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Industry Scale Evaluation of Maize Hybrids Selected for Increased Yield in Drought Stress Conditions of the U.S. Corn Belt

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Abbreviations:

CRM	Comparative Relative Maturity
ET	Evapotranspiration
FE	Favorable Environments
SNP	Single Nucleotide Polymorphism
TD_MET	Target Drought Multi-Environment Trial
TE_MET	Target Environment Multi-Environment Trial
WLE	Water Limited Environments

ABSTRACT

Maize is among the most important grains contributing to global food security. Eighty years of genetic gain for yield of maize under both favorable and unfavorable stress-prone drought conditions have been documented for the U.S. Corn Belt, yet maize remains vulnerable to drought conditions especially at the critical developmental stage of flowering. Optimum® AQUAmax® maize hybrids were developed for increased grain yield under drought and favorable conditions in the U.S. Corn Belt. Following the initial commercial launch in 2011 a large on-farm data set has been accumulated (10,731 locations) comparing a large sample of the AQUAmax hybrids (78 hybrids) to a large sample of industry leading hybrids (4,287 hybrids) used by growers throughout the U.S. Corn Belt. Following three years (2011 to 2013) of on-farm industry scale testing, the AQUAmax hybrids were on average 6.5% higher yielding under water-limited conditions (2,006 locations), and 1.9% higher yielding under favorable growing conditions (8,725 locations). In a complementary study, three years (2010 to 2012) of hybrid-by-management-by-environment evaluation under water-limited conditions (14 locations) indicated that the AQUAmax hybrids had greater yield at higher plant populations when compared to non-AQUAmax hybrids. The combined results from research (2008 to 2010) and on-farm (2011 to 2013) testing throughout the U.S. Corn Belt over the six year period from 2008 to 2013 indicate that the AQUAmax hybrids offer farmers greater yield stability under water-limited conditions with no yield penalty when the water limitations are relieved and growing conditions are favorable.

Maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) directly or indirectly provide approximately 60% of all human calories and will remain important due to high yield and relative ease of transport and storage (Cassman et al., 2003). While genetic improvement of yield under drought has been achieved (Barker et al., 2005; Cooper et al., 2014a; Duvick, 1977) maize remains sensitive to water-limiting conditions, especially around flowering time (Campos et al., 2006). Drought impacts nearly all crop production for at least a limited period of time across the U.S. Corn Belt. Total annual crop damage from drought in the U.S. has been estimated at $6 – 8 billion (FEMA, 1995). The estimate for the worst loss ever due to drought in the U.S. is $40 billion, which occurred in 1988 when widespread and severe drought hit the Central U.S. (NOAA Satellite and Information Service, 2014). In 2012, a drought of similar severity in the U.S. contributed to historically high grain prices (Boyer et al., 2013).

Improving yield potential, defined as the yield of a crop cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting, with pests and diseases controlled (Evans, 1993), has always been a goal of maize breeders in the U.S. Corn Belt. Campos et al. (2006) evaluated 18 Pioneer-brand hybrids released during the period 1953 – 2000 and reported a steady improvement of maize yields under environmental conditions that enabled expression of yield potential as well as yield under drought stress. Genetic improvement was conditional upon plant population indicating that in order to increase yield potential it is necessary to improve tolerance to abiotic stress. Breeders have long been selecting for tolerance to biotic and abiotic stress (Cooper et al., 2014a; Duvick et al., 2004; Hammer et al., 2009). Recently, transgenic approaches have been investigated for opportunity to maintain or increase maize yield (Castiglioni et al., 2008; Chang et al., 2014; Guo et al., 2013; Habben et al., 2014).

Improvements in maize yield production must continue in both irrigated and rainfed maize to meet the dual demands of global consumption and sustainable production. Drought events during the growing season and throughout history have been common (Boyer et al., 2013; Stambaugh et al., 2011) and may become more widespread due to climate change. Yield gains of grain crops in major grain-growing regions have been stagnating or are in decline (Deepak et al., 2012; Grassini et al., 2013; Hall and Richards, 2012). Reasons for a pessimistic view of progress for yield increases of the major crops include lack of commercial breeding efforts in selected geographic areas resulting in yield stagnation (Barerro Farfan et al., 2013); decades-long periods between inception and farmer implementation of innovations; lack of infrastructure and markets in select regions where large productivity gains are possible; considerations for intellectual property rights; and inconsistent global regulations (Hall and Richards, 2012). The current and future challenge of production agriculture is to not only meet demand for grains, but to do so in an environmentally sound manner on land that is currently devoted to agriculture rather than by expanding the cultivated area (Cassman and Liska, 2007; Tilman et al., 2011; West et al., 2014).

Optimum® AQUAmax® hybrid development is one example of on-going efforts to maintain or improve yield potential under good growing conditions, minimize risk to growers when moisture is limiting, and help meet global food and feed needs in a sustainable system. From such long-term research efforts it is important to evaluate industry scale impact of the research. Therefore, the objectives of the work presented here were: first, to obtain an industry scale measure of realized yield improvement under water-limited (drought) and non water-limited (favorable) growing conditions for a set of hybrids developed through breeding and selection for superior yield performance under drought and similar or better yield performance under favorable conditions; second, to understand the interaction of the newly developed drought tolerant AQUAmax hybrids with the key grower management practice of manipulating plant populations for various moisture conditions.

MATERIALS AND METHODS

Development of AQUAmax hybrids

The set of drought tolerant AQUAmax hybrids evaluated in the studies that are the focus of this paper were developed by DuPont Pioneer commercial maize breeding programs operating in the Western region of the U.S. Corn Belt. Selection for superior yield under drought involved a combination of yield and agronomic testing in drought managed-environments and a wide range of rainfed and limited-irrigation conditions sampled on research stations and growers fields in the Western region of the U.S. Corn Belt. The resulting drought data sets for the target traits were used to construct a sequence of training data sets that enabled genomic prediction for all stages of the breeding programs (Cooper et al, 2014a, 2014b; Desta and Ortiz 2014; Heffner et al. 2009). The key trait targets for precision phenotyping in managed-environments and genomic prediction within the breeding programs included yield performance under drought and favorable conditions, and important agronomics and disease resistance traits for the environments of the Western region of the U.S. Corn Belt; with emphasis on seedling emergence under stressful growing conditions, resistance to brittle snap, and resistance to Head Smut [*Sphacelotheca reiliana* (Kühn) G. P. Clinton] and Goss's Wilt [*Clavibacter michiganensis subsp. Nebraskensis* (Vidaver and Mandel) Davis et al.]. Suitable training data sets for genomic prediction were created for all traits.

The experiments used to construct the drought and target environment training data sets were obtained over multiple years from managed-environment stations and at several rainfed and limited irrigation locations in the Western U.S. Corn Belt. Maximum control of water supply to create a wide range of drought conditions was achievable at two Pioneer Research Stations:

Woodland, California, USA and Viluco, Chile (Cooper et al., 2014a). At both of these managed-environment locations, combinations of water-limited and fully-irrigated treatments were created by managing the timing and quantity of irrigation applied to the experiments. Utilization of managed-environment locations in the Northern (Woodland) and Southern (Viluco) hemispheres enabled the generation of two cycles of drought testing each year.

All experiments contributing to the data sets reported here were characterized for timing and intensity of water-deficit; referred to as an environmental stress characterization. The degree of water-deficit was quantified using the water supply/demand ratio methodology introduced by Muchow et al. (1996) and used by Chapman et al. (2000), Chenu et al. (2011), Hammer et al. (2014) and Harrison et al. (2014). A water supply-demand ratio of 1.0 indicates that the environmental conditions are suitable to meet the transpiration demand of the crop. The ratio decreases to 0.0 as the water-deficit increases and the transpiration demand is not met. Here water limited environments (WLE) were defined as those where there was a water supply/demand ratio of 0.66 or lower for one or more days during the flowering and/or grain fill periods of development, and the average yield of the hybrids was below 9.5 Mg ha^{-1} Those environments where the average yield of the hybrids was above 11.4 Mg ha^{-1} were classified as favorable environments (FE). Environments that did not fall in either of these two classes were unclassified and excluded from the analyses, since we could not establish whether water or other biotic or abiotic constraints were the main factors limiting yield. To implement this classification we used the maize crop model embedded in the EnClass® system (Löffler et al., 2005). The crop model was parameterized for hybrid maturity relevant to the experiment and soil and weather data were used as inputs to the model to calculate phenology for the supply/demand ratio. The characterization of the environments as WLE or FE enabled a breakout of the environments into the two contrasting groups on the basis of the presence or absence of drought stress. Grain yield and other traits were then analyzed on the basis of the drought stress breakout, as explained previously in this paragraph.. Hybrids could then be advanced on the basis of yield and other agronomic traits performance under combinations of water-limited and favorable environmental conditions.

The inbred parents of all of the candidate hybrids under evaluation in the breeding programs were genotyped with Single Nucleotide Polymorphism (SNP) markers. The SNPs were distributed across the genome to represent the characterized founder haplotype structure present in the parents (Cooper et al., 2014b). Analysis of the trait phenotypic data and the molecular marker fingerprints within the training data sets was conducted to construct trait genomic prediction models for use in the breeding programs (Cooper et al., 2014b, Heffner et al. 2009). The prediction models for the traits obtained from the training data sets were combined with the SNP fingerprints of the hybrid parents to enable genome-wide prediction for the candidate hybrids for all target traits at all stages of the breeding programs (Cooper et al., 2014b). All hybrids predicted to have desirable combinations of the target traits and superior yield under drought conditions and parity or superior yield under favorable environmental conditions in comparison to the industry checks were advanced into the Targeted Drought Multi-Environment Trial (TD_MET). All advanced hybrids were evaluated in the TD_MET for four years prior to commercial launch to assess their yield performance in comparison to a set of industry leading check hybrids widely adopted by growers. Experimental hybrids were advanced between evaluation stages based on a combination of superior yield and agronomic trait performance and superior predicted performance for all traits in comparison to a set of industry check hybrids with maturity similar to the experimental hybrids. Those hybrids that met all the defined performance criteria were advanced to commercial release as AQUAmax hybrids.

Hybrid Yield in Multi Environment Trials

Undertaking an industry level comparison between the AQUAmax hybrids that were developed by the procedures described above and an appropriate set of industry leading non-AQUAmax hybrids as comparator checks is challenging. For this purpose we examined three multi-year, multi-location data sets that are described below. Combined, these three data sets allowed comparison of 78 AQUAmax hybrids with 4,291 non-AQUAmax hybrids selected by growers (Table S1) over a total of 11,300 locations (Table S2) covering the six-year period from 2008 to 2013. Figure 1 provides a graphical view of the distribution of the experimental locations across the U.S. mainland states. The actual numbers of experimental locations by state in each year for each data set are summarized in Table S2. Detailed maps of the locations of each experiment by year are also presented in Figures S1 to S9. The individual location-year combinations for the three data sets were all classified as either WLE or FE based on a combination of the modeled water deficit and mean grain yield, following the procedures described above. For the small plot research experiments (2008-2010) the hybrids that became commercial AQUAmax hybrids were compared against key industry hybrid check comparators (Table S1). Figure 2 provides a graphical view of the incidence of the AQUAmax and non-AQUAmax hybrids in experiments by state for each year. The majority of the comparisons between the AQUAmax hybrids and the industry lead comparators used by farmers were in the on-farm experiments conducted from 2011 to 2013. Further details of the three data sets are given below.

Small Plot Research

Experimental hybrids were evaluated by DuPont Pioneer in an extensive TD_MET that involved increasing numbers of locations (108, 222, and 666, respectively for 2008, 2009, and 2010) as the experimental hybrids moved through the advancement process. Locations were weighed heavily towards the states in the Western Region, including KS, North TX, CO, NE, SD, and CA, but also included locations in MO, IA, IL, TN, GA, NC, IN and MI. (Table S2 and Figures S1 to S3). Plots were two-row, four-row, or eight-row, all with 76 cm inter-row spacing, and plot length ranged from 5.3 m long to 12.1 m long, depending on the advancement level of the hybrids being evaluated. One or two replications were established at each location in a randomized complete block design. In the rainfed locations, stress timing and intensity were determined by the water holding capacity of the soil, the initial soil moisture, timing of natural rainfall and the water demand for evapotranspiration (ET).

Plant density ranged from 54,362 plants ha^{-1} to 88,956 plants ha^{-1}, depending on the intended stress level and standard densities used in the area of each location. Typically, the lower densities were used in rainfed locations where yield targets are lower, due to the limited water availability at those locations. In contrast, at locations where irrigation was used or historical rainfall is typically high, the higher plant densities were used to provide the opportunity for maximum yields. Rainfed in this paper means farming practices that rely on rainfall only, as opposed to applying supplemental irrigation water when the crop shows stress.

In addition to the experiments conducted in the U.S. Corn Belt states, the two managed-environment locations of Woodland and Viluco were used to generate a diverse range of water regimes, extending from severe water deficit to high-input favorable water-regime. The procedures used to design the experiments conducted at Woodland and Viluco were described by Cooper et al. (2014a). The managed-environment experiments used sub-surface drip irrigation systems to target specific water deficit timing and drought stress intensities, with resulting yields ranging from five to eight Mg ha^{-1}. These included stress treatments that bracketed the critical stages of flowering, grain fill, or both development periods.

Experiments were also placed at fully irrigated locations throughout the Western Region of the U.S. Corn Belt, where yields typically exceeded 12 Mg ha^{-1}. Other rainfed locations receiving adequate to excessive rainfall for optimum yields were also included and contributed to the favorable environment classification breakout component of the data set (Figures S1 to S3 and Table S2).

At all locations, plots were harvested (both rows of two-row plots, middle two rows of four-row plots, middle four rows of eight-row plots) with research combines where harvest weight and moisture were measured. All yields were adjusted to standard grain moisture of 15.5%.

Population Density Study

A population density study was conducted from 2010 to 2012 to investigate the density response of a sample of AQUAmax and non-AQUAmax hybrids (Tables S1 and S2 and Figures S4 to S6). Four-row research plots (76 cm spacing) were planted and stands were thinned to target densities as needed by V6. A total of 14 locations in the Western Region were used for this study as follows: six in 2010 (Figure S4), two in 2011 (Figure S5), and six in 2012 (Figure S6), with three to four replications at each location. A split-plot design was applied to the population density experiment with plant density as the factor for the whole-plot treatment and hybrid as the factor for the split-plot treatment. A randomized complete block design was applied to the whole-plot level of the experiment at each location in each year.

On-farm Strip Trials

On-farm strip trials were conducted from 2011 to 2013 to compare a sample of 78 AQUAmax hybrids to a large sample of 4,287 commercial hybrids used by farmers (Tables S1 and S2 and Figures S7 to S9). In these trials, the grower chose their own industry standard hybrids to use as the commercial checks on the farm under their management practices. One or more AQUAmax hybrids were planted adjacent to the grower selected check hybrids to compare performance. Typically, all hybrids at a location were similar in relative maturity to minimize maturity interactions with timing of drought stress.

At all locations, strip trials were harvested by the growers and grain weights were recorded using weigh wagons. All yields were adjusted to standard grain moisture of 15.5%.

STATISTICAL ANALYSES

Small Plot Research

Data from the study based on small plot research experiments were analyzed in two stages. In the first stage, data were analyzed for each location with hybrid and rep (if there is more than one replication) as fixed effects and first order autoregressive (AR1) spatial correlation structure for residual effects. In the second stage, the best linear unbiased estimates of hybrid effect from the first stage were analyzed by a linear mixed-model-with-hybrid-group (AQUAmax vs Non-AQUAmax hybrids), year (n=3), environment classification (WLE vs FE), and their interactions defined as fixed effects, and hybrid (within AQUAmax and within Non-AQUAmax groups), the interactions of hybrid with environment and hybrid with year, the three-way interactions of hybrid with environment and year, and heterogeneous variance of locations for each environment defined as random effects. Heterogeneous residual variances were also fitted for each environment. Therefore, the mixed model equation was,

$$\begin{aligned} Y_{ijklm} = \mathrm{u} + \mathrm{GROUP}_i * \mathrm{YEAR}_j * \mathrm{ENV}_k + \underline{\mathrm{HYBRID}_l} + \underline{\mathrm{ENV}_k * \mathrm{HYBRID}_l} \\ + \underline{\mathrm{YEAR}_j * \mathrm{HYBRID}_l} + \underline{\mathrm{ENV}_k * \mathrm{YEAR}_j * \mathrm{HYBRID}_l} + \underline{\mathrm{at}(\mathrm{ENV}_k) * \mathrm{LOC}_m} \\ + \underline{\mathrm{at}(\mathrm{ENV}_k) * \mathrm{LOC}_m * \mathrm{HYBRID}_l} \end{aligned}$$

where u is the mean; the fixed effects are denoted without underbars and random effects are denoted with underbars; the $\mathrm{GROUP}_i * \mathrm{YEAR}_j * \mathrm{ENV}_k$ term includes the main-effects of each term, their two-factor interactions and their three-factor interaction; GROUP is with two levels (AQUAmax vs Non-AQUAmax); ENV_k represents the environment grouping (WLE vs FE); LOC_m represent the location m; $\mathrm{at}(\mathrm{ENV}_k) * \mathrm{LOC}_m$ and $\mathrm{at}(\mathrm{ENV}_k) * \mathrm{LOC}_m * \mathrm{HYBRID}_l$ represent the heterogeneous variance of locations and residual interaction of hybrid with location for each environment grouping.

Population Density Study

Data from the population density study were analyzed using random regression models (Cai et al., 2011). The mixed model equation was,

$$Y_{ijkl} = \mathrm{u} + \mathrm{LOCY}_i + \mathrm{OP2} + \mathrm{LOCY}_i * \mathrm{GROUP}_j * \mathrm{OP2} + \underline{\mathrm{LOCY}_i * \mathrm{REP}_k} +$$

$$\underline{\mathrm{LOCY}_i * \mathrm{REP}_k * \mathrm{fac(POP)}} + \underline{\mathrm{LOCY}_i * \mathrm{REP}_k * \mathrm{HYBRID}_l} + \underline{\mathrm{LOCY}_i * \mathrm{HYBRID}_l * \mathrm{us(OP2)}} +$$

$$\underline{\mathrm{LOCY}_i * \mathrm{ROW}} + \underline{\mathrm{LOCY}_i * \mathrm{COLUMN}} + \underline{\mathrm{at(LOCY}_i) * \mathrm{ar1(ROW)} * \mathrm{ar1(COLUMN)}},$$

where u is the mean; the fixed effects are denoted without underbars and the random effects are denoted with underbars. For convenience of notation, LOCY represents the combination of each location and each year with i from one to 14; GROUP is with two levels (AQUAmax vs Non-AQUAmax); REP represents the replications in each location; OP2 represents the first three (0, 1, and 2) orders of orthogonal polynomials of population density; fac(POP) is population density factor; ROW and COLUMN are row and column numbers of the field grid; $\mathrm{at(LOCY}_i) * \mathrm{ar1(ROW)} * \mathrm{ar1(COLUMN)}$ represents the AR1*AR1 (autoregressive correlation with order 1) spatial residual correlation structure for each LOCY. Therefore, fixed effects included LOCY and OP2 for each combination of group with LOCY. Random effects included the REP by LOCY and its interactions with plant density factor and with hybrid, the interactions of LOCY with row and column factors in each location, and OP2 for each combination of hybrid with LOCY. An unstructured covariance model was fitted among intercept, linear, and quadratic terms of OP2. The two-dimensional spatial residual structure with AR1 correlation for both row and column directions (Butler et al., 2009) were fitted for each LOCY.

On-farm Strip Trials

Data from on-farm strip trials were analyzed both by years and across years using linear mixed models. The fixed effects of the model for the by-year analyses included group (AQUAmax vs Non-AQUAmax hybrids), maturity (early, medium, and late), environment, and their interactions. The random effects included in the model were hybrid (within AQUAmax and within Non-AQUAmax groups), the interaction of hybrid with environment, and heterogeneous variance of locations for each environment. Heterogeneous residual variances were also fitted for each environment in the by-year analyses. The mixed-model for the by-year analyses was,

$$Y_{ijklm} = \mathrm{u} + \mathrm{GROUP}_i * \mathrm{MAT}_j * \mathrm{ENV}_k + \underline{\mathrm{HYBRID}_l} + \underline{\mathrm{ENV}_k * \mathrm{HYBRID}_l} + \underline{\mathrm{at}(\mathrm{ENV}_k) * \mathrm{LOC}_m} + \underline{\mathrm{at}(\mathrm{ENV}_\mathrm{k}) * \mathrm{units}}$$

where u is the mean; the fixed effects are denoted without underbars and the random effects are denoted with underbars; ENV_k represents the environment contrast (WLE vs FE); MAT represents maturity; the term $\mathrm{GROUP}_i * \mathrm{MAT}_j * \mathrm{ENV}_k$ includes the main-effects of each term, their two-factor interactions and their three-factor interaction; LOC_m represents the location m; $\mathrm{at}(\mathrm{ENV}_k) * \mathrm{LOC}_m$ and $\mathrm{at}(\mathrm{ENV}_k) * \mathrm{units}$ represent the heterogeneous variance of locations and residuals for each environment.

Besides the fixed and random effects in the by-year analyses, the model for the across-year analyses included year and its interactions with all the fixed effects in the by-year model as additional fixed effects, and the two-way interaction of year with hybrid and the three-way interaction of year with hybrid and environment as additional random effects. The heterogeneous location and residual variances were also fitted for each combination of environment and year in the across-year analyses. The mixed-model for the across-year analysis was,

$$\begin{aligned} \mathrm{Y_{hijklm}} = \ & \mathrm{u} + \mathrm{YEAR}_h * \mathrm{GROUP_i} * \mathrm{MAT_j} * \mathrm{ENV_k} + \underline{\mathrm{HYBRID_l}} + \underline{\mathrm{ENV_k} * \mathrm{HYBRID_l}} \\ & + \underline{\mathrm{YEAR_h} * \mathrm{HYBRID_l}} + \underline{\mathrm{ENV_k} * \mathrm{YEAR_h} * \mathrm{HYBRID_l}} + \underline{\mathrm{at(YEAR\_ENV)} * \mathrm{LOC_m}} \\ & + \underline{\mathrm{at(YEAR\_ENV)} * \mathrm{units}} \end{aligned}$$

where for convenience of notation, YEAR_ENV represents the combination of each year and each environment; $\mathrm{at(YEAR\_ENV)} * \mathrm{LOC_m}$ and $\mathrm{at(YEAR\_ENV)} * \mathrm{units}$ represent the heterogeneous variance of locations and residuals for each combination of environment and year.

All statistical analyses were implemented in the ASREML-R package (Butler et al., 2009). The method of restricted maximum likelihood was used to estimate the variance components. A t-test was used to test the difference between the AQUAmax and Non-AQUAmax groups after the mixed model analyses. P-values in all three tables in the paper were reported from the t-tests.

RESULTS AND DISCUSSION

Small Plot Research

Results from the mixed model analysis of AQUAmax and Non-AQUAmax hybrid comparisons evaluated in both managed-stress and U.S. Corn Belt environments (TD_MET) across three years 2008-2010 are summarized in Table 1. The data set consisted of 53 WLE locations and 502 FE conditions (Table S2, Figures S1 to S3). The average yield across the FE locations was 12.88 Mg ha^{-1}, and across the WLE locations was 7.79 Mg ha^{-1}, representing a 40% reduction in yield due to the characterized water limitations. All hybrids were in a maturity range of 107-113 comparative relative maturity (CRM), thus maturity differences were discounted as a reason for yield difference among the hybrids. In the WLE, the AQUAmax hybrids (six hybrids, Table S1) yielded 7.96 Mg ha^{-1} compared to 7.59 Mg ha^{-1} for the industry leading checks (10 hybrids, DuPont Pioneer and competitor hybrids, Table S1). In the FE, the AQUAmax hybrids yielded

13.00 Mg ha^{-1} compared to 12.69 Mg ha^{-1} for the industry leading check group. Thus, there was no yield penalty in the FE for the AQUAmax hybrids. Based on meeting the dual criteria of yield advantage under drought conditions and no yield penalty with adequate moisture, the first set of AQUAmax hybrids was advanced to commercial production. Eleven AQUAmax hybrids were advanced in the fall of 2010, and these hybrids were first sold commercially in 2011 (Table S1). In 2012, a total of 24 AQUAmax hybrids were included in on-farm trials. Additional hybrids were advanced and by the 2013 growing season, 61 AQUAmax hybrids were commercially available. In order to properly evaluate the AQUAmax offerings available for the period under study, a total of 78 AQUAmax hybrids (including those that had already been withdrawn from the market by 2013) were included in the on-farm trials, (Table S1).

Due to the complexities of drought, including timing, intensity, duration, and interactions with soil type, no one hybrid should be expected to perform optimally 100% of the time, and AQUAmax hybrids did not always provide a yield advantage when compared to commercially available non-AQUAmax hybrids chosen by farmers. For example, Roth et al. (2013) evaluated a sample of AQUAmax hybrids in small-plot trials over two years at one location in Northwest aIndiana and found no yield advantage. Their result is within the distribution of results observed in the industry scale study reported here (Fig 3). Based on the cumulative results of on-farm and small-plot research, additional effort is needed in managed stress environments to understand better the complexities of timing, duration, and intensity of drought, as well as management options such as plant population, irrigation management and soil fertility on hybrid yield and agronomic performance (Cooper et al., 2014a).

Population Density Study

Research on plant population by hybrid interactions was conducted in 2010, 2011, and 2012 for a total of 14 location-year combinations (Table S2, Figures S4 to S6). Samples of AQUAmax and non-AQUAmax hybrids (Table S1) were compared at plant population densities ranging from 19,768 to 69,188 plants ha^{-1} (Table 2). The yield of both the AQUAmax and non-AQUAmax groups of hybrids increased for each incremental increase in plant population but at a decreasing rate up to 59,304 plants ha^{-1}. At 69,188 plants ha^{-1}, the AQUAmax and non-AQUAmax hybrids were equal in yield to 59,304 plants ha^{-1}, but with AQUAmax hybrids maintaining an approximate 8% yield advantage over the non-AQUAmax hybrids at the higher population densities. At each plant population density above 19,768 plants ha^{-1}, the yield of the AQUAmax hybrids increased by a greater amount than for the non-AQUAmax hybrids. In these WLE a final plant population density of 49,420 to 59,304 plants ha^{-1} was indicated as a target for optimizing productivity with the AQUAmax hybrids.

The differences between the hybrid groups in response to changes in plant population density in this study conform well to results from experiments conducted by Texas A&M AgriLife Extension (Becker et al., 2012). In that study in north Texas, water use, as estimated by seasonal ET, remained constant across hybrids. The implication of this observation is that water productivity of AQUAmax hybrids is higher when compared to a non-AQUAmax commercial check (Becker et al., 2012). Because soil water content was measured to a depth of only 1.2 m and root water extraction in similar soils was documented to occur below 2 m (Tolk, 1998) it is not possible to rule out potential differences in total water uptake between the groups of hybrids. However, a study conducted in managed-environments where soil moisture measurements were collected down to a depth of 3.2 m demonstrated no differences in total water uptake between an

AQUAmax hybrid and a non-AQUAmax commercial check hybrid (Cooper et al., 2014a). However, differences were observed between the hybrids in the temporal pattern of water use rather than in the total water use. With the change in temporal pattern of water use the AQUAmax hybrid reached flowering time under a more favorable soil water environment, and utilized a larger fraction of the total water use to support kernel growth, than the non-AQUAmax commercial check hybrid (Cooper et al., 2014a). Limiting transpiration at high vapor pressure deficit, commonly referred to as a limited-transpiration trait, can determine the differences in water use pattern observed between AQUAmax and the non-AQUAmax hybrids. Lobell et al. (2013) showed that the often observed relationship between air temperature and yield (Schlenker and Roberts, 2009) is related to variations in vapor pressure deficit rather than temperature effects on growth and development in maize. Simulation studies for sorghum (Sinclair et al., 2005) and soybeans (Sinclair et al., 2010) indicate that the limited-transpiration trait and the associated changes in patterns of water use can improve crop yields under water-limited conditions. Based on recent studies, the limited-transpiration trait is ubiquitous in Pioneer drought tolerant hybrids (Gholipoor et al., 2013; Yang et al., 2012). This result suggests that limited-transpiration can underpin, at least in part, the observed increased levels of drought performance of the AQUAmax hybrids relative to non-AQUAmax hybrids.

Evaluation in On-farm Trials

Continual advancement and expansion of the AQUAmax hybrids allowed the number of on-farm comparisons to grow from 2,231 in 2011 (Figure S6) to 4,341 (Figure S9) comparisons in 2013 (Table S2, Figures S6-S9). Over the three-year period of the on-farm studies, 10,731 comparisons were documented, with 2,006 WLE comparisons and 8,725 FE comparisons (Table

3 and Table S2 and Figures S6 to S9). The drought of 2012, which impacted broad geographies of the U.S., allowed for a 2:1 ratio of FE to WLE, and 1,380 WLE comparisons were obtained (Table S2, Figure S7). At these on-farm locations, AQUAmax hybrids were compared to hybrids chosen by the farmer in an attempt to compare the farmer's current best practice hybrids with AQUAmax hybrids. Similar maturities (±4 CRM) were utilized for all comparisons. For some locations, the farmer made multiple non-AQUAmax hybrid comparisons to the AQUAmax hybrids, and in all cases identical management practices were used for all comparisons.

The average yield in the WLE breakout was less than six Mg ha^{-1} in 2011 and 2012, and over 6.5 Mg ha^{-1} in 2013, an indication of the severity of water limitations in the WLE. Conversely, in the FE breakout the average yield was always greater than 13 Mg ha^{-1}. When averaged across all three years, yields in the WLE averaged less than half that in the FE. The AQUAmax hybrid yield advantage in the WLE was 0.36, 0.53, and 0.22 Mg ha^{-1} over non-AQUAmax hybrids in 2011, 2012, and 2013, respectively. The largest yield advantage, 0.53 Mg ha^{-1}, was observed in the 2012 season, when the greatest number of comparisons was possible due to the severe and widespread drought.

The AQUAmax yield advantage under FE conditions was 0.50 and 0.40 Mg ha^{-1} in 2011 and 2012, respectively. The 0.04 Mg ha^{-1} yield difference between AQUAmax and non-AQUAmax hybrids was not significantly different in 2013 in the FE. When all comparisons over three years were combined, the average yield of the AQUAmax hybrids was 0.37 and 0.25 Mg ha^{-1} greater than the non-AQUAmax hybrids in the WLE and FE environmental breakouts, respectively. The consistent yield advantage of the AQUAmax hybrids over the non-AQUAmax hybrids in research testing (Tables 1 and 2) and on-farm testing (Table 3) over multiple years and agronomic management options, particularly increased population density, demonstrated repeatable improvement of yield and yield stability with AQUAmax hybrids in WLE when evaluated at industry scale.

SUMMARY/DISCUSSION

Hybrid maize production has continued to increase over time due to a combination of intensive breeding and improvements in agronomic practices (Duvick, 2005; Smith et al., 2014). An example of this success is the average U.S. maize yield in 2012, a severe drought year, which was equal to average maize yields in a favorable growing season less than 25 years ago (Boyer et al., 2013; United States Department of Agriculture, 2013). Historically common and severe periods of drought and the uncertainty of the effects due to climate change will be a constant challenge to the agricultural production community. Ongoing improvements in yield potential of the germplasm, combined with implementation of best management practices to protect yield potential while conserving valuable resources of soil and water are needed. In the industry scale hybrid comparison studies reported here the yield of the AQUAmax hybrids was two to three percent higher than that of the leading checks when grown in favorable environments and five to nine percent higher when grown in water-limited environments. Under water-limited conditions, the AQUAmax hybrids had greater yield at nearly all plant densities above 29,652 plants ha^{-1}, with the greatest difference at plant populations between 44,478 and 59,304 plants ha^{-1}. Additional seed cost for higher density seeding rates is a common and realistic concern for growers and may often be considered an unneeded risk (Grassini and Cassman, 2012). Returns from higher seeding rates will depend on cost of seed and other inputs and on the price a farmer is able to obtain for commodity maize. However, because there was no yield penalty, and often an advantage, in favorable environments, AQUAmax hybrids offer farmers greater flexibility and more options for risk management under water-limited conditions, while retaining the ability to maximize yield potential when moisture is more plentiful.

The 7.3%, 9.7% and 3.3% average yield advantage in 2011, 2012 and 2013, respectively, with AQUAmax hybrids over hundreds of locations under WLE across the U.S. Corn Belt represents a realistic industry level assessment of realized yield improvement achieved with a resource-intensive and long-term breeding effort. The eight percent AQUAmax yield advantage under the dual stress of water limitation (WLE) and high plant populations offers genotype-by-management-by-environment optimization opportunities and direction for future research. The challenge will be to continue evaluation of new AQUAmax hybrids in as many environments and against as many other hybrids as possible in appropriate on-farm management scenarios. The value of multidisciplinary research efforts that are focused on the needs of growers is demonstrated. The use of managed-environment research and new tools of genomics, genetic prediction, crop modeling and advanced phenotyping will continue to create new genetic gain opportunities (Araus and Cairns 2014; Cooper et al., 2014b; Hammer et al. 2006; Tardieu and Tuberosa 2010). The use of transgenic traits with these technologies has potential for even greater water productivity as water becomes more valuable. For example, Guo et al. (2013), Habben et al., (2014), Castiglioni et al. (2008) and Chang et al. (2014), have reported on transgenic research with various levels of success. New research and knowledge, from both private and public sector programs, will be necessary to improve breeding schemes and hybrid development for the next generation of products. Yield increases and more efficient use of resources under stress-prone growing conditions are possible through the use of improved breeding techniques, incorporating sound management practices, and working closely with growers and public research specialists to understand and implement those practices at industry scale.

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Figure Captions

Figure 1. Map of North America emphasizing the U.S. Corn Belt, with a summary of the distribution of experiments across U.S. states. Each state that had at least one experiment

comparing AQUAmax and non-AQUAmax hybrids contains a pie chart indicating the number of experiments (n) and breakout of water-limited and favorable environments. Across the states the pie charts are scaled in proportion to the total number of locations sampled across the small plot research and on-farm strip experiments (N). The proportions of the total number of locations within a state that were characterized as either Water Limited Environments (WLE) or Favorable Environments (FE) are indicated by the color segments. For visualization purposes the radius of the pie chart was scaled by $(n/N)^{0.2}$.

Figure 2. Graphical summary of the incidence of AQUAmax and non-AQUAmax hybrids in individual experiments by state within year for the three data sets reported in the paper; small plot, population density and on-farm. The vertical dimension represents the individual hybrids in the same order as they are listed in Table S1. The horizontal dimension represents an ordering of states within year. The vertical dashed line indicates the transition from small-plot research experiments to on-farm strip testing. The horizontal dashed line indicates the transition from the AQUAmax to non-AQUAmax hybrids.

Figure 3. Distribution of yield differences between Optimum® AQUAmax® hybrids and non-AQUAmax commercial hybrids in small plot research experiments and on-farm strip trials under water-limited (WLE) and favorable environments (FE).

Supplemental Material Description

The supplemental tables and figures provide a listing of all of the AQUAmax and non-AQUAmax hybrids used in the studies reported in this paper and distribution of the experiments in which the AQUAmax and non-AQUAmax hybrids were compared over the duration of the studies.

Supplemental Table Captions

Supplemental Table 1. List of the names of hybrids, AQUAmax or non-AQUAmax designation, and their company of origin included in the three data sets corresponding to the analyses reported in Tables 1, 2 and 3 in the main text. For each data set, identified by the Table number, the years that the hybrids were included in the study is indicated by a “yes”.

Supplemental Table 2. List of total number of experimental locations by state and year for the three data sets corresponding to the analyses reported in Tables 1, 2 and 3 in the main text. For each year and state in each of the three studies the experimental locations are allocated into either Water Limited Environments (WLE) of Favorable Environments (FE). For completeness the Viluco station experiments in Chile are also included in the table summary.

Supplemental Figure Captions

Supplemental Figure 1. Map of North America showing the location of the small plot research experiments conducted in 2008.

Supplemental Figure 2. Map of North America showing the location of the small plot research experiments conducted in 2009.

Supplemental Figure 3. Map of North America showing the location of the small plot research experiments conducted in 2010.

Supplemental Figure 4. Map of North America showing the location of the research plant population density experiments conducted in 2010.

Supplemental Figure 5. Map of North America showing the location of the research plant population density experiments conducted in 2011.

Supplemental Figure 6. Map of North America showing the location of the research plant population density experiments conducted in 2012.

Supplemental Figure 7. Map of North America showing the location of the on-farm experiments conducted in 2011.

Supplemental Figure 8. Map of North America showing the location of the on-farm experiments conducted in 2012.

Supplemental Figure 9. Map of North America showing the location of the on-farm experiments conducted in 2013.

Table 1. Yield performance of Optimum® AQUAmax® hybrids (6 hybrids) compared to non-AQUAmax hybrids (10 hybrids) in small plot research experiments across three years (2008, 2009 and 2010) in multiple water-limited and favorable environments.

Environment	Number of locations	AQUAmax	Non-AQUAmax	Difference	Difference %	Pvalue
		------------- *Mg ha^{-1}* -------------				
Water-limited	**53**	**7.96**	**7.59**	**0.37**	**4.9**	**0.059**
Favorable	**502**	**13.00**	**12.69**	**0.31**	**2.5**	**0.000**

Water-limited locations were concentrated in NE, CO, KS, OK, North TX, CA and Chile. Optimum® AQUAmax® hybrids were tested against leading commercially-available competitor and DuPont Pioneer non-AQUAmax hybrids. For a full list of all hybrids see Table S1. Water-limited environments are those in which the water supply/demand ratio during flowering or grain fill reached a value of less than 0.66 for at least one day and in which the average yield of the check hybrids was less than 9.5 Mg ha^{-1}. The water supply/demand ratio is measured on a 0-1 scale (1 = adequate moisture, 0 = no water available to meet transpiration demand) using DuPont Pioneer’s proprietary EnClass® system. Precipitation for each experiment was measured either at the experiment location or the nearest weather station. These data were collected in DuPont Pioneer research trials and were used to advance the first class of Optimum® AQUAmax® hybrids for sale in 2011.

Table 2. Yield performance of Optimum® AQUAmax® hybrids (14 hybrids) across three years (2010, 2011 and 2012), compared to non-AQUAmax commercial hybrids (19 hybrids) across a range of plant populations in water-limited environments.

Plant population	AQUAmax	Non-AQUAmax	Difference	Difference %	Pvalue
Plants ha^{-1}	---------------	*Mg ha^{-1}*	--------------		
19,768	4.60	4.66	-0.06	-1.2	0.585
29,652	5.21	5.11	0.10	2.0	0.205
39,536	5.67	5.44	0.23	4.3	0.001
49,420	5.99	5.65	0.34	6.0	0.000
59,304	6.16	5.72	0.43	7.6	0.000
69,188	6.18	5.68	0.50	8.8	0.000

Data are from a total of 14 location-year combinations (Table S2, Figures S4 to S6). For a full list of all hybrids included see Table S1.

Table 3. Yield performance of Optimum® AQUAmax® hybrids (78 hybrids) compared to non-AQUAmax commercial hybrids (4,287 hybrids) in on-farm strip trials across three years (2011, 2012 and 2013) in multiple water-limited and favorable environments.

Year	Environment	Number of locations	AQUAmax	Non-AQUAmax	Difference	Difference %	Pvalue
			-------------	*Mg ha^{-1}*	-------------		
2011	Water-limited	271	5.26	4.90	0.36	7.3	0.006
	Favorable	1960	13.26	12.76	0.50	3.9	0.000
2012	Water-limited	1380	6.06	5.53	0.53	9.7	0.000
	Favorable	2779	13.75	13.35	0.40	3.0	0.000
2013	Water-limited	355	6.94	6.72	0.22	3.3	0.000
	Favorable	3986	13.63	13.58	0.04	0.3	0.221
All	Water-limited	2006	6.07	5.70	0.37	6.5	0.000
	Favorable	8725	13.45	13.21	0.25	1.9	0.000

Water-limited locations were distributed across multiple growing areas of the U.S. For a summary of the geographical distribution of the water-limited and favorable environment breakouts by state across years see Figure 1 and for a breakout by state by year see Table S2 and Figures S7 to S9. Optimum® AQUAmax® hybrids were tested against commercially-available leading competitor and DuPont Pioneer hybrids selected by growers. For a full list of all hybrids included see Table S1. Water-limited environments are those in which the water supply/demand ratio during flowering or grain fill was less than 0.66 for at least one day and in which the average yield of the hybrids was less than 9.5 Mg ha^{-1}. The water supply/demand ratio is measured on a 0-1 scale (1 = adequate moisture, 0 =

no water available to meet transpiration demand) using DuPont Pioneer’s proprietary EnClass® system. Precipitation for each experiment was measured either at the experiment location or the nearest weather station. These data were collected in farmers’ fields in side by side strip trials.

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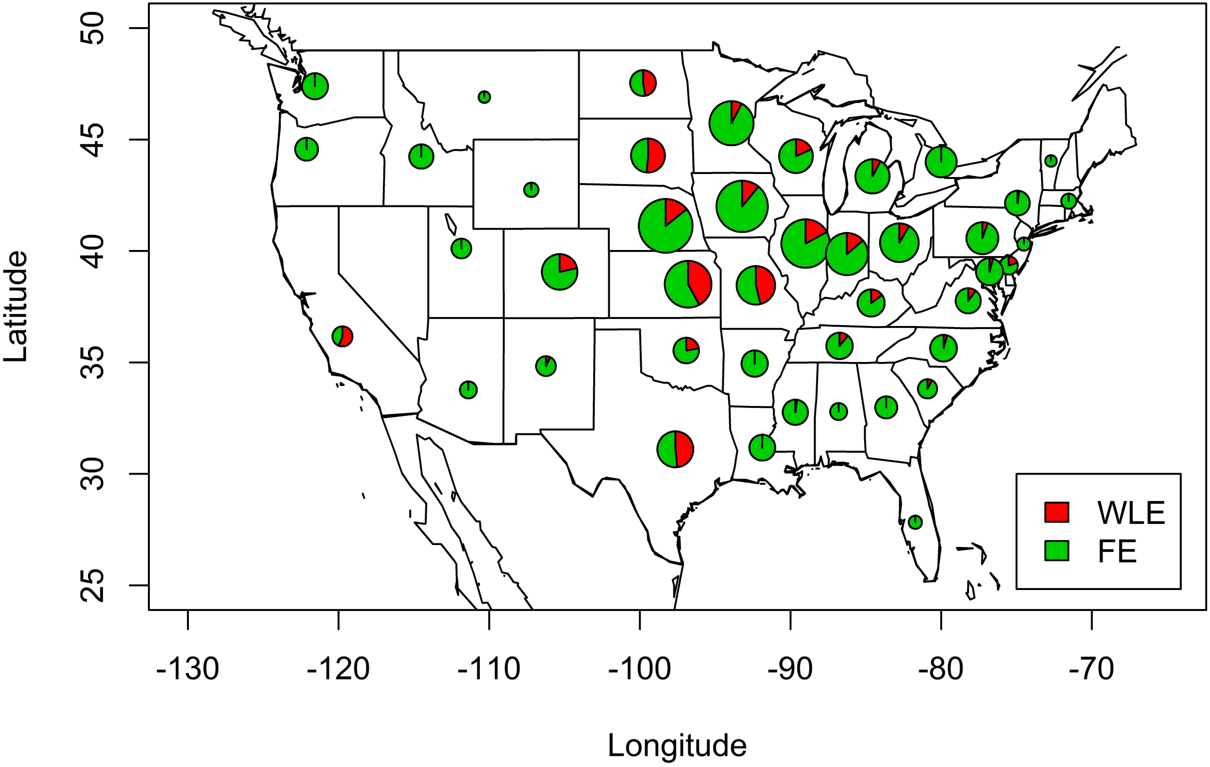

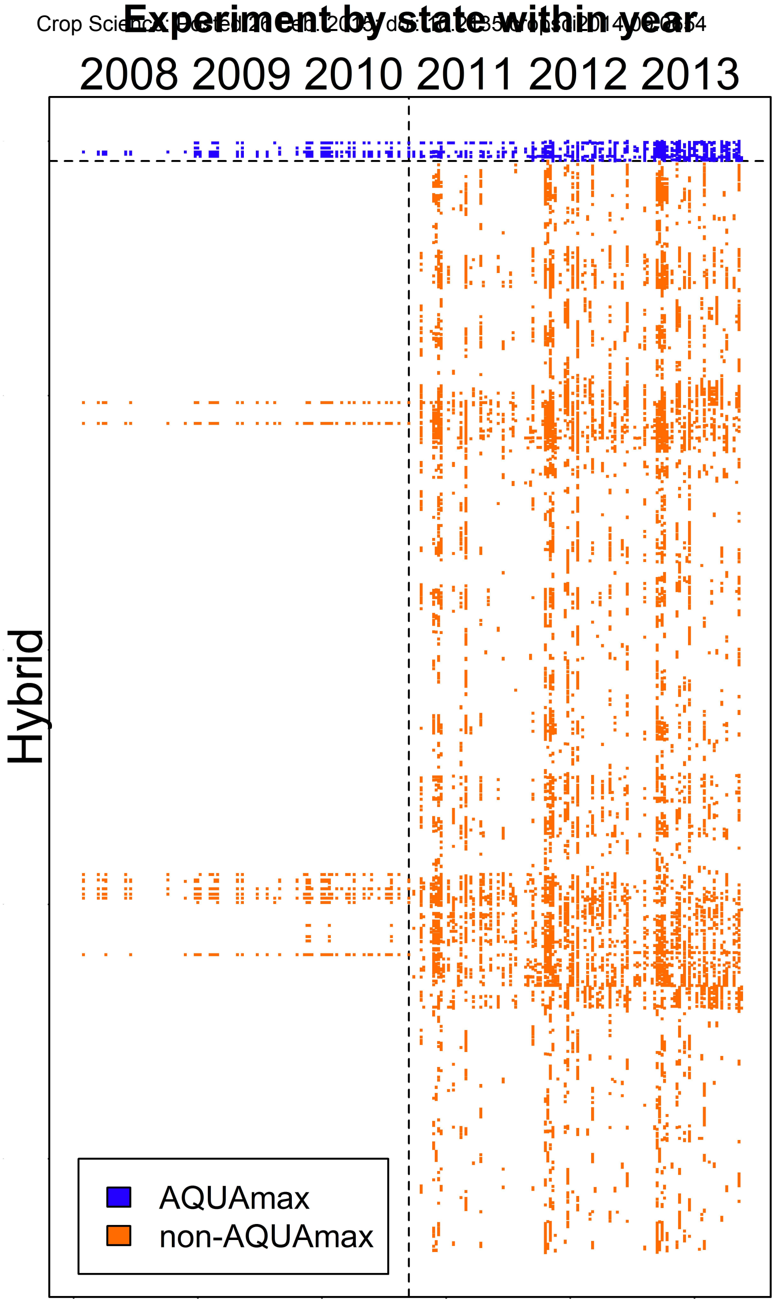
Experiment by state within year
2008
2009
2010
2011
2012
2013
Hybrid
AQUAmax
non-AQUAmax

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