiration over a convenient time-period. For sub-

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Abbreviations: AM, Aquamax drought-tolerant hybrid maize; DAP, days after planting; E, environment; ET_o , reference evapotranspiration; FI_{PAR} , fraction of canopy intercepted photosynthetically active radiation; G, crop genetics; H, high density (8.9 plants m⁻²); HL, mixed density (6.4 plants m⁻²); HI, harvest index; I_{PAR} , canopy intercepted photosynthetically active radiation; L, low density (4.6 plants m⁻²); M, management; NDVI, normalized difference vegetation index; PAR, photosynthetically active radiation; ROSI, return on seed investment cost; SW, Seedway non-drought tolerant hybrid maize.

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daily time frames, a suitable surrogate of that ratio is the canopy temperature depression (Idso et al., 1982), because as stomata close and latent heat transport is limited, the canopy temperature increases. The practical effect is that water stress can limit the growth or development of reproductive structures and severely reduce yield (Westgate and Boyer, 1985), especially when the vapor pressure deficit is high (the air is dry) and precipitation is low (Lobell et al., 2014; Hoffman et al., 2020). Similarly, excess water at key physiological stages may also negatively impact maize production by reducing yields, and traits related to stomatal closure and root structure are shown to mitigate these negative effects (Zaidi et al., 2003, 2004).

Maize yield is most responsive to water stress during flowering, when drought can lead to significant yield losses (Campos et al., 2006; Hoffman et al., 2020). Due to climate change, the Northeastern USA is expected to experience more days above 35 °C, increased spring precipitation, and more frequent short-term droughts during summer months, potentially leading to a wider gap between crop evapotranspiration (ET) demand (estimated here through the reference ET or ET₀; Allen et al., 1998) and soil plant available water (Kunkel et al., 2013; Wolfe et al., 2018). Producers started adapting tactically to drought stress by planting drought tolerant maize, which may be either conventionally bred (e.g., Pioneer Aquamax, used in this study), or genetically engineered (e.g., Bayer DroughtGuard). In 2016, approximately 22% of maize planted in the USA was a drought tolerant hybrid and this percentage is projected to have increased in subsequent years (McFadden, 2019). Aquamax maize mitigates the damage of drought on yield through improved root and silking traits (Gaffney et al., 2015; Pioneer, 2020). Conversely, genetically engineered drought tolerant maize is altered to suppress genes defensively expressed in response to drought stress, therefore allowing certain processes (particularly grain filling) to continue as if the stress were more moderate or non-existent (Castiglioni et al., 2008; McFadden et al., 2019).

Generally, and compared to non-drought tolerant hybrids, drought tolerant maize uses similar amounts or less water to produce grain, resulting in improved water use efficiency (Hao et al., 2015; Mounce et al., 2016; Zhao et al., 2018). In dry years and compared to non-drought tolerant hybrids, a yield increase of $\approx 6.5\%$ was observed in drought tolerant maize hybrids, with no negative impact in years with no water stress (Gaffney et al., 2015; Pioneer, 2020). However, seed of drought tolerant hybrids is expensive. In some years, traits for drought tolerance may not be needed and less expensive hybrids may yield similarly. One of our propositions here is that using mixtures of maize hybrids with both expensive and expensive may allow producers to reap the benefits of using expensive hybrids in years the trait is needed, while protecting return on seed investment by mixing with a lower priced seed in years when a particular trait is not needed.

Though in this research we focus on a hybrid marketed as drought tolerant, such a trait is just one of a suite of physiological characteristics of a given hybrid. Root system depth and distribution can play a role in not only water but also nutrient acquisition, thus aiding in overall production (Lynch, 2013). Differences in flowering characteristics between varieties can alter kernel set and thus yield (Lizaso et al., 2003). Additionally, resistance to heat stress is an important physiological parameter in growing regions where temperature during the growing season may exceed the optimal range (Alam et al., 2017; Cairns et al., 2013). Resistance to lodging can also affect the yield and profitability of maize production where resistance is related to stalk characteristics like plant and ear height (Ma et al., 2014). These characteristics may not necessarily be marketed as part of the germplasm and may allow maize to adapt to the production environment.

Altering plant density and plant spatial arrangement may provide an additional and more economical way for producers to mitigate the impact of low-production environments. The general response of crop yield to plant density is well understood: yield increases linearly with density at low densities and slowly tapers off, finally decreasing at high densities (Murphy et al., 1996; Assefa et al., 2016). The densities that

result in maximum yield depend on the G x E x M interaction. In locations with a long growing season and favorable water balance, maize grain yield is highly responsive to planting density with grain yields increasing with densities of up to ≈ 10 plants m⁻² (Tetio-Kagho and Gardner, 1988a; Begna et al., 1997; Li et al., 2015). With irrigation, grain yield can also increase with narrow row-spacing (i.e., from 0.7- to 0.5- or 0.35-m; Barbieri et al., 2008). When water supply varies among years, high planting densities may also contribute to intraspecific competition for water, lowering yield (Sangoi, 2001). In areas where the water regime is moist but punctuated by dry periods, like some parts of Pennsylvania, yields may be somewhat plastic with minor yield loss at moderately low densities (5.2 plants m⁻²) compared to higher densities (8 plants m⁻²; Van Roekel and Coulter, 2011).

In locations with lower average yields and predictable water shortages, recommended densities are lower, so that production costs are lower (reduced seed and fertilizer costs), light interception is reduced, and the use of water in the subsoil is delayed. The low density increases the probability that plants will have access to subsoil water and nutrients when reaching flowering and grain filling. Skip-row geometries play a similar role in dry conditions where soil water in the skipped row space becomes available to surrounding plants later in the growing season (Nielsen et al., 2018). In low productivity environments, plants can be planted in clumps to force early light competition and delay water use, resulting in more water available during grain filling and larger grain yields (Bandaru et al., 2006; Kapanigowda et al., 2010). Thus, maize planted at low densities may have more available water per plant in the event of a drought. However, it is not clear to what extent the yield penalty of low densities may result in yield and economic loss in good years in locations with variable water supply.

In the USA, seed is the second largest operating cost on farms growing maize, just behind fertilizer and other chemical inputs, and its cost vary considerably with incorporated traits and treatments (USDA Economic Research Service, 2020). In the Midwest USA, maize grain yield responds positively to increasing planting density up to 8.0 plants m⁻² when a plateau is reached (Van Roekel and Coulter, 2011). Further, planting density that maximizes returns falls below the density that maximizes yield, especially as cost for seed increases, but this is complicated by the price of grain at sale and highlights the need for sound planting decisions (Van Roekel and Coulter, 2011). Additionally, low to moderate density stands may intercept less photosynthetically active radiation (PAR) compared to higher density stands (Andrade et al., 1993), and may also alter the pattern of daily illumination in the interrow space (Allen, 1974; Timlin et al., 2014). Alternating rows of low- and high-density that produce a diurnally variable light penetration through the plant canopy may allow producers to consider, for example, using the light corridor to interseed cover crops, an option that might not be viable when crop density and shading are high (Youngerman et al., 2018).

The concept of mixing varieties for grain production is not new and has shown positive results for production (Tooker and Frank, 2012). Using winter wheat (*Triticum aestivum* L.) Baniszewski et al. (2021) showed that mixing cultivars may provide foliar disease suppression equivalent to the application of a fungicide while retaining yield and economic return. Another study in winter wheat showed that varietal mixtures, when chosen carefully, could mitigate frost and drought damage (Fletcher et al., 2019). Varietal mixtures of barley (*Hordeum vulgare* L.) were shown to not only resist diseases, but also decrease lodging and provide a more stable yield year to year, thus reducing uncertainty for producers (Creissen et al., 2016). However, the practice of mixing maize hybrids has not been studied despite its potential benefits.

We tested simple and cost-effective ways to stabilize yields and economic returns in maize production by combining drought-tolerant but costly genetics with innovative planting arrangements. To take advantage of both the higher yields of high-density plantings and the buffering capacity of low-density and non-uniform planting geometry (i. e., skip-row and clumping), we propose alternating rows of low- and high-density. Combining this approach with the use of maize hybrids marketed as both drought tolerant and non-drought tolerant may stabilize yields year-to-year and reduce seed cost associated with traits that can go unused in some years.

In two years in central Pennsylvania, we tested 10 treatments consisting of alternating low- and high-density rows with the same or two different maize hybrids (drought tolerant or non-drought tolerant). Our hypotheses were as follows. First, alternating rows of low- and highdensity will result in a minor yield penalty compared with highdensity, with a yield above the average of the low- and high-density stands. Second, mixing maize hybrids may influence yield and yield components, and potentially stabilize yield compared to pure stands due to a mixture of traits inherent to each hybrid. Third, mixing of hybrids whose seed have different costs may provide a higher partial economic return compared to that of using pure stands of each hybrid.

2. Materials and methods

2.1. Experimental site and design

Field experiments were conducted under rainfed conditions in 2018 and 2019 at the Penn State Russell E. Larson Agricultural Research Center at Rock Springs (40°42'58.3"N 77°56'05.3"W, elevation 330 m above sea level). Two maize hybrids were used each year, a drought tolerant hybrid (denoted Aquamax, AM, a Pioneer hybrid) and a nondrought tolerant hybrid suited to the area (denoted Seedway, SW, a Monsanto hybrid). The specific Aquamax and Seedway hybrids differed between years. Hybrid relative maturities were 100 days in 2018 and 105 days in 2019. Details provided in Supplemental Table 1S.

Ten treatments were tested in a fractional factorial design to avoid redundancy (Fig. 1 and Supplemental Table 2S), with the following conceptual framework. Within a plot, rows can be low-density or highdensity. A plot with a density between that of low- and high-density plots can be achieved by simply averaging the density per row or by alternating rows of high and low density. We opted for the second option since it preserves the intra-row spacing of low- and high-density rows. Additionally, within a plot, all rows can be of the same genotype or can alternate genotypes. The treatments are shown in a diagram in Fig. 1 and examples are shown in an aerial photo in Supplemental Fig. 1S. All possible combinations of hybrid and density were tested. Plots were 9-m wide by 10-m long with row spacing of 76 cm, resulting in 12 rows of maize per plot. Treatments were arranged in a randomized block design with five complete blocks.

Site management varied between 2018 and 2019 based on prior field management. Preceding crops were maize in 2018 and soybean in 2019. In both years, glyphosate, pendimethalin, and S-metolachlor were applied at label rates post-emergence. Maize was planted with a John Deere 1755 drawn planter. Treatments that required mixed density were planted at the high density and thinned by hand at V3. About 250 kg ha¹ of N was applied each year as UAN and ammonium sulfate (Supplemental Table 3S). The N fertilization rate was uniform across treatments. Additional field operations and site characteristics are listed in Supplemental Table 3S.

Reference evapotranspiration (ET_o) was calculated using the method in FAO Factsheet #56 for daily estimations (Allen et al., 1998). Daily weather data were obtained from the North American Land Data Assimilation System (NLDAS; Xia et al., 2012).

2.2. Light interception and vegetation index

Both light interception and green vegetation were quantified. Canopy intercepted photosynthetically active radiation (I_{PAR}) was estimated using radiation measurements above and below the canopy obtained with a ceptometer (Decagon AccuPar LP-80; Meter, Pullman, WA). Three sunlit and three under-the-canopy measurements per plot were



Fig. 1. Treatments with combinations of plant density (subscript L and H for high and low density), arrangement, and hybrid (AM and SW for Aquamax and Seedway) shown here for two rows that are 2-m long (repeated six times per plot). Overall, plots were 9-m wide by 10-m long. Treatments that include both a low (4.6 plants m⁻²) and high (8.9 plants m⁻²) density row, have a plot density of 6.4 plants m⁻².

taken 71 and 64 days after planting (DAP) in 2018 and 2019, respectively (August 15, 2018, and July 19, 2019). Normalized difference vegetation index (NDVI) was measured using a GreenSeeker (Trimble Inc., Sunnyvale, CA). Readings were taken three times throughout each growing season between 36 and 52 DAP, after which the crop was too tall to take NDVI measurements. The sensor was held approximately 30 cm above the crop canopy and measurements were taken over a single row for 8 m. Data available in the accompanying Supplemental material.

2.3. Biomass

Biomass samples were taken by hand from adjacent rows in the plot center when maize reached physiological maturity. For each treatment, an equal number of rows were harvested to ensure a representative sample. In plots where rows 1 and 2 were identical (e.g., both rows Aquamax high-density), two 1-m rows were sampled for yield and biomass. If a plot contained different hybrids and/or densities in rows 1 and 2 (e.g., row 1 Aquamax, high-density; row 2 Aquamax, low-density), two 1-m samples of each row were harvested to ensure a similarly representative sample of each hybrid x density. Rows with lodged plants were avoided during harvest. No plant had more than one ear at harvest. Harvested stalk samples were weighed fresh, chopped using a silage chopper, subsampled, and weighed again. Ears were harvested and weighed fresh. All samples were dried at 50 °C until their mass was stable (10-14 days) and weighed. Once dry, maize ears were shelled by hand. Total aboveground biomass and grain yield were estimated, and harvest index (HI) calculated as the ratio of grain to total aboveground biomass. The number of kernels per m², kernels per ear, and kernel size (mg kernel⁻¹) were calculated. Kernels per m² was calculated using the yield per m² and the kernel size. Kernels per ear was calculated using the total plot weight harvested, number of ears harvested, and kernel size. Kernel size was calculated from the weight of a 500-grain subsample.

2.4. Data analysis

For statistical analysis we used SAS (v9.4, SAS Inst., Cary, NC). For linear models we used PROC GLM, for normality tests we used PROC UNIVARIATE on residuals, and for mixed models we used PROC MIXED. To assess the impact of individual components of treatments on biomass, grain yield, and HI, we used linear models and regressed these variables against plant arrangement, plant density per m^2 , and the Aquamax fraction. Biomass and grain yield were natural log transformed, so that the model coefficients yielded slopes in relative change of the predicted variable per unit of change of the predictor. Means of dry biomass and grain mass m^2 and HI were calculated for all treatments using PROC Mixed sliced by year and are reported in Supplemental Table 4S. Additionally, the treatment effect on yield components (yield, kernels m^2 , kernels per ear, and kernel size) was assessed using PROC GLM with the hybrid and density of the row and neighboring row as predictors and reported in Supplemental Table 5S.

We estimate the partial economic returns using the seed cost for each treatment and the price of maize grain at the point of sale based on historically available USDA data. All other costs—fixed and variable—were assumed to be comparable between treatments and are therefore not included in the analysis (only yield-dependent costs like grain drying or transportation would have varied slightly). Per plot revenue was calculated using the mean grain yield of each treatment multiplied by the Pennsylvania-based grain prices in 2018 and 2019 (\$3.85 bu⁻¹ in 2018 and \$4.33 bu⁻¹ in 2019). Returns on seed cost investment were calculated by subtracting seed cost from revenue. Revenue and returns on seed cost investment were analyzed using PROC MIXED sliced by year.

Plot averages of radiation interception both above and below the canopy for each day of measurement were used to calculate the fraction of the incoming PAR that was intercepted by the canopy (FI_{PAR}). The

 FI_{PAR} and NDVI were analyzed using linear models with the regressors plant arrangement (categorical, two rows with either similar or different densities), plant density (the overall density in plants m⁻²), and Aquamax fraction (Aquamax plants divided by total plants in the stand). Residuals from NDVI readings were tested for normality using the Shapiro-Wilk test. A supporting analysis using a mixed effects model for FI_{PAR} can be found in Supplemental Table 6S.

3. Results

3.1. Weather

In 2018, precipitation was 60% greater than ET_o , making this a comparatively wet year. Total precipitation through the growing season (May-September) was 891 mm and ET_o was 562 mm (Supplemental Table 7S). In 2019, precipitation and ET_o were better matched, and conditions were drier with precipitation totaling 474 mm and ET_o 660 mm (May-September). In 2019, precipitation was less than ET_o from June to August. The federal drought monitoring did not indicate drought conditions in 2019 (National Drought Mitigation Center, 2020). Thus, none of the years experienced a mild or severe drought.

3.2. Biomass, yield, yield components, and harvest index

Treatment effects taken as the complete package of arrangement, plant density, and fraction of Aquamax, were significant for yield and its components (kernels per m², kernels per ear, and kernel size) in each year except for yield in 2019 (Table 1). In 2018, the highest yields were achieved with AM_H, AM_H-AM_L, and AM_H-SW_H (i.e., there was always an AM_H row in the highest yielding treatments). In 2019, the highest yields were achieved with the same treatments, but the effect was not statistically significant.

Kernel size was mostly stable in 2018 (190–225 mg kernel⁻¹) and 2019 (250–310 mg kernel⁻¹) except for Aquamax at low density in 2019 for which the kernel size was large (377 mg kernel⁻¹; Table 1). In general, low-density plantings had the greatest individual kernel size and a greater number of kernels per ear compared to high-density plantings. As expected, high- and medium-density plantings had the greatest number of kernels per m² (Table 1).

Linear regression of biomass and grain yield revealed nuanced effects of plant density, plant arrangement, and hybrid mixtures (Supplemental Table 8S), even though biomass and grain yield did not differ when treatments were assigned to classes rather than using density as a covariable (P = 0.14 and 0.35, respectively, Supplemental Table 4S). Aboveground biomass in 2018 was significantly affected by planting density (P < 0.0001). Both aboveground biomass and grain yield increased by 2% for each additional 1 plant m^{-2} , and by 6% as the fraction of Aquamax increased from 0 to 1 (Supplemental Table 8S). No significant effects on biomass or grain yield were detected in 2019 (Supplemental Table 8S). Further, removing arrangement from the linear regression for biomass, grain yield, and HI rendered similar Pvalues and standard errors compared to the model that included arrangement (most of the variability went to the error term; Supplemental Table 9S), signaling that our analysis catches some effect of different planting arrangements, and not only that of density.

Regressing the yield and yield components of each individual row (expressed on a per m² basis) against the hybrid and density of the row and that of the neighboring row revealed further information. The yield components considered were kernel size (mg kernel⁻¹), ear size (kernels ear⁻¹), and kernels m⁻². The density of both the row and neighboring row had the strongest effect on kernel size, ear size, and kernels m⁻² across both years. Including a high-density row increased the number of kernels m⁻² and grain yield (Supplemental Table 5S). The hybrid of the row affected all yield components (P < 0.05), except for kernels m⁻² in 2018. For ear size, the number of kernels per ear was greater in stands of pure hybrids compared to mixed hybrid counterparts, and the effect was more

Table 1

ANOVA (mean effect) and least squares means (LS means) of grain yield (yield), kernels per meter squared (No. kernels), kernels per ear (ear size), and kernel size (mg kernel⁻¹) in 2018 and 2019. Data for all variables except yield were missing for two SW_H plots in 2018. Bottom of table shows LS mean across all five blocks for each hybrid in a mixture, with data corresponding to the bolded hybrid within the row (i.e., $AM_H AM_L$ denotes LS means for the Aquamax high density (AM_H) within the AM_HAM_L treatment). DF = degrees of freedom. Within a column, the same letter indicates no statistically significant differences.

		2018				2019				
		Yield kg m ⁻²	No. kernels kernels m ⁻²	Ear size kernels ear ⁻¹	Kernel size mg kernel ⁻¹	Yield kg m ⁻²	No. kernels kernels m ⁻²	Ear size kernels ear ⁻¹	Kernel size mg kernel ⁻¹	
Source of Variation	DF									
Block	4	0.03	399	35.6	9.6	0.368 ^a	974 ^a	138 ^b	38.4 ^a	
Treatment	9	0.09^{b}	1129 ^c	197 [°]	39.9 ^a	0.131	853 ^a	191 [°]	51.8 ^c	
Error	36	0.06	4533	46.5	12.6	0.153	498	76.1	17.1	
Treatment least square means										
Aquamax										
AM _H		0.88a	4750a	518e	186e	1.18	4267ab	427 <i>de</i>	277 <i>def</i>	
AML		0.77bc	3559def	721a	218bc	1.16	3448cd	603 <i>ab</i>	378a	
AM _H AM _L		0.83abc	4467 <i>ab</i>	608cd	198 <i>de</i>	1.21	3987b	516bcd	304bc	
Seedway										
SW _H		0.79bc	4173abcd	441g	190 <i>de</i>	1.21	4653a	465 <i>cde</i>	259f	
SWL		0.76c	3168f	625bc	239a	1.11	3873bcd	678a	286cde	
SW _H SW _L		0.78bc	3472ef	516 <i>ef</i>	218bc	1.14	4152ab	539bc	276 <i>def</i>	
Mixtures										
AM _H SW _H		0.84 <i>ab</i>	4269abc	457 <i>fg</i>	195 <i>de</i>	1.08	4089abc	396e	265ef	
AM _H SW _L		0.77bc	3928bcde	566 <i>de</i>	2012cde	1.07	3750bcd	486 <i>c</i> de	282de	
AM _L SW _H		0.78bc	3835cde	537e	204cd	1.15	4060abc	506bcd	290bcd	
AM _L SW _L		0.80bc	3437ef	673ab	234ab	1.04	3361 <i>d</i>	588ab	309b	
By hybrid										
$AM_H AM_L$		1.01	4927	617	181	1.41	4686	456	300	
AM _H AM _L		0.64	2965	600	216	1.01	3288	575	309	
SW _H SW _L		0.93	4255	465	218	1.34	5002	500	268	
SW _H <i>SW_L</i>		0.62	2877	567	215	0.94	3301	578	283	
AM _H SW _H		0.76	4108	464	186	1.12	3944	384	283	
АМ _Н <i>SW_H</i>		0.91	4431	450	204	1.05	4233	407	247	
AM_H SW _L		0.90	4927	533	182	1.26	4306	413	290	
АМ _н <i>SW</i> _L		0.65	2929	580	221	0.88	3194	559	274	
AM_L SW _H		0.58	2793	560	208	0.97	3113	536	315	
AM _L <i>SW_H</i>		0.98	4878	514	200	1.34	5008	476	266	
AM_L SW _L		0.80	3564	693	225	1.09	3223	564	338	
AM _L <i>SW_L</i>		0.80	3310	652	243	0.98	3498	612	279	

^a =significant at P < 0.01

 $^{\rm b}$ =significant at P < 0.05

^c =significant at P < 0.0001

marked for Aquamax (Fig. 2, indicated by points mostly falling below the 1:1 line in both 2018 and 2019). Kernel size was relatively stable across hybrids (P > 0.05; Fig. 2 and Supplemental Table 5S).

Aquamax fraction increased the HI in 2018 (P < 0.0001) and decreased it in 2019 (P < 0.01; Supplemental Table 8S). The planting density decreased the HI significantly only in 2018 (P < 0.01). Arrangement of plants in the plots, either with identical rows or alternating rows of low- and high-density, did not affect the HI in either year. The mean HI was 0.55 and 0.61 kg kg⁻¹ in 2018 and 2019, respectively (Supplemental Table 4S).

3.3. Costs, revenue, and economic return

On average across two years, the highest return on seed investment generally resulted from low- or medium-density plantings, due to higher seed costs at higher planting densities (Table 2). Seed costs were approximately 25% greater for Aquamax compared to Seedway when planted at the same density (Table 2). In all treatments, revenues were greater in 2019 than in 2018 (Table 2 and Fig. 3), the result of a greater yield in 2019. In 2018 when growing conditions were sub-optimal due to excess precipitation, mixing low-density rows of Aquamax and Seedway (AM_L SW_L) was the most profitable treatment (Table 2 and Fig. 3). However, in 2019 when growing conditions were more favorable, single hybrid plantings of maize were more profitable.

3.4. Light interception and canopy closure

Increasing plant density increased FI_{PAR} significantly in both 2018 and 2019 (P < 0.0001 for both years, Supplemental Table 10S), although the effect magnitude decreased as the season progressed and leaf area increased. Increasing plant density by 1 plant m⁻² resulted in a 1.7% and 2.8% increase in FI_{PAR} in 2018 and 2019, respectively. Plant arrangement and Aquamax fraction were statistically significant only in 2018 (P < 0.05 and P < 0.001, respectively). Alternating rows of lowand high-density led to a 1.8% increase in FI_{PAR} compared to nonalternating rows, although caution is needed in the interpretation as this response is confounded with the increased plant density. Planting all Aquamax rather than all Seedway led to a 3.1% decrease in FI_{PAR}. Means by treatment for FI_{PAR} are reported in Supplemental Table 6S.

Factors significantly affecting NDVI differed between years, and within year by DAP (Supplemental Table 11S). In both years and at each DAP, the NDVI increased as the planting density increased (P < 0.05) where increasing plant density by 1 plant m⁻² increased the NDVI by 0.016–0.034, depending on the DAP and year (this is similar to the FI_{PAR} response). In 2018, increasing the Aquamax fraction from 0 to 1 lowered NDVI by 0.129 (38 DAP) and 0.077 (52 DAP, Supplemental Table 11S). The Aquamax fraction did not have any effect on NDVI in 2019. The plant arrangement affected NDVI only in 2019; rows with different plant arrangement increased the NDVI by 0.045 (36 DAP) and 0.091 (43 DAP).



Fig. 2. Comparison of pure stands (one hybrid) to mixed stands (two hybrids) for ear size (kernels ear⁻¹; top panels) and kernel size (mg kernel⁻¹; bottom panels) by hybrid (Aquamax and Seedway). Line represents a 1:1 ratio. Points that fall below the line indicate better performance in pure hybrid stands while points that fall above the line indicate better performance in mixed hybrid stands. LD = low density (4.6 plants m⁻²); HD = high density (8.9 plants m⁻²). Mixed density stands not shown.

4. Discussion

4.1. Is alternating low- and high-density rows economically beneficial?

This work tested whether maize plants would exploit the virtues of both low- and high-density plantings when planting alternating rows of each density or penalize yields compared with either low- or highdensity. We expected benefits of using low- and high-density rows to accrue in a dry year, but neither 2018 nor 2019 had drought conditions.

Table 2

Cost, revenue, and return on seed investment cost (ROSI) of each treatment. The top maize performers within each year with respect to return on seed investment cost are bolded and italicized. Within each year for revenue and ROSI, letters indicate statistically similar results. The two-year arithmetic mean for return on seed investment cost of each treatment is calculated. Treatment shorthand is the same as in Fig. 1.

Treatment	Density	Yield		Seed Cost		Revenue		ROSI		
		2018	2019	2018	2019	2018	2019	2018	2019	Mean
	plants ha ⁻¹	ton ha ⁻¹		USD ha ⁻¹						
Aquamax										
AM _H	89,000	8.8	11.8	\$430	\$436	\$1,338	\$2,017ab	\$908	\$1,581 <i>ab</i>	\$1,245
AM_L	46,200	7.7	11.6	\$223	\$226	\$1,171	\$1,982ab	\$947	\$1,756a	\$1,352
$AM_H AM_L$	63,900	8.3	12.1	\$327	\$331	\$1,255	\$2,064a	\$928	\$1,733a	\$1,331
Seedway										
SW _H	89,000	7.9	12.1	\$341	\$351	\$1,200	\$2,055a	\$859	\$1,704a	\$1,282
SW_L	46,200	7.6	11.1	\$177	\$182	\$1,147	\$1,891ab	\$972	\$1,708a	\$1,340
$SW_H SW_L$	63,900	7.7	11.4	\$259	\$267	\$1,174	\$1,942ab	\$915	\$1,675ab	\$1,295
Mixtures										
AM _H SW _H	89,000	8.3	10.8	\$385	\$394	\$1,266	\$1,849ab	\$890	\$1,455b	\$1,173
AM _H SW _L	63,900	7.7	10.7	\$304	\$309	\$1,167	\$1,821ab	\$865	\$1,512ab	\$1,189
$AM_L SW_H$	63,900	7.8	11.5	\$282	\$289	\$1,182	\$1,968ab	\$900	\$1,679ab	\$1,290
AM _L SW _L	46,200	8.0	10.4	\$200	\$204	\$1,217	\$1,775b	\$1,017	\$1,570ab	\$1,294

We found that row arrangement (alternating or same rows of low or high density) did not have an impact on grain yield in the years we tested, which had adequate or excess moisture supply (Supplemental Table 8S). Thus, at the very least, there is no yield penalty associated with this row arrangement. The overall planting density had a greater impact on both grain yield and aboveground biomass in 2018, an extremely wet year of relatively low average yields in the region.

We found that the top two performing treatments with respect to return on seed investment cost in each year were low-density or medium density obtained by mixing low- and high-density rows (4.6 and 6.4 plants m⁻²; SW_L and AM_LSW_L in 2018 and AM_L and AM_HAM_L in 2019; Table 2), indicating that an overall low planting density may be closer to the economically optimal density (Table 2). The economic optimum planting density is lower than the agronomic optimum planting density respectively.



Fig. 3. Return on seed investment cost per treatment. Yields were greater in 2019 than 2018, explaining the increased return on seed investment cost. Vertical line shows the average cost for each year; horizontal line shows the average return on seed investment cost for each year. Top left quadrant indicates top performing treatments in terms of cost and return on seed investment; lower right quadrant indicates bottom performing treatments.

(Lacasa et al., 2020), but the results of our experiment indicate that well-managed low- or medium-density stands may be much preferable from an economic perspective even when considering that compared to 2019, lower maize yields in 2018 due to greater precipitation may have reduced the yield response to plant density.

Plant density also affects the HI, with the HI decreasing in a sigmoid pattern as the plant density increases, and a mild negative slope between 5 and 10 plants m⁻² (Li et al., 2015). Other authors have found either a decrease of HI with increasing plant density or no response (Tetio-Kagho and Gardner, 1988b). Boomsma et al. (2009) reported low response of HI to density when N fertility sufficed. In agreement with these reports, we found a mild negative response of HI to plant density in one year (2018, the extremely wet year), but no response in the following year. It is possible that the densities were well within the neutral zone of roughly 5–10 plants m⁻² as noted by Li et al. (2015). We would expect that the combination of low and high densities may mute any response of HI to plant density.

4.2. How does the use of diverse hybrids influence yield, yield stability, and yield components?

We tested a drought tolerant and non-drought tolerant hybrid with the goal of (at least) partially shielding production from drought, but neither year was dry. However, incorporating drought tolerant seed was beneficial in a wet year for causes that might be unrelated to drought tolerance traits. Further, mixing hybrids appears to be advantageous under some conditions, resulting in a yield intermediate of either pure stand, but care is needed for the mixture should be made of compatible hybrids.

In 2018, the very wet year, we found a 6% increase in grain yield as the Aquamax fraction increased from 0 (no Aquamax) to 1 (all Aquamax), and no impact on grain yield in 2019 (Supplemental Table 8S). A comprehensive evaluation of Aquamax yields in the USA Corn Belt that used industry trial data found a 6.5% increase in yields with Aquamax under water-limited conditions and a nearly 2% increase under favorable conditions (Gaffney et al., 2015), with the most yield increase correlated with areas of higher ET (Adee et al., 2016). This is consistent with the two-year averages for the Aquamax high density in the experiment (AM_H; 10.3 Mg ha⁻¹) and Seedway high density plots (SW_H; 10.0 Mg ha⁻¹), in which Aquamax had a 3% yield increase over Seedway (Table 2). Similarly, genetically engineered drought tolerant maize increased yield by 6% in water-limiting conditions over conventional hybrids (Nemali et al., 2015). Furthermore, Hao et al. (2019) found not only large increases in yield with drought tolerant seed under severe stress compared to non-drought tolerant hybrids, but also concluded that greater yield stability was obtained with high-density plantings of drought tolerant hybrids (Hao et al., 2019). However, due to seed cost, it is not clear if this yield gain is also an economic gain. Other reports indicate that under well-watered conditions, drought tolerant hybrids do not have increased grain yield compared to non-drought tolerant hybrids (Roth et al., 2013; Nemali et al., 2015; Mason et al., 2018) and may not always out-perform non-drought tolerant hybrids in droughty years (Roth et al., 2013). It is likely that these results are hybrid dependent. Considering reports of drought tolerant maize under all conditions and with respect to the data presented here, it seems clear that incorporating drought tolerant hybrids does not reduce yields and may potentially provide added benefit in dry conditions.

In 2018, mixed seed plots resulted in an intermediate yield compared to the single hybrid plots at the same overall density (e.g., $AM_HSW_H v$ AM_H and SW_H ; Table 1). However, a most curious result was obtained in 2019, when some hybrid mixtures (i.e., AM_LSW_L) had a lower yield (1.04 kg m⁻²) than the average of the corresponding pure stands (i.e., AM_L and SW_L ; average 1.14 kg m⁻²). Both the kernel size and the kernels per ear in the mixture were lower than the average of the corresponding pure stands (308 vs 318 mg kernel⁻¹, and 588 vs 641 kernels ear⁻¹), explaining the yield depression (Table 1). The observed depression in

kernel size may have been a result of negative xenia effects, where the genetics of the pollen affected the mass of set seed (Seka and Cross, 1995). It must be noted that on occasion, higher yields have been reported from cross pollination (Weingartner et al., 2002), but this was not the case in our experiment.

Regression analysis revealed that kernel size and number of kernels m⁻² were strongly influenced by the hybrid of the row, but not the hybrid of the neighboring row (Supplemental Table 5S). However, the interaction of the hybrid of the row and neighboring row was significant for ear size (number of kernels ear⁻¹) and kernels m⁻² in 2019, the same year in which we observed a yield depression. Thus, the ear size and kernels per m⁻² are influenced by hybrid selection and hybrid mixture. Due the synchrony in cycle between hybrids and the similar height, we do not expect that the yield depression was driven by shading of one hybrid over the other (without compensation) or differing flowering time between the two hybrids, both of which may result in decreased seed set or kernel size. The observed yield depression in one year highlights the importance of choosing compatible hybrids, a subject that deserves further research. As early as 1716, Cotton Mather recognized that maize pollen dispersion extends about six rows downwind (Zirkle, 1935). Thus, planting alternating pairs of rows of distinct hybrids may temper vield depression from cross pollination but also the benefits of compensatory growth by the more adapted hybrid to the stresses of a given year.

The response of the HI to an increased fraction of the Aquamax hybrid varied across years (Supplemental Table 8S). In 2018, HI increased with the Aquamax fraction in the stand, but the reverse happened in 2019. It is not clear what were the mechanisms behind the response each year, but late lodging of the Seedway hybrid in 2018 may hint at wet conditions being more stressful for such hybrid, possibly affecting grain filling and HI (Supplemental Fig. 2S). Aquamax, a drought tolerant hybrid, did well in a very wet year, showing that the vigor and adaptability of this hybrid extend beyond drought conditions and might be due to traits other than those related to drought tolerance.

Combined with previous work that found significant grain yield increases with Aquamax under drought conditions (Gaffney et al., 2015), we conclude that mixing a drought tolerant hybrid, like Aquamax, with a compatible non-drought tolerant hybrid may help stabilize yield when the weather pattern is not predictable.

4.3. Can return on seed investment cost be protected by using hybrids of varying costs?

Yield stability across years may also stabilize return on seed investment cost. When seed cost is high (like Aquamax) or the grain price is low, the ideal planting density decreases considerably and is much lower than the density needed to achieve maximum yield (Van Roekel and Coulter, 2011). Our overall goal was to develop a climate- and economic- resilient maize system by using plant density, plant arrangement, and hybrid mixtures. Though we were unable to test the system under drought conditions, we found that in the two years tested, relatively low-density plantings are often the most advantageous. Low-density plantings mixing hybrids may provide benefits, though further research is needed to exploit this technology.

Treatment taken as a whole (hybrid + density + arrangement) did not have a significant effect on revenue or return on seed investment cost (ROSI) in 2018, the very wet year (P > 0.05; Table 2). In 2019, AM_HSW_H resulted in a lower ROSI than single hybrid treatments, a result of the yield depression combined with high seed costs (Supplemental Tables 2, 12S, and 13S). However, when analyzed as a regression on each factor, plant density and fraction of maize that is Aquamax appeared to have an effect in 2018 where increasing plant density by 1 plant m⁻² resulted in an increase of 22 USD ha⁻¹ for revenue but decreased ROSI by 19 USD ha⁻¹; planting all Aquamax increased revenue by 83 USD ha⁻¹ (Supplemental Table 13S).

Despite a lack of significance in one of the two years, we detected a

general trend in the economic data, which may become more discernable under more contrasting environments. In general, and as expected, while the greatest revenues were found with the highest yields in the high-density plantings of 8.9 plants m⁻², the maximum partial net returns were mainly found at lower densities. This is similar to the results of Stanger and Lauer (2006), who reported that increasing planting density from 7.4 to 10.2 plants m⁻² (+ 38%) produced a mere 4.2% increase in grain yield. Reports show that economically optimal planting densities are somewhere between 6% and 15% lower than the planting density for maximum grain yield (Coulter et al., 2010).

In our work, the maximum return on seed investment costs were achieved with low-density mixtures of Aquamax mixed with Seedway (AM_LSW_L) in 2018 and Aquamax planted at low density (AM_L) in 2019 (Table 2). We found that mixing Aquamax and Seedway at low density appeared to capitalize on the superior performance of both seeds in 2018, the year that experienced stress as a result from excess precipitation. However, in 2019 when precipitation was timely, this mixture resulted in mediocre return on seed investment cost with all plots containing one seed hybrid outperforming the mixture due the already discussed yield depression (Fig. 3 and Table 2). In both 2018 and 2019 and in terms of the mixtures of seeds, AM_LSW_H (shaded triangle, lower (2018) and upper (2019) left quadrant in Fig. 3) proved to be a superior combination compared to AM_HSW_L (shaded triangle, lower right quadrant) with greater yield at a modest cost increase.

It appears that across both years, producers may obtain the highest economic return by mixing seed, as done with AM_LSW_L or AM_LSW_H , only if overcoming the yield depression by combining compatible hybrids. Even with a yield depression, these hybrid mixtures avoided the worst economic result. Across both years these mixtures resulted in economic returns that were within 60 \$ ha⁻¹ of the highest and 130 \$ ha⁻¹ of the lowest return on seed investment cost treatment. In general, the difference in return on seed investment cost among treatments in years that either experienced stress (like excess precipitation) or lacked stress highlights the importance of G x E x M interaction and the importance of diversifying management within the field.

4.4. Other considerations

Part of the benefit of mixing hybrids lies with the unpredictability of yearly weather. Heavy rains that may prevent planting in the ideal window are also expected to be more common (Wolfe et al., 2018). Revenue loss can be expected when planting outside the ideal window, but this is genotype specific and dependent on G x E x M interactions (Baum et al., 2020). Using a mixture of high-performing and well-adapted hybrids may help alleviate such losses, but the concept needs to be refined. Planting hybrid mixtures may provide other benefits that can have economic and ecological implications-like tolerance to disease-that could keep yields, and thus return on seed investment cost, stable between years (Browning and Frey, 1969; Mundt and Leonard, 1986; Mundt, 2002; Baniszewski et al., 2021). However, compatible hybrids must be used when mixing hybrids to avoid yield depression, which may negate any derived benefit of mixtures. Otherwise, just planting half the field with one hybrid and half with the other may provide enough buffer.

Further, adopting a variety of hybrids and densities may provide additional cropping system and environmental benefits, depending on producer goals. Higher densities may result in faster and more complete canopy closure and competition with weeds (less use of agrochemicals), while lower densities may allow for light to penetrate through the canopy and enable the adoption of green practices like interseeding cover crops. Aquamax appeared to intercept less radiation than Seedway (Supplemental Tables 6S and 10S). Morphological differences may explain these results as Aquamax hybrids showed smaller, more erect leaves and may reduce total radiation capture and reduce the irradiance per unit of leaf area (due to more erect leaves), thus reducing transpiration (Sinclair and Lemon, 1974; Chapman and Edmeades, 1999; Ribaut et al., 2009). In years drier than the ones in this experiment, the morphology of this hybrid may increase water use efficiency and potentially yield (Ribaut et al., 2009). Faster canopy closure and greater light interception may partition more of the latent heat flow to transpiration instead of soil water evaporation, therefore using more of the water for crop growth. This is the efficient use of water, as opposed to water use efficiency, postulated by Blum (2009). However, by the same token, this approach can use more water earlier in the season and reduce both the available water during grain filling and the HI if the water deficit is severe (Sinclair et al., 1990; Sadras and Connor, 1991; Kemanian et al., 2007).

Additionally, high plant densities can result in fast canopy closure and reduce inter-row weed growth by reducing the available PAR that reaches through the canopy (Murphy et al., 1996). However, if weed pressure is under control, the PAR that reaches through the canopy when plant density and FIPAR are lower, like in the low and mixed densities used here, may enable adopting green practices like interseeding cover crops which would be otherwise difficult to establish (Baributsa et al., 2008). If monetized through green payments, producers may be enticed to adopt cover cropping to mitigate negative externalities of maize production and add monetary benefit to farm production without sacrificing much yield of the main crop, especially if timing of establishment avoids high competition for resources (Curran et al., 2018). A careful balance between density, arrangement, and hybrid choice may enable producers to conserve water until grain filling, prevent evaporative losses to the environment, and enable timely cover crop establishment while controlling weeds.

We found low planting densities to be most advantageous, and that mixing drought tolerant Aquamax and a non-drought tolerant Seedway hybrids has potential for added benefits. Studies that used Aquamax maize and found positive impacts on yield were primarily carried out in the Midwestern USA on productive soils (Roth et al., 2013; Gaffney et al., 2015). Areas that may experience periodic droughts, or fields with low water holding capacity soils like the often shallow and stony soils in the Northeast USA, may benefit from mixing Aquamax with low-cost hybrids. Even though we did not try mixing seeds in the row (i.e., in the planting bin instead of alternating rows), that approach may also work if the hybrids are compatible. Additionally, low-density plantings of the hybrid mixture (4.6 plants m⁻²) appear to provide the greatest economic return on average across both years compared to other mixed-seed treatments. Producers may choose to increase the density via the hybrid of lowest cost. If producers are looking to expand the genetic diversity in the field, a low to medium density of diverse hybrids including a proportion of a drought tolerant hybrid may be a suitable choice.

5. Conclusion

We found that adopting a diversified management approach can provide both economic and environmental cropping system benefits. First, alternating rows of low- and high-density maize may not penalize yield, but the benefits of such practice need to be further explored. Differences between stands with rows of alternating density versus uniform density may be more pronounced in growing seasons drier than those experienced in our experiment. Second, in a year where maize was potentially stressed by excess precipitation, the low cost of Seedway and the high yield of Aquamax rendered an economically resilient mixture. Third, increasing planting density in maize beyond 4.6 plants m⁻² only marginally increased grain yield and often decreased return on seed investment cost. Lower planting densities than those often recommended in the area (8 pl m⁻²) were economically advantageous. Finally, future work may focus on alternating rows of low- and high-density maize, which increases the light penetration through the maize canopy, and the potential for interseeding cover crops.

Future research needs to address potential yield depression or stimulation from mixing hybrids, as well as quantify the benefits of these practices in drier climates or shallower soils, either experimentally or via modeling. We conclude that when seeking economically and climate resilient management strategies, producers can benefit from mixing hybrids and using low to medium densities (approximately 4.6–6.4 plants m⁻²), perhaps alternating low- and high-density rows. These approaches balance the benefits of new but expensive genetics with no-cost and low-risk adjustments in planting geometry and density.

CRediT authorship contribution statement

ABB and ARK conceived the hypotheses and experiments, discussed the analyses, and co-edited the manuscript. ABB managed the experiment, conducted the statistical analyzes, and wrote the first manuscript draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126472.

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