

Agronomic and Physiological Responses of Modern Drought-Tolerant Maize (*Zea mays*
L.) Hybrids to Agronomic Production Practices

DISSERTATION

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ABSTRACT

The potential of climate change to impact crop production has increased farmer interest in new drought-tolerant maize (*Zea mays* L.) hybrids. However, limited research on the physiology and agronomic management of these hybrids has been published. Three separate field studies were conducted from 2012 through 2014 at Hoytville, South Charleston, and Wooster, OH to evaluate the physiological and morphological responses of two non-transgenic drought-tolerant hybrids (P0210 and P1352) and two conventional hybrids (P0448 and P1184) to nitrogen application rate (0, 67, 134, 202, and 269 kg N ha⁻¹), plant population (59,000, 74,000, 89,000, 104,000, and 124,000 plants ha⁻¹), planting date (May or June), and watering treatment (rainfed plus irrigation and water exclusion). Studies in which gas exchange was measured on P1352 and P1184 found drought-tolerant hybrid maintained or increased net photosynthetic rates relative to the conventional hybrids while reducing or maintaining a similar level of stomatal conductance. The ratios of chlorophyll fluorescence were greater for the drought-tolerant hybrid during the vegetative growth stages. The drought-tolerant hybrid also exhibited a greater leaf area index (LAI) and specific leaf area (SLA) as compared the conventional hybrid. The relative chlorophyll content (RCC) was lower in the drought-tolerant hybrids, and P1352 exhibited lower chlorophyll and ear-leaf N concentrations compared to P1184 as was hypothesized. The drought-tolerant hybrids also exhibited a shorter anthesis-

silking interval (ASI). The grain starch content was generally similar in the drought-tolerant hybrids compared to the conventional hybrids, but the drought-tolerant hybrids had greater oil content and less protein content than the conventional hybrids. All hybrids exhibited similar responses to population, including a decrease in RCC and an increase in ASI and LAI, and similar changes in grain protein, oil, and starch content. The grain yield at the agronomic optimum nitrogen rate (AONR) was similar regardless of hybrid (<5%), but the AONR was greater (3-11%) for the drought-tolerant hybrids. This higher N rate determined from regression analysis may be due to an increase in water use efficiency, which could decrease N use efficiency. The optimum plant population (OPP) was lower by 3,600 plants ha⁻¹ and maximum yield at the OPP was greater by 0.2 Mg ha⁻¹ for the drought-tolerant hybrids compared to the conventional hybrids when planted in May across all locations. When planting was delayed until June the maximum yield was 0.1 Mg ha⁻¹ less but the OPP was 16,400 plants ha⁻¹ less for the drought-tolerant hybrids compared to the conventional hybrids. When the conventional hybrid yield was less than 12.2 Mg ha⁻¹, a yield advantage was observed in 66% of our observations when a drought-tolerant hybrid of similar maturity was grown under identical conditions. However, when the conventional hybrid yielded greater than 12.2 Mg ha⁻¹, 60% of the time the drought-tolerant hybrid produced less grain than the conventional counterpart. These results suggest that the physiological traits of drought-tolerant hybrids may positively contribute to yield when yield potential is depressed, but under favorable conditions the traits of the conventional hybrid may be more advantageous for yield production.

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CHAPTER 1: LITERATURE REVIEW

Maize (*Zea mays* L.) is an important grain crop grown throughout the world, with 39.2 million ha planted in 2012 in the United States alone (USDA-NASS, 2012). Maize (also known as corn) is a monoecious warm-season grass originating in the tropical regions of Central America that has been domesticated as a source of food, and more recently, fuel, along with multiple other uses (Hoeft et al., 2000). The breeding process in the U.S. has allowed for marked improvements in the modern maize varieties that are commercially available over the open-pollinated maize varieties common in the early 1900's. Researchers in the early 1930's observed the offspring from crossed open-pollinated varieties could substantially improve plant vigor, beginning with a double cross, and eventually proceeding to a single cross hybrid that is planted today (Troyer, 2006). Researchers were able to improve maize yields through multiple selection events and inbred line development mainly by improving flowering synchrony and increasing kernel weight, rather than increasing the number of ears per plant, ear length, or harvest index (Hay and Porter, 2006). This process led to the development of three main heterotic groups from which most of the modern maize hybrids are developed in the U.S. (van Heerwaarden et al., 2012). The breeding process in the U.S. has further improved most recently by using genomic techniques to assist in the breeding process, and has produced

hybrids capable of withstanding stress conditions in an ever-changing climate (Edmeades et al., 2000; van Heerwaarden et al., 2012).

Current crop yield losses due to abiotic stresses have been estimated to be anywhere from 40 to 65% of the maximum potential yield (Bray et al., 2000). Multiple climate change models have predicted that precipitation events will become more sporadic as global temperatures rise (Lobell et al., 2008), which may result in longer and/or more frequent drought events during the growing season (Kunkel et al., 1999). One method of adapting to changing climatic conditions is using crops or varieties with increased tolerance to environmental stress (Wall and Smit, 2005), including drought stress. Multiple researchers have defined drought tolerance as a plant's ability to endure drought periods with low internal water levels, which differs from drought avoidance (high internal water levels during drought periods) (Levitt, 1972; Fischer and Maurer, 1978). Examining relative yield performance across both favorable and drought-stressed environments has been commonly used to select for drought tolerance traits (Clarke et al., 1992). One of the major objectives of breeding can be described as the ability to minimize the grain yield reduction under water stress conditions when compared to favorable growing conditions (Blum, 1973). Yield stability across drought-stressed and favorable environments has been a focus of previous breeding efforts (Finlay and Wilkinson, 1963; Cooper et al., 2014). Modern breeding approaches have also incorporated genetic evaluation and selection in addition to yield stability evaluation to further improve drought tolerance (Cooper et al., 2014). Some companies have relied on molecular-marker tools and streamlined data analysis in conjunction with phenotypic

screening to identify drought-tolerant maize lines, with improvements in leaf area duration, root architecture, and ability to flower during water stress suggested as contributors to yield stability under stress (Butzen et al., 2009). Other companies have focused on developing transgenic hybrids that constitutively express inserted cold-shock proteins from multiple bacterial species (Fernandez, 2010). However, there is little published research that identifies the physiological mechanism(s) underlying the drought tolerance of the hybrids developed using these advanced techniques (Roth et al., 2013).

Drought stress can be measured in multiple ways, but is related to plant water content. Leaf water potential (Ψ_w), or the chemical potential of water divided by the partial molal volume of water (Cosgrove and Holbrook, 2010), as described by Bray et al. (2000) is a common way to measure plant moisture status, and is the sum of four components:

$$\Psi_w = \Psi_m + \Psi_g + \Psi_p + \Psi_s$$

where Ψ_m is the matric potential (how solid surfaces interact with water), Ψ_g is the gravitational potential (gravity pulling on water; negligible at distances less than 5-10 m), Ψ_p is the pressure potential (physical forces such as tension will be negative, whereas the force of turgor will be positive), and Ψ_s is the solute potential (concentration of cytoplasmic solutes in water). In maize, the contributions of Ψ_m and Ψ_g are minimal, and are typically removed from the equation. Water content (fresh weight-dry weight/dry weight) can be calculated (Beadle et al., 1993), but the relative water content (RWC) as

described in Poorter and Garnier (2007) allows for a more accurate measure of plant water status and is calculated:

$$\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) * 100$$

where the fresh weight is the weight of the tissue removed from the plant, turgid weight is determined after the tissue sample has been floated on water at the light compensation point until a constant weight is reached, and dry weight is determined after tissue has been dried to remove the water from the tissue. While these methods for measuring tissue water content are destructive, non-destructive methods to measure plant water status are available by examining physiological process on living tissue, including measuring net photosynthesis rate [carbon assimilation per unit leaf area (PS_a) or per unit leaf mass (PS_m)], transpiration rate, stomatal conductance, and visual ratings for phenotypic drought response (e.g., leaf color change, leaf rolling) (Poorter and Garnier, 2007).

There are many physiological pathways that are activated by plants under drought stress. Some of these pathways are dependent on the plant signaling hormone abscisic acid (ABA), and others are activated independently of ABA (Shinozaki and Yamaguchi-Shinozaki, 1999). The products of genes influenced by ABA are categorized into two areas: 1) stress tolerance proteins or metabolites (e.g., chaperones, water channel proteins, small sugar transporters); and 2) signal transduction protein factors, such as the ABA-responsive element that upregulates ABA responsive genes (Shinozaki and Yamaguchi-Shinozaki, 1999; Yokota et al., 2006). Abscisic acid is produced from

carotenoids in root tip cells or vascular bundle parenchyma cells and is transported through the plant in the xylem alone or conjugated with sugars. In isohydric plants (such as maize) ABA is transported through the xylem from the roots (feedforward control), and the xylem sap pH is also increased which reduces the ability of the leaf mesophyll cells and the epidermal cells to sequester the hormone apoplastically (Lambers et al., 2008c). The hormone initiates multiple activities in stomatal guard cells to enable them to close, including the outflux of K^+ ions, Ca^{2+} release from vacuole to cytosol, and influences protein phosphorylation (Blatt et al., 1999; Yokota et al., 2006). Stomata can close even if the roots have sufficient water as the leaves sense vapor pressure difference, which is a form of feedback control (Assmann et al., 2000; Lambers et al., 2008c). As the stomata close, carbon reduction and oxidation decreases, electrons in photosystem I reduce oxygen when NADPH accumulates, the ATP/ADP ratio increases, and the pH of the lumen decreases (Yokota et al., 2006). As the pH lowers in the lumen, zeaxanthin is formed and prevents the energy from auxiliary pigments from entering the P680 reaction center of photosystem II by converting it to heat and fluorescence.

The responses that are ABA-independent include upregulation of dehydration responsive element binding transcription factors (DREBs). The DREB2 protein class is responsible for inducing many pathways to increase plant tolerance to high salinity and water stress (Liu et al., 1998; Lata and Prasad, 2011). Another response is the synthesis and accumulation of compatible solutes (also known as osmolytes or osmoprotectants), such as proline or glycine betaine (Yokota et al., 2006). These small molecules are important in drought response because: 1) they have high solubility in water and can act

as a substitute for water molecules released from leaves; 2) they can act as reducers of singlet oxygen species to minimize peroxide formation; 3) they help increase the osmotic pressure of the cell and allow it to retain its turgor pressure and prevent further water loss; and 4.) they may surround proteins and nucleic acids in the absence of water to help retain structural integrity by maintaining the hydrogen bonds. An additional mechanism for protecting against drought stress is through the production of heat shock proteins (Sharkey and Schrader, 2006). These proteins are thought to aid in protein folding and recovery of these proteins after stress and maintaining membrane stability (Wang et al., 2004).

In addition to changes in physiology due to drought stress, plants may also respond morphologically. Previous literature has demonstrated that morphological traits correlated to yield under stress conditions have varied by inbred line and hybrid (Bolaños and Edmeades, 1996; Cox, 1996), and response to water stress is more prominent in aboveground tissue as compared to the roots (Lambers et al., 2008c). Morphological parameters examined in previous studies have included ASI, leaf senescence after pollination, plant height, and leaf number, but there has been limited emphasis on physiological parameters such as transpiration rate, leaf water potential, stomatal conductance, and chlorophyll fluorescence (Bolaños and Edmeades, 1996; Edreira and Otegui, 2012; Escobar-Gutiérrez and Combe, 2012). Adaptive changes have occurred for some plants over time, but plants can also respond to stress through avoidance or tolerance mechanisms (Bray et al., 2000). Plants grown under moisture stress can have smaller stomata at a greater density that respond more rapidly to changes in humidity

(Yokota et al., 2006). Non-permanent leaf rolling can occur when stomata are closed by lowering the relative water content of bulliform cells, and can help protect the photosystems from photooxidative damage (Yokota et al., 2006; Lambers et al., 2008c). The relative growth rate (RGR) of plants is also influenced by environmental conditions (Poorter and Garnier, 2007). Higher RGR can be observed under low levels of stress, but moisture or nutrient stress can decrease RGR. This change is largely caused by alterations in the specific leaf area (SLA), or the ratio of leaf area to leaf mass.

Water stress can reduce the assembly of amino acids into proteins and inhibit auxin production, which can limit cell enlargement and result in plants with smaller growth habits (Treshow, 1970b). Environmental stress can cause SLA to be negatively related to leaf lifespan, and plants with lower SLA tend to be more competitive in stressed environments (Poorter and Garnier, 2007) and have lower levels of leaf N (Lambers et al., 2008b). Smaller leaves or leaves experiencing rolling could reduce the radiation use efficiency (change in dry matter per unit photosynthetically active radiation absorbed per unit time), and ultimately limit grain yield by reducing photosynthate production during grain fill and the harvest index (Earl and Davis, 2003). Leaf retention after pollination in maize, while related to nutrient status (Cirilo et al., 2009), is also related to heat stress and moisture availability (Edreira and Otegui, 2012; Escobar-Gutiérrez and Combe, 2012).

Chlorophyll fluorescence can also indicate moisture stress prior to expression of visually observable symptoms (Escobar-Gutiérrez and Combe, 2012). Dividing the variable fluorescence (F_v), calculated by subtracting the minimal initial fluorescence at

open PSII centers (F_o) from the maximal fluorescence at closed PSII reaction centers (F_m), by F_m provides a ratio that correlates well with photoinhibition (Bolhàr-Nordenkampf and Öquist, 1993), which can be observed at moderate or low light if photosynthesis is restricted by another stress. Fluorescence of PSII should be examined because activity of PSI can continue during stress because of cyclic electron transport (Yokota et al., 2006). The quantum efficiency of PSII (Φ_{II}) can be measured under light adapted conditions by subtracting the steady-state fluorescence (F_s) under light from the maximum fluorescence after a pulse of saturating light (F_m') and dividing by F_m' , and has been shown to relate to leaf photosynthetic capacity (Earl and Tollenaar, 1999). Alterations in photosynthetic efficiency related to elevated temperature are due to slower enzymatic rates in the “dark reactions” and because of an increased affinity of RuBisCO for oxygen, which can increase photorespiration (Lambers et al., 2008b). In lower leaves of the plant canopy where light may be limiting, the C₄ carbon concentrating method will decrease but the biochemical efficiency (the ATP cost of gross assimilation) is maintained at low light (Bellasio and Griffiths, 2014). Similarly under drought conditions, plants will reduce their photosynthetic capacity in order to maximize both the dark and light reactions (Lambers et al., 2008b).

While breeding has been the main source of increased maize yields in the U.S., the second most impactful process to increase yields over time has been agronomic management (Duvick, 2005). Managing fertilizer application, plant density (number of plants per hectare), and planting date all affect tolerance of maize to stress (Cox, 1996; Otegui and Melón, 1997; Cirilo et al., 2009). The addition of phosphorus fertilizer has

been shown to influence stomatal density in cowpea (Sekiya and Yano, 2008), which can influence gas exchange and water use. In the post-World War II era, on-farm synthetic fertilizer use increased dramatically, with 97% of the U.S. maize hectares being fertilized in 2010 with nitrogen (USDA-NASS, 2011). However, the increase in fertilizer use has led to unintended environmental consequences. Hypoxia in the Gulf of Mexico has been partially attributed to nitrate leaching from agricultural fields in the Midwestern agricultural states (Turner and Rabalais, 2003). One method of reducing off-target nitrogen losses has been to breed for increased nutrient use efficiency in crops (Below and Haegerle, 2012).

Retaining leaves is essential to the grain fill period (Cirilo et al., 2009), and drought-tolerant hybrids may be able to delay leaf senescence to allow for a longer grain fill period. Maximum photosynthetic assimilation increases with leaf N per unit area (Lambers et al., 2008b), and decreasing leaf N can inhibit the plant's ability to synthesize chlorophyll *a* and *b* (Crawford et al., 2000). More chlorophyll *b* may enable more efficient light capture in the light harvesting protein complexes, which is the first step in photosynthesis (Eggink et al., 2001). Altering pigmentation could reduce the plant's photosynthetic ability as well as its ability to defend against photooxidation, resulting in more rapid leaf senescence. Maize hybrids with high nitrogen use efficiency in sub-Saharan Africa exhibited a shorter ASI (enhanced flowering synchrony) and less leaf senescence after pollination compared to hybrids with less efficient nitrogen use (Worku et al., 2012). Rossini et al. (2012) attributed lower yields from nitrogen deficiency to an

inability of the plant to allocate nitrogen into developing grain, which increased kernel abortion.

Increasing root depth or changing rooting angle can lengthen the grain fill period because of the increased access to deeper soil water reserves (Hammer et al., 2009), and if drought-tolerant maize hybrids produce large root systems with a steeper rooting angle that penetrate deeply into the soil profile they may be able to access more soil moisture and increase the amount of nitrate absorbed in the soil solution (Havlin et al., 2005). A higher root mass ratio (RMR) has been observed in plants adapted to low soil moisture conditions, but C₄ plants have lower RMR as compared to C₃ plants (Lambers et al., 2008c). However, water use efficiency and photosynthetic nitrogen use efficiency have been shown to be inversely related (Lambers et al., 2008b). Potassium and calcium levels in the plant may also influence its ability to withstand drought due to their roles in stomatal control and plant osmotic potential (Epstein and Bloom, 2005). The transpiration rate and stomatal conductance may influence nitrogen uptake by changing the amount of water absorbed, and therefore the amount of nitrate absorbed by the roots. Nitrate can influence carbon assimilation by redirecting metabolic pathways to produce amino acids containing nitrogen by upregulating phosphoenolpyruvate carboxylase rather than starch by down-regulating ADP glucose pyrophosphorylase (Crawford et al., 2000). If drought-tolerant hybrids more efficiently absorb applied nitrogen from the soil or can use nitrogen more efficiently, the optimum nitrogen application rate may be changed with these hybrids.

Another aspect of agronomic management is plant density. The number of plants within a unit area, or plant density, can drastically affect plant production both on a field basis and a per plant basis. The plant density necessary to maximize maize grain yield per unit area has increased over time, with current recommendations at between 81,000 and 89,000 plants ha⁻¹ (Butzen and Jeschke, 2014). However, yield per plant can decrease as intraspecific competition increases as a function of plant density (Weiner, 1990). This may be explained by a decrease in plant height that occurs as density increases, and because the total leaf area per unit ground area, or leaf area index (LAI), does not increase at the same rate as plant density (Cox, 1996; Boomsma et al., 2009). Stomata are responsive to light (blue light stimulates stomatal opening) and intercellular CO₂ concentration (Lambers et al., 2008c). Both blue light and internal CO₂ levels can be influenced by plant density, and may influence photosynthesis and transpiration. Increasing plant population can affect the light quantity absorbed by the canopy (Van Roekel and Coulter, 2011), which could reduce the quantity and quality of light reaching lower leaves during the grain fill period. Decreases in irradiance through the plant canopy can be explained using an exponential equation (Lambers et al., 2008b):

$$I = I_0 e^{-kL}$$

where I is the irradiance below the canopy, I_0 is the irradiance above the canopy, k is the extinction coefficient (dependent on leaf angle, and is typically 0.3-0.5 for grasses), and L is the LAI. Under low light conditions, photosynthesis is inhibited and leaves that

develop in shade have fewer chloroplasts than those that develop in full sun (Treshow, 1970a), but plants will optimize the efficiency of carbon assimilation when adapted to low-light conditions ($<600 \mu\text{mol m}^{-2} \text{s}^{-1}$; Bellasio and Griffiths, 2014). The ratio of red to far red light could influence phytochrome activity, and at higher plant densities could trigger a shade avoidance response (Lambers et al., 2008a). Shaded leaves and plants typically have a lower chlorophyll *a:b* ratio because there are fewer PSII systems relative to PSI and/or the light harvesting complexes may be larger with a low chlorophyll *a:b* ratio (Porra, 2002), which may also occur in maize grown at high plant densities.

Additionally, the light within the canopy can be variable which can lead to differences in individual leaf contributions to yield (Treshow, 1970a), as was observed by Tanaka and Yamaguchi (1972) in maize. Leaves above the ear that were removed resulted in significantly lower grain yield as compared to when leaves below the ear were removed, suggesting the leaves above the ear are contributing to carbohydrate accumulation more than the leaves below the ear. The increasing grain yield is not due to remobilization of carbon resources, but rather is due to the direct transport of fixed carbohydrate to the sink from the five leaves at or above the ear. In addition to light, CO_2 within the canopy may also be limiting (Treshow, 1970a). Research has also demonstrated longer ASIs can occur as densities increase over 79,000 plants ha^{-1} (Boomsma et al., 2009; Otegui, 1997), leading to asynchrony in pollen shed and silk emergence. Most of the studies examining plant density effects on physiology demonstrated hybrids differed in their response to density. Increasing plant density will increase intraspecific competition for resources including water, nitrogen, and light, and

the drought-tolerant hybrids may be able to maintain yields at higher plant populations as compared to other maize hybrids grown under similar situations. Butzen et al. (2009) mentions drought-tolerant hybrids may silk more quickly under water stress conditions, which may reduce asynchrony in pollination and improve yields at higher plant densities.

Altering planting date can prevent stresses that may occur early in the growing season, but may result in greater stress during flowering. Previous studies have demonstrated the loss of growing degree days due to late planting can be offset by adequate weather conditions (Imholte and Carter, 1987; Otegui et al., 1995; Otegui and Melón, 1997), even when delayed by up to three months. However, grain yield can be reduced 25 to 30%, depending on tillage, as heat unit accumulation before and/or after silking decreases (Imholte and Carter, 1987; Hoefl et al., 2000). Early-season heat unit accumulation may also influence maize grain yield in cooler climates (Van Roekel and Coulter, 2011). Delaying planting date may decrease the number of leaves produced (Birch et al. 1998) and total biomass of maize (Tsimba et al., 2013), but planting date effects on LAI are more variable (Maddoni and Otegui, 1996; Van Roekel and Coulter, 2011). Delaying planting in a cool year may decrease grain yield for all hybrids, but the magnitude of the decrease can vary by hybrid because of differences in their time to physiological maturity (Nielsen et al., 2002). Maize hybrids will differ in the number of heat units required to achieve physiological maturity (Hoefl et al., 2000). Physiological maturation can be determined by measuring the number of growing degree days accumulated prior to silk elongation or prior to physiological maturity, using 10°C as the minimum and 30°C as the maximum temperature (Hoefl et al., 2000).

As temperatures rise and precipitation events become more erratic in Ohio due to climate change, growers will need to use management strategies to minimize their risk in crop production. Evaluating drought-tolerant maize hybrids in Ohio will provide growers the necessary information to make informed decisions about using these as a risk management tool. Much of the research examining plant characteristics associated with drought tolerance has been conducted on tropical maize lines in the Southern hemisphere or tropical environments (Bolaños and Edmeades 1996; Otegui, 1997; Otegui and Melón, 1997), and limited work has been conducted with commercially available hybrids in the U.S. (Boomsma et al., 2009; Roth et al., 2013). While tropical maize lines and U.S. developed hybrids are related, their response to stress conditions may not be consistent due to their different genetic backgrounds (Parentoni et al., 2001; Edreira and Otegui, 2012). Most of the drought-tolerant hybrids have been developed for use in the Western U.S. Corn Belt. There is very limited research on these hybrids in the Eastern U.S. Corn Belt where interest in drought-tolerance is increasing. The drought-tolerant hybrids should be evaluated for yield stability in non-stressed environments in addition to drought conditions. There may be a yield penalty associated with drought tolerance traits when growing conditions are adequate for susceptible hybrids to succeed (Bolaños and Edmeades, 1996; Cattivelli et al., 2008). Evaluating these hybrids under both adequate moisture and drought conditions will elucidate any yield advantage or penalty associated with drought tolerance, and help determine proper agronomic management practices to maximize grain yield. Maximizing the efficiency of fertilizer inputs is also important to minimize the environmental impacts of agricultural production, and drought-tolerant

maize hybrids may enable growers to achieve the goal of maximizing yield while minimizing loss. This research will help establish Ohio as a global leader in agricultural sustainability by decreasing fertilizer applications and using management tools to mitigate some of the challenges that will occur because of climate change.

This research will allow growers to maximize grain yield of drought-tolerant hybrids in Ohio by manipulating planting date and density. Additionally, this investigation will help minimize the negative environmental impacts of management by measuring the lowest nitrogen fertilization rates necessary to achieve the maximum yield of drought-tolerant hybrids in Ohio. This research will also help to identify traits that can be targeted by breeders during phenotypic evaluations to select for future hybrids that will perform well in Ohio. **The overall hypothesis of this research is that drought-tolerant hybrids will produce greater yield than conventional hybrids under drought conditions without suffering a yield penalty under adequate growing conditions, and will be more tolerant of high plant densities by (i) reducing transpiration rates during stress periods throughout the season and (ii) by altering leaf characteristics to maximize photosynthetic efficiency.**

CHAPTER 2: DROUGHT-TOLERANT MAIZE (*ZEA MAYS* L.) HYBRID RESPONSE TO NITROGEN APPLICATION RATE IN OHIO

INTRODUCTION

Annual crop yield losses due to abiotic stress have been estimated to range from 40 to 65% of the maximum potential yield (Bray et al., 2000). Several climate change models predict that as global temperatures rise, changes in precipitation frequency will be geographically dependent (Lobell et al., 2008). Changes in distribution of precipitation and increases in extreme rainfall events has been occurring in the Upper Midwestern U.S. (Morton et al., 2015), and these changes could reduce infiltration and may result in longer and/or more frequent drought events (Kunkel et al., 1999). One method of adapting to changing climatic conditions is by planting varieties with increased tolerance to environmental stress (Wall and Smit, 2005), including drought stress. Drought tolerance, which has been defined as the ability of a plant to endure drought periods with low internal water levels (Levitt, 1972; Fischer and Maurer, 1978), has historically used yield stability across favorable and stressed environments as a selection parameter (Finlay and Wilkinson, 1963). A new generation of drought-tolerant maize hybrids is currently being marketed in the United States from multiple companies. These hybrids have been developed from both improved selection techniques (Cooper et al., 2014) and through

transgenic efforts (Deikman et al., 2012). While used widely in the Western U.S. Corn Belt, there is little published research investigating the agronomic production requirements for these hybrids in the Eastern U.S. Corn Belt as well as the physiological mechanism contributing to the observed tolerance (Roth et al., 2013).

Managing agronomic factors such as fertilizer application, plant population (number of plants ha^{-1}), and planting date can affect the tolerance of maize to stress (Cox, 1996; Otegui and Melón, 1997; Cirilo et al., 2009). In the post-World War II era, on-farm synthetic fertilizer use increased dramatically, with 97% of the U.S. maize crop being fertilized in 2010 with N (USDA-NASS, 2011). However, the increase in fertilizer use has led to unintended environmental consequences. Hypoxia in the Gulf of Mexico has been partially attributed to nitrate leaching from agricultural fields in the Midwestern agricultural states (Turner and Rabalais, 2003). One method of reducing off-target N losses is to utilize varieties with improved nitrogen use efficiency in systems with optimized N rates applied in the proper form at the proper time and placement for crop use (Raun and Johnson, 1999; Fageria and Baligar, 2005).

Modern drought-tolerant hybrids should be evaluated for their yield response to N application because water use efficiency and photosynthetic N use efficiency are inversely related (Fredeen et al., 1991). Differences in soil moisture at flowering through grain fill indicate drought-tolerant hybrids may utilize water differently than conventional hybrids (Cooper et al., 2014). Additionally, plants with lower specific leaf area (SLA, ratio of leaf area to leaf mass) tend to be more competitive in stressed environments (Poorter and Garnier, 2007) and have lower levels of leaf N (Lambers et al., 2008b).

Decreasing leaf N can inhibit the plant's ability to synthesize chlorophyll *a* and *b* (Crawford et al., 2000), which could reduce the plant's photosynthetic ability as well as its ability to defend against photooxidation. Smaller leaves may limit grain yield by reducing photosynthate production during grain fill and harvest index (Earl and Davis, 2003). The overall N requirement for drought-tolerant hybrids may be lower than for more drought-susceptible hybrids because leaf N and area may be less, but this may also result in less yield. Additionally, hybrid maturity should be evaluated because previous research in Indiana found short-season maize hybrids (≤ 105 d) are less responsive to N application compared to longer-season hybrids (Tsai et al., 1984, 1992).

Nitrogen uptake and utilization can influence grain quality. Previous research from Ohio and Indiana observed that grain protein increased with N application, but starch content decreased (Tsai et al., 1992; Thomison et al., 2004). Additionally, grain protein content can be influenced by environmental stress (Genter et al., 1956). If the N requirements differ for drought-tolerant hybrids, the grain quality profile may differ from conventional hybrids resulting in changes that may be of interest to processors.

Drought-tolerant hybrids may have a lower overall yield potential under adequate growing conditions, or a differential response to N application compared to conventional hybrids. Additionally, different maturities of drought-tolerant maize hybrids may respond differently to N application. The objective of this study was to evaluate the response of drought-tolerant hybrids to N application compared to conventional hybrids, and evaluate the effect on ear-leaf N and grain protein, oil, and starch content.

MATERIALS AND METHODS

Study Sites and Experimental Design

A field experiment was established in 2013, and repeated in 2014 at the Northwest Agricultural Research Station (NWARS) in Hoytville, OH (41°13'N, 83°45' W; 212 m elevation), and the Western Agricultural Research Station (WARS) in South Charleston, OH (39°51'N, 83°40' W; 333 m elevation). The soil at NWARS was a Hoytville clay loam (fine, illitic, mesic Mollic Epiaqualfs) in 2013 and a silty clay loam in 2014. The same field was used in both years at WARS and was characterized as a Kokomo silty clay loam (fine, mixed, superactive, mesic Typic Argiaquolls). Soil chemical properties are listed in Table 1. Crop staging was conducted using the method described by Abendroth et al. (2011). The experiment was conducted as a randomized complete block design with four replications at each location.

Treatment design was a full factorial of hybrid with four levels (Table 2) and sidedress N application rate with five levels (0, 67, 134, 202, and 269 kg N ha⁻¹). The hybrids were all Pioneer® brand, and were selected because of their relative maturities (RM) and drought tolerance. P0210 and P0448 were selected as an early maturing hybrid pair for Ohio environments, and P1184 and P1352 were selected as a later maturing pair. One of the hybrids within each pair was a non-transgenic drought-tolerant hybrid marketed as an Optimum® AQUAmax® hybrid, and had a greater drought tolerance rating from Pioneer as compared to the conventional counterpart (9 to 1 scale, 9 = high drought tolerance). The hybrids were also rated by Pioneer for susceptibility to mid-season brittle stalk (9 to 1 scale, 9 = minimal brittle stalk).

The field at NWARDS was planted on 6 May following fallow in 2013 and on 13 May following winter wheat in 2014. At WARS, the plots were planted following maize each year on 15 May 2013 and 20 May 2014. Plots at NWARDS (12.2 x 3.1 m in 2013 and 19.8 x 3.1 m in 2014) consisted of four maize rows (76-cm spacing) and were planted using a four-row maize planter to 77,000 plants ha⁻¹. At WARS in both years, plots (12.2 x 3.1 m) were four rows (76-cm spacing) planted at 89,000 plants ha⁻¹. To achieve the target populations, stand counts were collected at V4 on all rows and were thinned as necessary to achieve desired plant populations. Management strategies to minimize interference from weeds and insects were implemented each year.

The five levels of sidedress N application rate were selected to provide adequate resolution for a yield response curve, and ranged from a rate that should cause deficiency symptoms (0 kg N ha⁻¹) to a rate that should be N non-limiting for most Ohio environments (269 kg N ha⁻¹). Sidedress applications were made at the V4 growth stage using 28% urea-ammonium nitrate (28N-0P-0K). Pre-sidedress soil nitrate test (PSNT) samples were collected prior to sidedress to measure initial soil nitrate levels by homogenizing six 30-cm deep cores for each plot (Magdoff, 1991). End of season soil nitrate samples were collected by homogenizing six 20-cm cores per plot.

Plant Growth, Grain Yield and Grain Quality Measurements

Aboveground plant growth was measured at the V7 and R2 growth stages. At V7, plant height was measured to the uppermost extended leaf on four plants per plot, and plant height was measured to the uppermost leaf collar at R2. Ten ear-leaf samples were

collected from the harvest rows of each plot at the R2 growth stage, dried for 7 d at 60°C, weighed for biomass measurement and analyzed using the total combustion method for ear-leaf N concentration and content. On 10 July 2013, a severe storm producing straight-line winds created brittle snap damage and root lodging at both locations. Brittle snap is defined as complete stalk breakage below the primary ear shoot, and root lodging is defined as lodging caused by the inability of the root system to maintain plant erectness. Counts of root lodged and brittle snapped plants were collected one week after the wind storm. Relative chlorophyll content was measured non-destructively on ten plants per plot at V7, R2 and R5 using a SPAD 502c meter (Konica Minolta, Chiyoda, Japan). All plots were compared to the treatment with the highest SPAD value within each environment (site- year). At V7, the uppermost fully collared leaf was measured, but at R2 and R5 the uppermost ear leaf was measured.

Approximately 1 to 3 wk following R6, stalk nitrate samples were collected from each plot (Binford et al., 1990). Eight 20-cm stalk samples were cut starting 30 cm above the soil surface per plot, and analyzed for stalk nitrate content. All plots were visually rated for stalk lodging (stalk breakage below the ear) at harvest. Grain yield was collected from the center two rows of each plot using a combine, and grain moisture at harvest was also collected. Reported yields have been adjusted to 155 g kg⁻¹ moisture. Subsamples of the grain from each plot was evaluated for protein, oil, and starch content using an Infratec NIR grain analyzer (Foss Tecator, Höganäs, Sweden) and are reported on a dry matter basis.

Statistical Analysis

Data were analyzed using PROC MIXED in SAS 9.4. Each site-year (environment, E) was analyzed as a fixed factor to observe consistent treatment effects across environments. Each significant E x factor interaction was investigated to identify the cause of the interaction. End of season soil nitrate, stalk nitrate levels, percent brittle snapped plants and percent root lodged plants were log-transformed to meet the normality assumption, and data are presented as the untransformed means with the transformed statistical interpretation. When the Global *F*-test was found to be significant ($\alpha = 0.05$), means separation was conducted using Fisher's protected LSD. Single degree of freedom contrasts were conducted to compare drought-tolerant maize hybrids (DT) to the conventional hybrids (Conv.). Linear regression was used to investigate the effect of N application on brittle snap at NWARS in 2013 using the REG procedure, with slope comparisons made using single degree of freedom contrasts. Grain yield response curves to determine the agronomic optimum N rate (AONR), economic optimum N rate (EONR), agronomic optimum maximum yield (AOMY) and economic optimum maximum yield (EOMY) were generated using a quadratic plus plateau model as previously described by Bast et al. (2012) in Ohio environments using the NLIN procedure. The CORR procedure was used in SAS to generate Pearson's correlation coefficients to compare grain protein, oil, and starch production.

RESULTS AND DISCUSSION

Weather Conditions

Above average temperatures were recorded in May (8-17%) and June (1-7%), but below average temperatures were recorded in July by 1-10% (Table 3). August, September, and October temperatures were close to the 30-yr average. Precipitation in May was 25% greater than the 30-yr average only at WARS in 2014, but June precipitation was greater than average for all environments by 6-71%. July precipitation was greater than average by 21-150% in 2013, but was below average by 20-45% in 2014. Additionally on 10 July 2013, strong winds caused brittle snap and root lodging at both locations. August precipitation was also 39-88% below average except at WARS in 2014 when precipitation was 12% above average. The NWARS was the only site-year that received above average rainfall in September of 2014 (48%). Even though precipitation was less than the 30-yr average at most locations during August in both years and in July 2014, the plants did not display stress symptoms (i.e., leaf rolling, wilting, etc.) visually (data not shown). Below average temperatures during July and above average precipitation in June may have contributed to the lack of plant stress observed.

Plant Morphological Differences

The E x H interaction observed for RCC at the V7 growth stage in Table 4 was caused by specific year differences. Each year, RCC for P0210 and P0448 was greater than P1184 and P1352. In 2013, RCC at V7 was similar for P1184 and P1352, but in 2014 P1184 was greater than P1352 at each site (data not shown). The E x H interaction for RCC at R2 was not significant, but an E x H interaction for RCC at R5 was observed.

P1352 always had the lowest values. Hybrid P1184 consistently had the greatest RCC at R5, with P0448 not being statistically different. Hybrid P0210 was typically similar to both P1184 and P1352, regardless of environment (data not shown). The interaction of E x H for ear-leaf biomass was caused by variation in P1184 and P1352 ear-leaf biomass at each environment. Consistently, P0210 produced the lowest ear-leaf biomass whereas P0448 produced the greatest biomass (data not shown). While every parameter measured for plant growth exhibited a response to N rate unique to each environment (E x NR), the lack of H x NR and E x H x NR interactions indicated the hybrids exhibited similar responses to N application in each environment. Because there were consistent trends across environments (with the exception of the interactions discussed above), the main effect of hybrid is presented in Table 5.

Plant height varied by hybrid at V7, with P1184 being shorter than the other hybrids (Table 5), but the drought-tolerant hybrids were taller than the conventional hybrids at R2. This height difference along with the similar ear-leaf biomass of the drought-tolerant hybrids suggests the tolerance mechanism for these hybrids is not due to a change in plant architecture. The RCC was consistently less in the drought-tolerant hybrids than the conventional hybrids at all stages, and the RCC in late-maturing hybrids was less than the early-maturing hybrids regardless of drought tolerance except at R5. The drought-tolerant hybrids also had a lower ear-leaf N concentration and content at R2 as compared to the conventional hybrids, which may explain the lower RCC values. The decrease in RCC and leaf N concentration could be related to the differences in water use efficiency (Fredeen et al., 1991). An increased water use efficiency (less water used per

unit biomass produced) could reduce the amount of N removed from the soil, resulting in less accumulation in the leaf tissue at the R2 growth stage. Lambers et al. (2008b) suggests that plants that are more stress tolerant can have lower levels of leaf N, and decreasing leaf N reduce chlorophyll production (Crawford et al., 2000). The RCC in the drought-tolerant hybrids was still less than the conventional hybrids at R5, suggesting a delay in N uptake was not responsible for the differences observed in tissue N content at R2.

Grain Yield, Harvest Moisture, Soil Nitrate, and Stalk Nitrate

The E x H interaction for yield in Table 4 was caused by the brittle snap event at NWARS in 2013. Table 6 shows that P1184 experienced greater brittle snap than the other hybrids (16.0% vs. 2.2-4.5%), and grain yield was reduced compared to the other hybrids (8.1 vs. 8.9-9.2 Mg ha⁻¹). Root lodging at NWARS in 2013 was < 3% for all hybrids and N rates (data not shown) and was considered non-significant. As listed in Table 2, P1184 received the lowest brittle snap rating (rating of 3) compared to the other three hybrids (ratings of 4 or 6). When N was applied (≥ 67 kg N ha⁻¹), the yield loss caused by brittle snap was significantly greater than when 0 kg N ha⁻¹ was applied. While the slope calculated for yield loss due to brittle snap at 67 kg N ha⁻¹ was almost twice that of higher N rates, the slope was not significantly different than the slopes for 134-269 kg N ha⁻¹ ($P > 0.05$). Wilhelm et al. (1999) also observed an increase in brittle snap with N application (8% at 0 kg N ha⁻¹ to 24% at rates above 80 kg N ha⁻¹). At WARS in 2013 brittle snap was < 5% for all hybrids and N rates, and root lodging among hybrids was

non-significant (data not shown). The difference in damage severity between the two locations may have been related to the development stage (Wilhelm et al., 1999), with plants at NWARDS being at a more susceptible stage compared to those at WARS as a result of different planting dates and heat unit accumulation.

The H x NR interaction was not significant indicating all hybrids had a similar yield response to N application (Figure 1), but the E x NR interaction indicated the response was unique in each environment. Previous research has also demonstrated that hybrids with unique characteristics (e.g., modified kernel composition, non-transgenic drought tolerant hybrids) respond similarly to N application, but the response can differ by environment (Thomison et al., 2004; Miao et al., 2006; Shepard et al., 2011).

The E x NR interaction for yield was primarily driven by the results from WARS in 2013, which had an overall AOMY of 11.93 Mg ha⁻¹, which was greater than any other environment (8.84-10.18 Mg ha⁻¹; Table 7). At WARS in 2013, the conventional hybrids had a greater AOMY than the drought-tolerant hybrids (12.63 vs 11.74 Mg ha⁻¹) because the yields were 1.4 Mg ha⁻¹ greater at the 0 kg N ha⁻¹ application rate compared to the drought-tolerant hybrids (Fig. 1). Consequently, the AONR and EONR for the conventional hybrids at WARS in 2013 were 75% and 40% greater, respectively, than for the drought-tolerant hybrids. At all other sites, the drought-tolerant hybrids had 3 to 11% greater AONR and 2 to 10% greater EONR than the conventional hybrids, but the AOMY and EOMY for drought-tolerant hybrids were within 3 to 5% of maximum yield for conventional hybrids.

The AONR across hybrids at WARS in 2013 was 244 kg N ha⁻¹, which was greater than any other environment (222-226 kg N ha⁻¹; Table 7). However, the EONR across hybrids were similar for each environment, ranging from 184 to 199 kg N ha⁻¹ with EOMY ranging from 8.72 to 11.7 Mg ha⁻¹. Current N recommendations for Ohio range anywhere from 180 to 246 kg N ha⁻¹ applied following a non-legume for a yield potential of 8.78 to greater than 11.3 Mg ha⁻¹ (Thomison et al., 2005), which is similar to the values observed for EONR and EOMY. The EONR across sites was 16% less than the AONR, which resulted in the EOMY being reduced by 1.3% compared to the AOMY.

The interaction of E x H observed for grain moisture at harvest was driven by the magnitude of difference between the hybrids. The harvest moisture differences were associated with comparative relative maturity rather than drought tolerance (Table 7). P0210 consistently had the lowest harvest moisture across environments, with P1352 having the greatest harvest moisture. The harvest moisture for the conventional hybrids was between the two drought-tolerant hybrids, with P0448 generally closer to P0210 and P1184 closer to P1352. A H x NR interaction was not observed for harvest moisture, and grain moisture at harvest was not affected by N application rate across hybrids at NWARS in 2013 and WARS in both years. At NWARS in 2014 harvest moisture was greatest at 0 kg N ha⁻¹ (228 g kg⁻¹) and decreased with N application rate to 196-201 g kg⁻¹ until N rates were equal to or exceeded 134 kg N ha⁻¹ (data not shown).

The soil nitrate level at the time of N application was similar for all hybrids across environments as indicated by the PSNT results (Table 8). The end of season soil nitrate

increased with N application rate across environments, and P0448 had the greatest end of season soil nitrate (11.4 mg kg^{-1}) relative to the drought-tolerant hybrids ($8.8\text{-}8.9 \text{ mg kg}^{-1}$). Hybrid P1184 end of season soil nitrate (9.7 mg kg^{-1}) was similar to both drought-tolerant hybrids and P0448. Stalk nitrate (across hybrids) increased with N rate, but the magnitude of the change differed by environment resulting in an E x NR interaction (data not shown). Stalk nitrate increased for each hybrid as N application rate increased. However, stalk nitrate was only found to be excessive ($> 2000 \text{ mg kg}^{-1}$ as described by Binford et al., 1992) in P0448 at the highest N rate, which contributed to the difference in stalk nitrate levels between drought-tolerant and conventional hybrids. These results suggest luxury consumption of N in the stalk was an uncommon occurrence, and was more likely to occur with P0448 than the other hybrids.

Differences in Grain Quality

Hybrid P0210 contained the lowest level of protein compared to the other hybrids at each environment and most N rates (Fig. 2). Conversely, when N was applied P1352 had the highest concentration of protein in the grain with the exception of 67 kg N ha^{-1} applied at WARS in 2014. The protein in the two conventional hybrids was more variable with N application, but P1184 had a greater response than P0448 when N application exceeded 202 kg N ha^{-1} . Protein concentration tended to increase for all hybrids as additional N was applied, but the magnitude of the increase varied by hybrid (resulting in a significant H x NR interaction). However, a decrease in protein concentration was observed at 202 kg N ha^{-1} at WARS in 2013.

Differences in grain oil content (Fig. 3) were more consistent with the drought tolerance designation of the hybrid. Both the drought-tolerant hybrids exhibited greater oil content at N rates ≤ 134 kg N ha⁻¹. Hybrid P0448 consistently exhibited the lowest oil concentration of all hybrids evaluated. At N rates > 134 kg N ha⁻¹, the differences between hybrids were less pronounced. Variation in oil content caused by drought tolerance has been documented previously (Ali et al., 2010), but other researchers observed N application having little to no effect on oil concentration (Mason et al., 2002; Thomison et al. 2004). The H x NR interaction indicates the response of grain oil to N application may be hybrid specific. Starch content (Fig. 4) tended to be greater in the conventional hybrids at N rates ≤ 202 kg N ha⁻¹ than the tolerant hybrids, with the exception of WARS in 2014 where starch levels were greater in P1352 than P0448 at 67 kg N ha⁻¹, but hybrid differences were minimal above 134 kg N ha⁻¹.

The compensatory relationship for each hybrid seemed to differ. For all hybrids, a strong negative correlation existed between protein and starch content ($r > |-0.87|$, $P < 0.001$), which indicated that as protein increased starch content decreased. This relationship has been documented by previous researchers (Miller and Brimhall, 1951; Cook et al., 2012). However, the relationship between oil and starch as well as oil and protein differed for each hybrid. Both the conventional hybrids P0448 and P1184 exhibited a negative correlation between oil and starch ($r = -0.436$ and $r = -0.253$, respectively, $P < 0.001$), with no significant relationship between oil and protein content. This relationship between starch and oil has also been observed in multiple genotypes (Cook et al., 2012). Conversely, P0210 of the drought-tolerant hybrids had a significant

positive correlation between oil and starch ($r = 0.254$, $P = 0.023$). P1352 did not have a significant correlation between oil and starch content. The drought-tolerant hybrids did exhibit significant ($P < 0.005$) negative correlations for oil and protein (P0210: $r = -0.542$; P1352: $r = -0.316$). Previous research has typically identified a positive correlation between protein and oil content in maize grain (Miller and Brimhall, 1951; Cook et al., 2012), but these results indicated the opposite trend. The negative correlations may be attributed to the magnitude of the oil decrease when N application increased from 0 to 67 kg N ha⁻¹ for the drought-tolerant hybrids in both years at NWARS and at WARS in 2013. However, these results may indicate a physiological difference in the drought-tolerant hybrids which may contribute to the documented tolerance.

CONCLUSIONS

The RCC and ear-leaf N content were less in the drought-tolerant hybrids, which may have resulted in slightly greater AONR and EONR as compared to the conventional hybrids (3-11%). However, the hybrid grain yield response to N application was similar for all hybrids evaluated with grain yields being within 3-5% at the optimum N rate. Grain quality differences were observed with the four hybrids, but were not consistently associated with drought tolerance. At N rates less than 202 kg N ha⁻¹, grain oil content was greater in drought-tolerant hybrids, and grain starch was reduced in drought-tolerant hybrids compared to conventional hybrids. The negative correlations observed between oil and protein content in the drought-tolerant hybrids may indicate a metabolic difference in grain allocation, but future research is needed to investigate these results.

The AONR and EONR to maximize yield were marginally greater for drought-tolerant hybrids than conventional hybrids in non-drought conditions. Reducing the AONR by 16% (on average) to achieve the EONR only resulted in a 1.3% yield reduction, and resulted in N rates that are more reflective of recommendations for Ohio.

TABLES AND FIGURES

Site	Year	OM	CEC	pH	P	K
		g kg ⁻¹	cmol(+) kg ⁻¹		-----mg kg ⁻¹ -----	
NWARS	2013	17.0	14.1	6.4	37	203
	2014	40.0	17.3	6.7	30	177
WARS	2013	25.0	15.3	6.1	53	138
	2014	31.0	15.0	6.2	37	119

Table 1. Soil characteristics of organic matter (OM), cation exchange capacity (CEC), pH, P (Bray P₁), and K (ammonium acetate method) for Northwest Agricultural Research Station (NWARS) and Western Agricultural Research Station (WARS) each year.

	Hybrid			
	P0210	P0448	P1184	P1352
GDUs ¹ to silk (°C)	730	705	770	740
GDUs to maturity (°C)	1405	1390	1470	1430
Comparative Relative Maturity	102	104	111	113
Drought Tolerance ²	9	7	7	9
Mid-Season Brittle	4	6	3	6
Stalk Technology Trait ³	YXR, YXR	YXR, YXR	XR, XR	YXR, CYXR

Table 2. Characteristics of the four hybrids evaluated.

¹ GDUs: Growing degree units in Celsius with 30°C upper limit and 10°C lower limit.

² Ratings are listed as reported in industry literature where 9=most tolerant and 1=most susceptible.

³Technology trait for each year is separated by a comma, with 2013 trait listed followed by the 2014 trait. YXR: Contains YGCB (YieldGard® Corn Borer insect trait), HXX (Herculex® XTRA insect trait), LL (LibertyLink® herbicide resistance), and RR2 (Roundup Ready® Corn 2 herbicide resistance). XR: Contains HXX, LL, and RR2. CYXR: Contains YGCB, HXX, LL, RR2, and Agrisure® RW trait.

Month	Average Temperature						Total Precipitation					
	NWARS			WARS			NWARS			WARS		
	2013	2014	30-yr avg.	2013	2014	30-yr avg.	2013	2014	30-yr avg.	2013	2014	30-yr avg.
-----°C-----						-----mm-----						
May	18.1	16.8	15.5	17.8	17.3	16.0	40.4	51.8	95.5	77.0	147	118
June	21.2	22.2	20.9	21.2	22.0	21.0	98.8	137	79.8	111	132	105
July	22.6	20.7	22.8	22.3	20.4	22.7	252	55.4	101	143	94.0	118
Aug.	21.1	21.7	21.3	21.3	21.9	21.9	36.1	10.9	92.5	50.0	91.4	81.8
Sept.	17.9	17.5	17.6	18.3	17.6	17.9	51.1	102	69.3	79.8	34.3	83.8
Oct.	12.1	11.1	11.1	11.9	11.5	11.5	161	63.2	71.1	118	68.6	72.6
Entire Season	18.8	18.4	18.2	18.8	18.4	18.5	639	420	509	579	567	581

Table 3. Average monthly temperature and total monthly precipitation for the 2013 and 2014 growing seasons at the Northwest Agricultural Research Station (NWARS) and the Western Agricultural Research Station (WARS).

Parameter	Stage	Source of Variation						
		E	H	E x H	NR	E x NR	H x NR	E x H
df		3	3	9	4	12	12	36
Plant height	V7	<0.001	<0.001	0.380	0.032	0.155	0.339	0.406
	R2	<0.001	<0.001	0.176	<0.001	<0.001	0.248	0.719
Relative Chlorophyll Content	V7	<0.001	<0.001	0.006	<0.001	<0.001	0.068	0.683
	R2	0.047	<0.001	0.080	<0.001	<0.001	0.742	0.966
Ear-leaf biomass	R5	<0.001	<0.001	0.011	<0.001	<0.001	0.430	0.168
	R2	<0.001	<0.001	0.004	<0.001	<0.001	0.060	0.338
Ear-leaf N concentration	R2	0.002	<0.001	0.275	<0.001	<0.001	0.498	0.642
Ear-leaf N content	R2	0.051	0.007	0.338	<0.001	<0.001	0.393	0.640
PSNT	V4	0.004	0.814	0.892	0.852	0.212	0.403	0.128

continued

Table 4. Significance of environment (E), hybrid (H), nitrogen rate (NR), and their interactions for each parameter measured.

Table 4. continued

Parameter	Stage	Source of Variation						
		E	H	E x H	NR	E x NR	H x NR	E x NR x H
End soil nitrate	R6	<0.001	0.033	0.587	<0.001	<0.001	0.171	0.084
Stalk nitrate	R6	0.002	<0.001	0.885	<0.001	<0.001	0.193	0.362
Grain Yield		<0.001	0.863	0.004	<0.001	<0.001	0.772	0.294
Harvest Moisture		<0.001	<0.001	<0.001	0.005	<0.001	0.074	0.256
Protein		0.063	<0.001	0.578	<0.001	<0.001	0.015	0.839
Oil		<0.001	<0.001	<0.001	<0.001	0.014	<0.001	0.014
Starch		0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.340

Type	H	Plant Height		RCC ⁴			ELB	ELN	ELN
		V7	R2	V7	R2	R5	R2	R2	R2
		cm		%			g leaf ⁻¹	g kg ⁻¹	mg N leaf ⁻¹
DT	P0210	89.4	232	97.7	89.1	78.2	4.08	26.57	110.4
	P1352	93.7	239	88.1	85.3	72.9	4.46	24.42	111.8
Conv.	P0448	94.4	226	97.9	91.0	79.6	4.49	25.34	115.5
	P1184	85.9	235	90.5	89.6	83.4	4.21	27.61	118.1
	LSD _{0.05} ⁵	2.2	3.3	1.5	1.7	2.6	0.10	0.81	4.79
	DT	91.6	236	92.9	87.2	75.5	4.27	25.50	111.1
	Conv.	90.2	231	94.2	90.2	81.5	4.35	26.46	116.7
	H	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	DT vs Conv.	0.120	<0.01	0.079	<0.01	<0.01	0.064	0.002	<0.01
	NR	0.032	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	H x NR	0.339	0.248	0.068	0.742	0.430	0.060	0.498	0.393

Table 5. Plant height, relative chlorophyll content (RCC), dry ear-leaf biomass (ELB), ear-leaf nitrogen (ELN) concentration (Conc.), and ELN content (Cont.) for drought-tolerant (DT) and conventional (Conv.) hybrids (H) across sites and nitrogen rates (NR).

⁴ Relative chlorophyll content determined by dividing each value by the greatest treatment value for the specific environment using the values from the SPAD 502c meter.

⁵ Means of each hybrid within a column are not different if within one least significant difference (LSD) of one another.

H	NR	Brittle Snap	Grain Yield	Yield loss (Y) as influenced by brittle snap (X)
	kg N ha ⁻¹	%	Mg ha ⁻¹	
P0210		4.5	9.15	
P1352		5.3	8.89	
P0448		2.2	9.06	
P1184		16.0	8.05	
LSD _{0.05}		1.8	0.42	
	0	1.0	3.99	Y = 0.403X + 3.60, P = 0.058 R ² = 0.234
	67	5.1	7.84	Y = -0.142X + 8.57, P = 0.006 R ² = 0.455
	134	8.7	10.14	Y = -0.076X + 10.80, P = 0.003 R ² = 0.486
	202	10.4	10.90	Y = -0.074X + 11.65, P < 0.001 R ² = 0.740
	269	9.9	11.07	Y = -0.086X + 11.92, P < 0.001 R ² = 0.640

continued

Table 6. Percent brittle snap (stalk breakage below the primary ear) and grain yield for each hybrid (H), and yield loss due to brittle snap for each N application rate (NR) across hybrids at NWARS in 2013.

Table 6. continued

H	NR	Brittle Snap	Grain Yield	Yield loss (Y) as influenced by brittle snap (X)
	kg N ha ⁻¹	%	Mg ha ⁻¹	
LSD _{0.05}		2.1	0.47	
H		0.004	<0.001	
NR		<0.001	<0.001	
H x NR		0.074	0.026	

Site, Year	Hybrid	Moisture	AONR	AOMY	EONR	EOMY
		g kg ⁻¹	kg N ha ⁻¹	Mg ha ⁻¹	kg N ha ⁻¹	Mg ha ⁻¹
NWARS, 2013	P0210	150	241	9.12	202	8.99
	P1352	164	221	9.29	195	9.20
	P0448	156	215	9.34	190	9.26
	P1184	159	237	7.97	193	7.81
	LSD _{0.05}	3.7				
	DT	157	229	9.20	198	9.09
	Conv.	157	223	8.67	192	8.56
	Overall	157	226	8.93	195	8.82
NWARS, 2014	P0210	178	235	9.06	196	8.92
	P1352	227	234	9.12	197	8.99
	P0448	211	237	8.90	200	8.77
	P1184	214	190	8.46	160	8.36
	LSD _{0.05}	9.2				
	DT	203	235	9.09	196	8.96
	Conv.	213	211	8.62	178	8.51
	Overall	208	222	8.84	186	8.72

continued

Table 7. Harvest moisture, agronomic optimum N rate (AONR), maximum yield at AONR (AOMY), economic optimum N rate (EONR) and maximum yield at EONR (EOMY) for each drought-tolerant (DT) and conventional (Conv.) hybrid in each environment. The EONR and EOMY were calculated using \$1.10 kg N⁻¹ and \$157 Mg⁻¹ grain price.

Table 7. continued

Site, Year	Hybrid	Moisture	AONR	AOMY	EONR	EOMY
		g kg ⁻¹	kg N ha ⁻¹	Mg ha ⁻¹	kg N ha ⁻¹	Mg ha ⁻¹
WARS, 2013	P0210	140	223	11.71	178	11.56
	P1352	175	193	11.80	159	11.68
	P0448	158	454	13.00	278	12.38
	P1184	171	309	12.53	212	12.19
	LSD _{0.05}	7.4				
	DT	158	206	11.74	168	11.60
	Conv.	165	360	12.63	236	12.19
	Overall	162	243	11.93	184	11.72
WARS, 2014	P0210	200	222	9.88	199	9.80
	P1352	229	235	10.24	206	10.14
	P0448	217	214	10.16	191	10.08
	P1184	217	228	10.50	203	10.41
	LSD _{0.05}	9.3				
	DT	215	229	10.04	202	9.95
	Conv.	217	221	10.32	197	10.24
	Overall	216	224	10.18	199	10.10

Type	Hybrid	N Rate	PSNT (V4)	End of Season Soil Nitrate (R6)	Stalk Nitrate (R6)
		kg N ha ⁻¹		mg kg ⁻¹	
DT	P0210	0	6.2	2.8	46.9
		67	6.3	4.3	101.9
		134	5.6	10.3	105.4
		202	6.3	11.1	478.6
		269	6.1	16.3	1123.1
		Avg.	6.1	8.9	371.2
	P1352	0	5.8	2.4	35.5
		67	6.0	4.0	68.6
		134	6.8	8.3	171.6
		202	6.4	12.5	122.7
		269	6.1	16.9	1283.1
		Avg.	6.2	8.8	336.3
Conv.	P0448	0	6.1	2.5	49.3
		67	5.8	6.0	134.7
		134	5.7	7.0	555.0

continued

Table 8. Pre-sidedress soil nitrate test (PSNT) results, end of season soil nitrate levels, and stalk nitrate levels at R6 for each drought-tolerant (DT) and conventional (Conv.) hybrid (H) and N rate (NR).

Table 8. continued

Type	Hybrid	N Rate	PSNT (V4)	End of Season Soil Nitrate (R6)	Stalk Nitrate (R6)
		kg N ha ⁻¹	mg kg ⁻¹		
		202	5.9	15.3	496.7
		269	6.8	26.0	2220.7
		Avg.	6.1	11.4	691.3
	P1184	0	6.2	2.5	36.3
		67	5.9	4.6	39.4
		134	5.9	8.4	410.1
		202	6.1	11.8	175.0
		269	5.9	20.9	889.5
		Avg.	6.0	9.7	310.1
		LSD _{0.05} ¹	NS	2.0	182.0
		H	0.814	0.033	<0.001
		DT vs Conv.	0.442	0.226	0.018
		NR	0.852	<0.001	<0.001
		H x NR	0.403	0.171	0.193

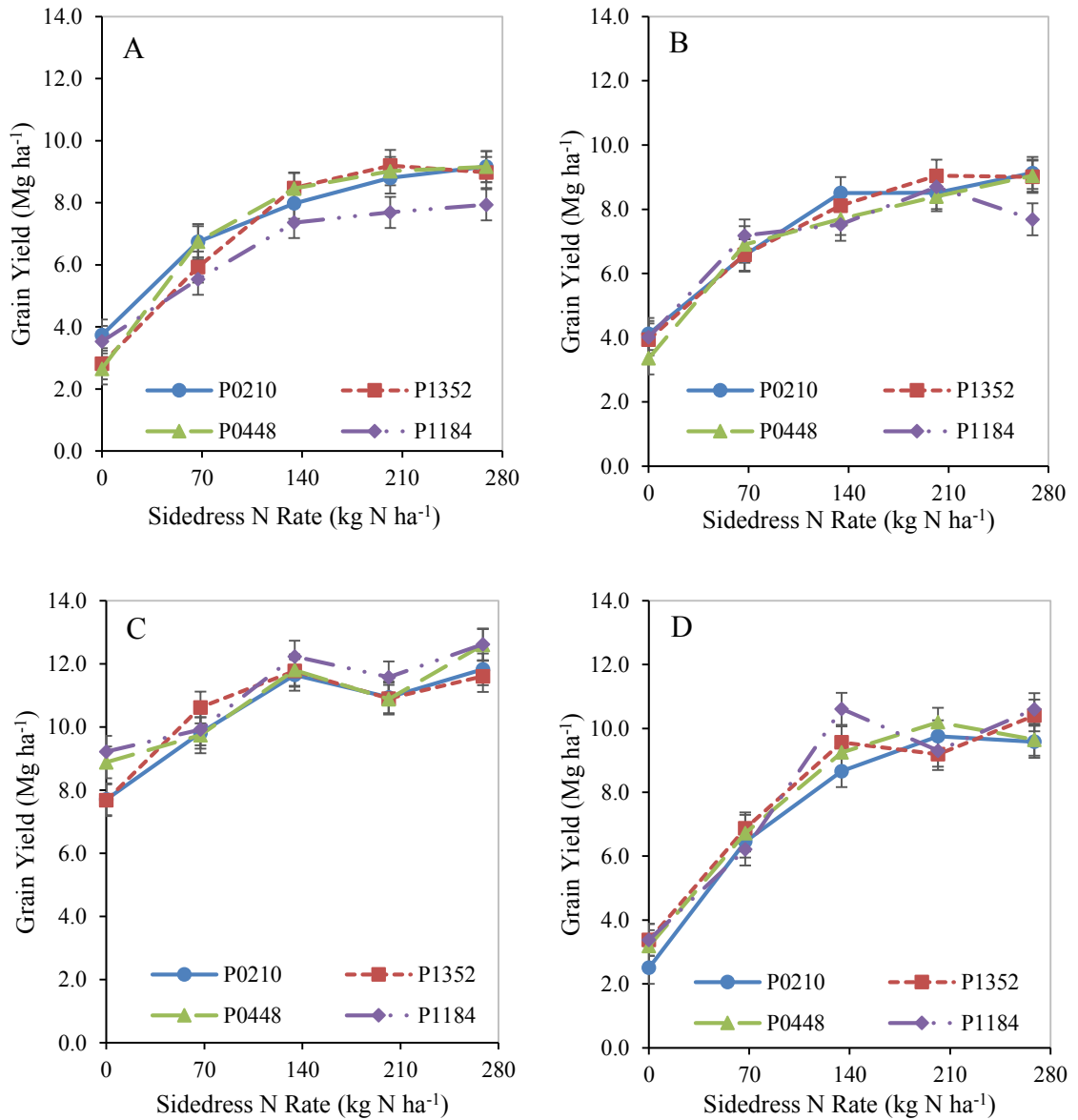


Figure 1. Grain yield response curves for each hybrid at NWARS in 2013 (A) and 2014 (B), and WARS in 2013 (C) and 2014 (D).

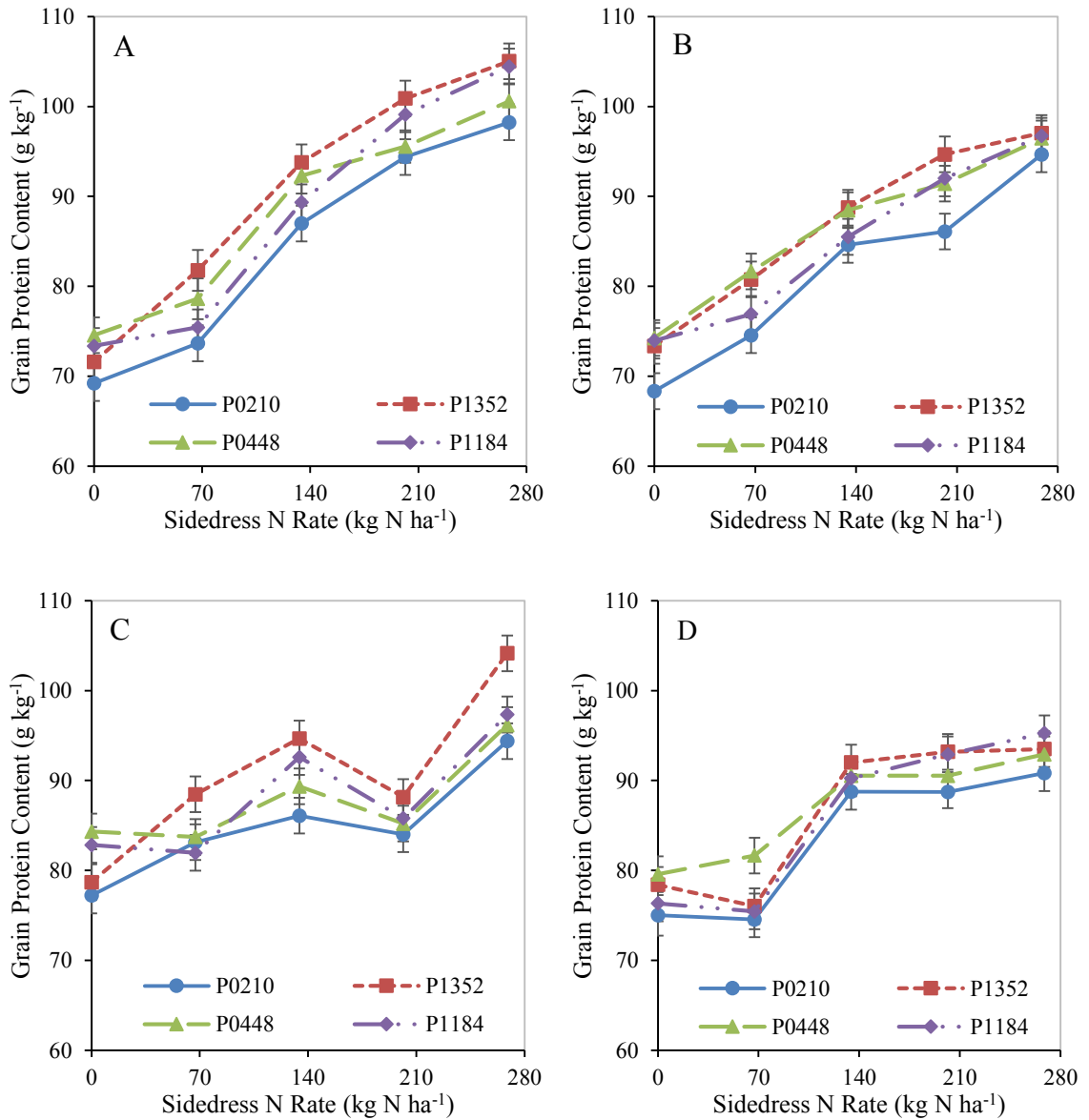


Figure 2. Protein content on dry matter basis from NWARS in 2013 (A) and 2014 (B), and WARS in 2013 (C) and 2014 (D).

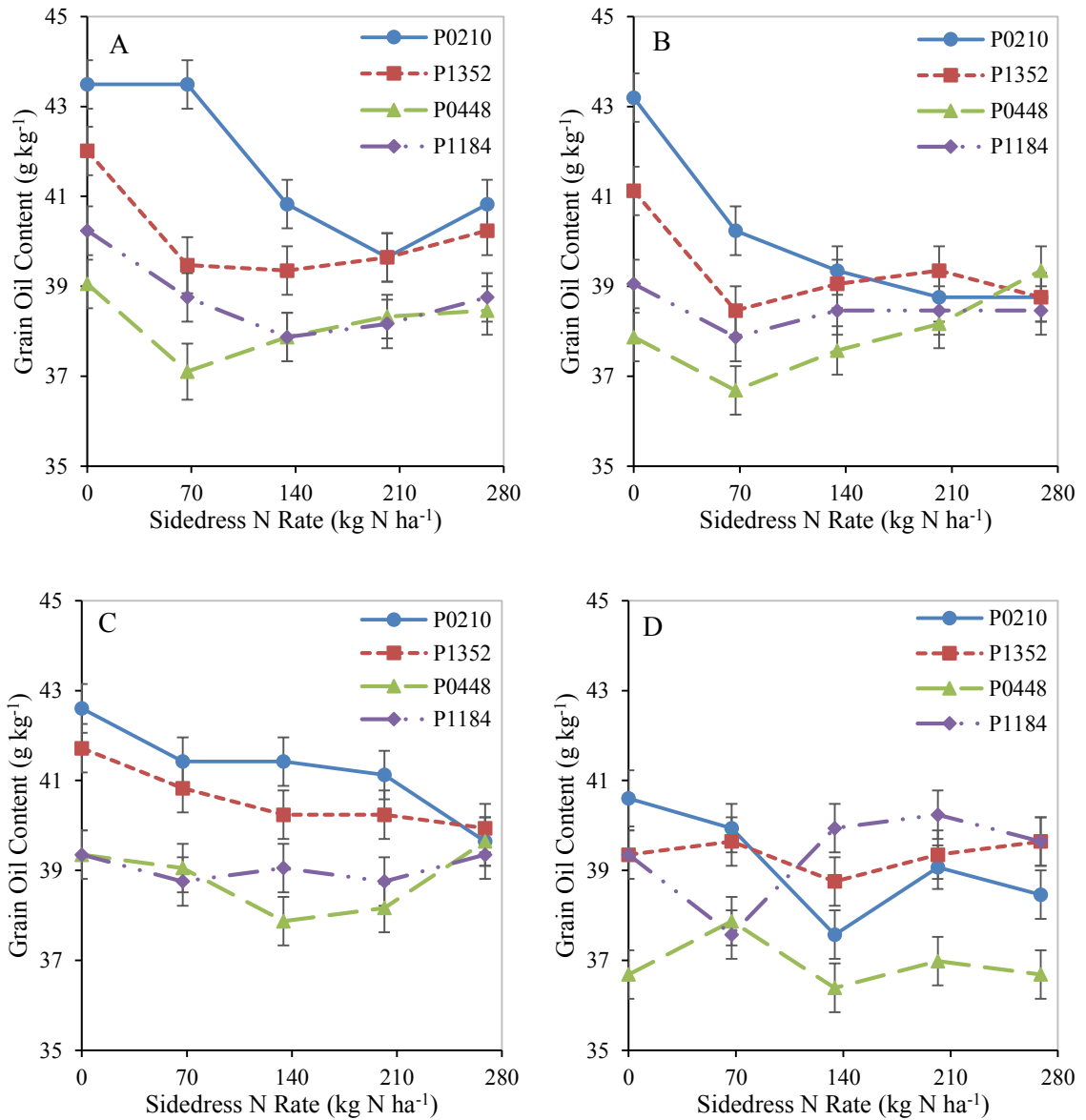


Figure 3. Oil content on dry matter basis from NWARS in 2013 (A) and 2014 (B), and WARS in 2013 (C) and 2014 (D).

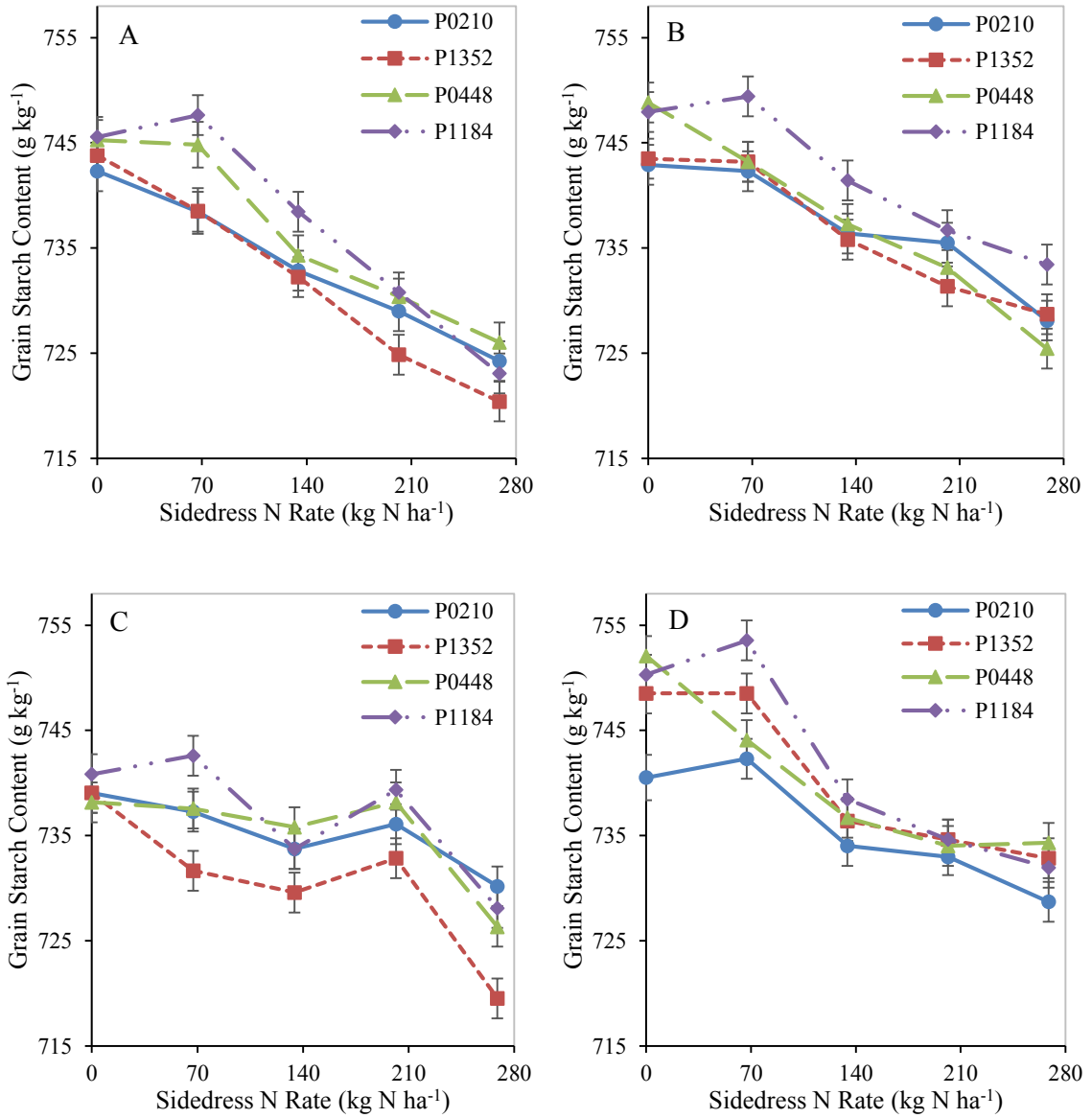


Figure 4. Starch content on dry matter basis from NWARS in 2013 (A) and 2014 (B), and WARS in 2013 (C) and 2014 (D).

CHAPTER 3: PHYSIOLOGICAL RESPONSE OF A DROUGHT-TOLERANT AND
CONVENTIONAL MAIZE (*ZEA MAYS* L.) HYBRID TO PLANT POPULATION AT
TWO SOIL MOISTURE LEVELS

INTRODUCTION

Recent climate change models predict that increasing global temperatures will affect precipitation differently based on geography (Lobell et al., 2008). The upper Midwestern U.S. has experienced a change in precipitation distribution during the growing season, and a 37% increase in extreme precipitation events over the last 45 years (Morton et al., 2015). Increases in extreme events could decrease water infiltration, and changes in precipitation distribution could increase the incidence of drought events (Kunkel et al., 1999), resulting in anywhere from 40 to 65% decrease in yield potential (Bray et al., 2000). For instance, drought in 2012 reduced maize (*Zea mays* L.) grain yields in Ohio by 25% compared to 2010 and 2011, which resulted in an economic loss to farmers of over \$700 million (USDA-NASS, 2015). Growers have been adapting to changing climatic conditions to minimize yield loss from environmental stress by using stress-resistant or tolerant varieties (Wall and Smit, 2005).

A new generation of non-transgenic drought-tolerant maize hybrids has been released for commercial use. Drought tolerance has been previously described as the ability for a hybrid to survive periods of drought while maintaining low levels of water in

the tissue (Levitt, 1972), and has been bred for by assessing for yield stability across both drought stressed and favorable environments (Finlay and Wilkinson, 1963; Clarke et al., 1992). Recent research has shown that breeders may have been selecting for traits allowing for improved water use efficiency, or using less water to produce a similar or great amount of biomass and yield as a conventional hybrid (Cooper et al., 2014). However, research on the physiology of these hybrids as well as their agronomic production in the Eastern U.S. Corn Belt is limited (Roth et al., 2013).

Evaluating phenotypic and physiological characteristics of drought-tolerant hybrids may provide insight into the traits contributing to the minimization of the yield decrease. Previous researchers have identified multiple phenotypic traits associated with improved stress tolerance, including anthesis-silking interval (ASI), leaf senescence after pollination, plant height, and leaf number, but there has been limited emphasis on transpiration rate, stomatal conductance, chlorophyll fluorescence, and chlorophyll concentrations (Bolaños and Edmeades, 1996; Crawford et al., 2000; Edreira and Otegui, 2012; Escobar-Gutiérrez and Combe, 2012; Roth et al., 2013). Much of the research examining plant characteristics has been conducted on tropical maize lines in the Southern hemisphere or tropical environments (Bolaños and Edmeades, 1996; Otegui, 1997; Otegui and Melón, 1997). Limited research has been conducted with commercially available hybrids in the U.S. (Boomsma et al. 2009; Roth et al., 2013), and more research is needed to identify plant characteristics that contribute to observed drought tolerance.

Measuring the response of a hybrid to drought stress can be conducted through experimental design using water manipulation. Many researchers have used deficit

irrigation to induce moisture stress in crops (Lafitte, 2002; Jia et al., 2012), but in a rainfed environment such as Ohio, limiting irrigation may not be sufficient to induce moisture stress. Use of water exclusion structures has proven effective at reducing precipitation (Upchurch et al., 1983; Changnon and Hollinger, 2003), but one limitation of using permanent structures is that radiation reaching the crop canopy is reduced if permanently installed (Hudak and Patterson, 1996). The need for automation or labor to install and remove non-permanent structures between rain events (Johnson and Kanemasu, 1982; Sullivan and Teramura, 1990) could be cost prohibitive for small-plot research, and may not enable researchers to achieve adequate statistical replication at a single test site. Water exclusion has been successfully achieved using black plastic mulch in soybean (Mederski and Jeffers, 1973; Thomison et al., 1987; Fredrick et al., 1991), but its utilization in maize has been mainly for water conservation (Lu et al., 2014) and weed control (Rajablarijani et al., 2014). The objectives of this research were to: 1) observe the physiological response of a drought-tolerant hybrid and a conventional hybrid to plant population and water exclusion; 2) correlate any physiological differences to differences in yield; and 3) determine if black plastic can be used in maize as a successful method to exclude moisture throughout the growing season.

MATERIALS AND METHODS

Site Characteristics and Plot Establishment

A field experiment was established in 2013, and repeated in 2014 at the Western Agricultural Research Station in South Charleston, OH (39°51'N, 83°40' W; 333 m

elevation). The soil each year was a silt loam Strawn- (fine-loamy, mixed, active, mesic Typic Hapludalfs) Crosby (fine, mixed, active, mesic Aeric Epiaqualfs) complex with 2-6% slope in 2013 and 0-2% slope in 2014. Previous crop each year was pumpkins, and the site was prepared for planting with tillage in the fall and spring. Initial soil properties each year are listed in Table 9.

The field in 2013 and 2014 received a spring preplant anhydrous ammonia (82N-0P-0K) N application of 202 kg N ha⁻¹. Forty-five kg N, 20 kg P, and 37 kg K ha⁻¹ was applied at planting using starter fertilizer in 2013, and was reduced to 30 kg N, 27 kg P, and 0 kg K ha⁻¹ in 2014. The experiment was planted on 20 May 2013 and 30 May 2014. Crop staging was conducted using the method described by Abendroth et al. (2011). The experiment was conducted as a split-plot randomized complete block design with two replications of the whole plot factor in 2013 and three replications of the whole plot factor in 2014.

Treatment Implementation

Each sub-plot was 7.6 x 3.1 m and consisted of four rows (76-cm spacing). Every sub-plot factor combination of hybrid and population was completely randomized within each whole-plot treatment. The first sub-plot factor consisted of two Pioneer® brand hybrids, P1184 and P1352, with similar common relative maturities (111 and 113, respectively) adapted for Ohio environments. Hybrid P1352, marketed as an Optimum® AQUAmax® hybrid (non-transgenic drought-tolerant), had a greater drought tolerance rating (9 out of 9) from Pioneer as compared to the non-AQUAmax P1184 (conventional

hybrid, 7 out of 9) where 9 is the greatest drought tolerance and 1 is the least tolerance. The herbicide and insect resistance traits in both years for P1184 were Herculex® XTRA insect trait, LibertyLink® herbicide resistance, and Roundup Ready® Corn 2 herbicide resistance. Hybrid P1352, in addition to the above listed traits, included the YieldGard® Corn Border insect trait (YGCB) in 2013 and both the YGCB and Agrisure® RW trait in 2014. The second sub-plot factor was plant population. Populations of 74,000 and 124,000 plants ha⁻¹ were selected because the first is a common population used by growers in Ohio, and the latter was considered an extremely dense population that may elicit stress in the plants. Plots were initially planted using a 124,000 plants ha⁻¹ seeding rate, and stand counts collected at V4 on all four rows were used to thin the plots as necessary to achieve desired plant populations.

The whole plot factor was watering regime with two levels (rainfed plus irrigation or water excluded). The watering regime was determined prior to planting, but the irrigation and water exclusion treatments were not implemented until canopy closure at V6 (24 June 2013 and 27 June 2014) to minimize temperature effects and to initiate drought at the late vegetative stages. Each whole plot (30.5 x 3.1 m) consisted of four rows at 76-cm spacing. Black 4-mil polyethylene plastic (0.9-m wide) sheets were laid at ground-level between each maize row within the plot and outside the first and fourth row. Additionally, plastic sheeting was extended 6 m beyond the whole plot end to provide additional buffer. Plastic was secured around the plants by stapling the edges of each sheet 3-cm from the soil surface to 30-cm stakes placed in the row spaced every 40 cm. Plastic was also used in the rainfed plus irrigation treatment, but one 30-cm slit was cut

horizontally in the plastic every 20-cm to allow for water infiltration. Drip tape (2.5-cm width) irrigation with openings every 30-cm was installed halfway between each row below the plastic. Soil tensiometers (112-R Irrrometer, Irrrometer Company Inc., Riverside, CA) were installed in each irrigated treatment, and plots were irrigated as necessary to maintain readings below 0.04 MPa. Watering regimes were separated spatially by 2.3 m (four rows, three inter-row spaces) of maize within each replication in which the ground received no cover. Replication of the whole-plot factor was also separated by four rows of border receiving the same treatment. Temperatures at 9- and 20- cm soil depths were recorded in the whole-plot treatment and the uncovered border to observe effects of the plastic cover on soil temperature throughout the season.

Within Season Measurements

Soil moisture measurements were collected at V6, V8, V14, R2, R5, and R6 growth stages at 10-cm depth increments to 60 cm below ground level using a portable capacitance probe (Diviner 2000, Sentek Technologies, Stepney, Australia). At the V2 growth stage, 70-cm long polyvinyl chloride tubes (5-cm wide) were installed using the soil slurry procedure halfway along the sub-plot length and 7.5-cm inside one harvest row. Holes for installation were created in 2013 using a 10-cm auger, and a 15-cm automated auger in 2014. Volumetric water content reported by the probe was correlated to gravimetric water content samples collected at 10-cm and 20-cm depth intervals at both V6 and R6 growth stages. Since volumetric water content is equal to the gravimetric water content multiplied by the soil bulk density divided by the density of water (Scott,

2000), a linear regression was conducted to determine soil bulk density each year (Table 9), and the volumetric measurements were converted to gravimetric values using the bulk density. Field capacity and the permanent wilting point were determined using a pressure plate (Klute, 1986) with set points of 0.033 MPa for field capacity and 1.5 MPa for permanent wilting point. Soil moisture results are reported as percent plant available water content ($1.0 \text{ m}^3 \text{ m}^{-3}$ = field capacity, $0.0 \text{ m}^3 \text{ m}^{-3}$ = permanent wilting point).

Aboveground plant growth was measured at V8, V14, R2, and R5. On 10 July 2013, a severe storm producing straight-line winds created significant root lodging (defined as lodging caused by the inability of the root system to maintain plant erectness) in all plots. This caused the V14 measurements in 2013 to be delayed until the V17 growth stage. Counts of root lodged plants were collected one week after the wind storm.

At the V8 and V14 timing, plant height was measured to the tallest extended leaf on five plants per plot, and plant height was measured to the uppermost leaf collar at R2. Two consecutive plants from one non-harvest row were collected at V8, V14, and R2 to measure dry biomass production. Plants collected at R2 were measured for number of green leaves and surface area of these leaves prior to drying using an LI-3000 area meter (Li-Cor Biosciences, Omaha, NE), and stalk diameter measured on the internode above the brace roots (147, General Tools and Instruments, New York, NY). Samples were dried for 7-14 days at 60°C prior to measuring biomass. Leaf area index (LAI) was calculated by dividing total leaf area by the area of ground each plant occupied at the measured plant population (Westgate et al., 1997), and specific leaf area (SLA) was calculated by dividing total leaf area by the dry leaf biomass (Poorter and Garnier, 2007).

The dried leaf tissue collected at R2 was ground with a Udy Cyclone Mill (Udy Corporation, Fort Collins, CO) and analyzed for chlorophyll *a*, *b*, and total carotenoid pigment content using the procedure described by Wellburn (1994). Leaf tissue (10 mg) was extracted with 5 mL dimethyl-formamide overnight. Samples were centrifuged for 15 min to remove particulates, and supernatant was analyzed using a UV spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) spectrophotometer. Absorption maxima were set at 663.8 nm (chlorophyll *a*), 646.8 nm (chlorophyll *b*), and 480 nm (total carotenoids). Concentration was calculated using the described formulas for each pigment. The leaf tissue was also analyzed for total N concentration through total combustion with a Leco Total N analyzer (Leco Corporation, St. Joseph, MI).

Percent total light intercepted (or interception efficiency) was measured using a 1-m long Line Quantum Sensor (Li-Cor Biosciences, Omaha, NE) to measure canopy light absorbance. The fraction of light intercepted (*I*) was calculated for each plot using the formula described by Westgate et al. (1997):

$$I = (R - R_t) / R$$

where *R* is the incident radiation reading for the plot, and *R_t* is the radiation that was transmitted beneath the canopy. The quantum sensor was oriented diagonally between the center rows to ensure only the interception by the center two rows was measured. Leaf greenness was measured on 10 plants in each plot using the youngest fully collared leaf at

V8 and V14 and the uppermost ear-leaf at R2 and R5 using a SPAD 502c meter (Konica Minolta, Chiyoda, Japan).

Plant physiological activity at V8, V14, R2, and R5 using a Li-Cor 6400XTF (Li-Cor Biosciences, Omaha, NE) to measure gas exchange and light-adapted chlorophyll fluorescence ratios on three plants per plot at each growth stage. At V8 and V14, the youngest collared leaf was measured, and at R2 and R5 the uppermost ear leaf was measured similar to Roth et al. (2013). Rather than implement an isolation technique and a saturating light intensity to measure gas exchange (Earl and Tollenaar, 1999; Roth et al., 2013), a lower light intensity was used to better mimic ambient conditions at the ear-leaf within the canopy, minimize acclimation time, and reduce neighboring plant destruction. The photosynthetic photon flux density was set at $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, the sample chamber CO_2 held constant by the CO_2 mixer at $400 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$, flow was maintained at $500 \mu\text{mol air s}^{-1}$, and relative humidity of the sample chamber was maintained between 55-65% using the dessicant. Light-adapted chlorophyll fluorescence was measured concurrently with gas exchange using the multiphase flash method (Loriaux et al., 2013). Chlorophyll fluorescence can also indicate moisture stress prior to expression of visually observable symptoms (Escobar-Gutiérrez and Combe, 2012). Dividing the variable fluorescence (F_v), calculated by subtracting the minimal initial fluorescence at open photosystem II (PSII) centers (F_o') from the maximal fluorescence at closed PSII reaction centers (F_m), by F_m' provided a ratio ($F_v' F_m'^{-1}$) describing the efficiency of open PSII centers for excitation capture (Lu et al., 2003), which can be observed at moderate or low light if photosynthesis is restricted by another stress. The

quantum efficiency of PSII (Φ_{II}) was measured under light adapted conditions by subtracting the steady-state fluorescence (F_s) under light from F_m' and dividing by F_m' , and has been shown to relate to leaf photosynthetic capacity (Earl and Tollenaar, 1999).

The ASI of each plot was measured from VT-R1. Anthesis was evaluated by counting the number plants in the center two rows exhibiting tassels, and silking was evaluated by counting the number of plants in the center two rows with silks emerged. Each plot was counted at least three times for tassel and silk emergence. The data from each plot was used to develop a linear regression for tassel emergence and silk emergence, and the slope of these equations were used to compute the calendar date when 50% tassels had emerged and when 50% of the silks had emerged. The difference in calendar dates after 50% anthesis until 50% silking was considered the ASI for each plot.

After R6 (physiological maturity), all plots were rated for stalk lodging at harvest. Grain yield was collected from the center two rows of each plot using a combine, and grain moisture at harvest was also collected. Reported yields have been adjusted to 155 g kg⁻¹ moisture. Grain from each plot was evaluated for protein, oil, and starch content using an Infratec NIR grain analyzer (Foss Tecator, Höganäs, Sweden) and kernel weights. Six ears from consecutive plants from one non-harvest row were evaluated for ear yield components, including ear length, unfilled tip length, number of kernel rows, number of kernels per row, and were visually rated for abnormalities such as missing or aborted kernel rows and poor basal kernel fill.

Statistical Analysis

Data were analyzed using the MIXED procedure in SAS 9.4 (SAS Institute., Cary, NC). Each year (Y) was analyzed as a fixed factor with replication (rep) nested within year as the error term to observe trends across years. Water (W) treatment was set as the whole-plot factor using W*rep(Y) as the error term, and hybrid (H) and population (P) were each considered sub-plot factors. Each significant Y x factor interaction was investigated to observe the cause of the interaction (magnitude, direction, or combination) and is explained in the text. When the Global *F*-test was significant ($\alpha = 0.05$), means separation was conducted using Fisher's protected LSD. To calculate ASI, each plot was analyzed using the REG procedure to fit a linear model to determine 50% anthesis and 50% silking. The CORR procedure was used to determine which Pearson's correlation coefficients were significant between grain yield and each measured parameter. Principle component analysis was conducted using the PRINCOMP procedure. The tested variables included all of the variables tested for correlation to yield, and yield was included in the analysis. The objective of explaining 70% of the variation in the study. When the eigenvector exceeded $|0.23|$ for each variable, the variable was considered a strong contributor to the principle component (PC). Ordination plots were developed using the principle component analysis.

RESULTS AND DISCUSSION

Growing Conditions and Soil Moisture

May and June in each year had average temperatures above the 30-yr average (Table 10). However, July and August were below the 30-yr average temperature each year. September and October in 2013 were above average, but in 2014 were below or equal to the 30-yr average temperature. Consequently, the 2013 season was 0.3°C above average and 2014 was 0.1°C below average. Measured soil temperatures were slightly elevated due to the presence of the plastic throughout the season (data not shown). Across both soil depths, the soil temperatures under water exclusion were the greatest (20.3°C) and were similar to the irrigated plots with plastic mulch (20.0°C). The uncovered border had an average soil temperature of 19.8°C, which was not different than the irrigated control but was significantly less than the water exclusion treatment. Regardless of watering treatment, the addition of plastic increased soil temperatures from the uncovered control by 0.2-0.5°C (1-3%).

Precipitation in 2013 was less than average in May, but above average in June and July (Table 10). An irrigation event occurred mid-July based on soil tensiometer readings to maintain soil moisture near field capacity. August precipitation was below average, but one irrigation event raised total precipitation to the average. September precipitation was similar to the 30-yr average, with a single irrigation event increasing the total to exceed the average. October received above-average rainfall. In 2014, both May and June exceeded the 30-yr average rainfall totals. July precipitation was below average, but two irrigation events improved precipitation to above average levels. Precipitation in August exceeded the 30-yr average, but based on soil tensiometer readings three irrigation events were necessary to maintain soil moisture levels below 0.04 MPa. September and October

were both below average precipitation, but soil moisture was not decreased enough to require an irrigation event.

The above average precipitation in May and June in 2014 prior to implementation of the exclusion treatment at V6 resulted in a smaller difference between treatments in 2014 and caused significant Y x W interactions observed at select timings and depths for soil moisture (data not shown). Because the interaction was of magnitude, not direction, means are presented across years (Figure 5). Treatment differences were more evident at soil depths above 50 cm, with the major differences being driven by population and watering treatment. Across depths and water exclusion at V8, soil moisture at 124,000 plants ha⁻¹ was 9% less when compared to 74,000 plants ha⁻¹ and soil moisture was greater by 10% for P1184 compared to P1352. Plant available soil moisture at V14 was greater (0.57 m³ m⁻³) at 74,000 plants ha⁻¹ compared to 124,000 plants ha⁻¹ (0.51 m³ m⁻³). However, after V14 soil moisture was most influenced by water exclusion. The plant available soil moisture was 16, 34, and 25% less under water exclusion compared to the rainfed plus irrigation treatment at R2, R5, and R6, respectively.

Plant Morphology

At V8, V14, and R2, no difference was recorded in plant height due to water exclusion (data not shown). Hybrid P1184 was taller at R2 compared to P1352 in 2013 (248 cm vs. 229 cm, respectively), but heights were similar between the hybrids in 2014 (294 cm and 301 cm, respectively). Both hybrids at 124,000 plants ha⁻¹ were 5% taller than those at 74,000 plants ha⁻¹ at V8 in both years, but were 6 to 10% shorter at V14 and

R2 in 2013. Boomsma et al. (2009) also measured a height decrease for multiple hybrids as population increased, but there was no difference in height due to population in 2014 (data not shown). The observed difference in height in 2013 may have been related to the root lodging event on 10 July and the recovery of those plants. All treatments were root lodged greater than 90% when measured one week after the event. Hybrid P1352 root lodging (98%) was greater than P1184 (90%) ($P = 0.100$), but the difference in lodging from population (91% vs. 96% for 74,000 and 124,000 plants ha^{-1} , respectively) was non-significant ($P = 0.206$). However, by R3 root lodging was not evident aside from curvature of the stem at the soil surface. Similar recovery has been observed with 100% root lodging at V13-14 in Wisconsin (Carter and Hudelson, 1988), with a 5 to 15% yield reduction for all hybrids compared to the non-lodged control. Mid-season root lodging was not observed in 2014, and there were no recorded height differences between any of the treatments.

Total biomass per plant was greater for P1352 (23.5 g) than P1184 (20.8 g) at V8, but the hybrid biomass difference was not evident at V14 and R2 (data not shown). Total biomass decreased as population increased from 74,000 to 124,000 plants ha^{-1} for all growth stages. Across hybrids and water exclusion treatments, the biomass decreased from 24.1 to 20.3 g at V8, from 92 to 66 g at V14, and from 172 to 128 g at R2 as population increased. At V14, a significant Y x W x H interaction was observed. Under water exclusion, the biomass of P1184 decreased to 72 g compared to the irrigated treatment (106 g) in 2013, but this trend was not observed with P1352 in 2013 (84 g in both treatments) or both hybrids in 2014 (67 to 73 g for all treatments). Stalk diameter

decreased from 2.42 cm to 2.09 cm as population increased from 74,000 to 124,000 plants ha⁻¹, and was a similar reduction regardless of year, watering regime, and hybrid (data not shown).

Water exclusion did not affect light interception at all stages, LAI, or SLA (Table 11). The H x P interaction for SLA was a result of P1352 increasing SLA with increasing population, but no response to population for P1184. Additionally, P1352 across water and population treatments had greater SLA than P1184. Previous research has demonstrated improved drought tolerance can be associated with lower SLA (Poorter and Garnier, 2007), but these results suggest the drought tolerance of this hybrid was not conveyed by a permanent morphological adaptation. The H x P interaction for LAI was due to a response of greater magnitude in P1352 compared to P1184 to increasing population. Green leaf number was similar for all treatments (Table 11), which indicated the increase in LAI was driven by leaf area. Light interception at all timings was greater at 124,000 than 74,000 plants ha⁻¹, and greater for P1352 than P1184. However, at 74,000 plants ha⁻¹ the LAI for both hybrids was above the critical level of 3.5 as denoted by Westgate et al. (1997), and the increases at 124,000 plants ha⁻¹ may not have improved light interception (> 94% at R2 for all treatments).

Plant Physiology

Photosynthesis and stomatal conductance was not influenced by water exclusion at any physiological growth stage (Table 12). Population only influenced photosynthetic rates at the V8 growth stage, with greater levels observed at 124,000 plants ha⁻¹ compared

to 74,000 plants ha⁻¹. Consistent hybrid differences related to photosynthesis and stomatal conductance were observed at all growth stages. At V14, stomatal conductance values were similar for both hybrids, but photosynthesis for P1352 was greater than for P1184. At V8, R2, and R5 the photosynthetic rates were similar for both hybrids but P1352 consistently exhibited lower stomatal conductance values. The net photosynthetic rates reported in this study were lower than what previous researchers have reported (Trouverie et al., 2003; Zegada-Lizarazu et al., 2012; Roth et al., 2013), and could be attributed to the non-saturating light intensity selected for measurements. However, biochemical efficiency is maintained even in lower maize leaves acclimated to low light conditions (Bellasio and Griffiths, 2014). These results suggested P1352 was able to maintain photosynthetic rates while using less water, or able to increase photosynthesis at similar stomatal conductance compared to P1184. Improved water use efficiency has been proposed as a mechanism for drought tolerance in AQUAmax hybrids on a biomass and yield level (Cooper et al., 2014), and these results suggest water use efficiency was improved for P1352 compared to P1184 at the cellular level. Under ambient light conditions that occurred midday in Maryland, lower stomatal conductance for hybrids exhibiting drought tolerance were observed (Bunce, 2010). However, other researchers who used a saturating light intensity did not detect photosynthetic and transpirational differences related to hybrid or drought tolerance (Roth et al., 2013).

A W x H x P interaction was observed at V8 for $F_v' F_m'^{-1}$, which was caused by the fluorescence ratio increasing for P1352 under water exclusion and the ratio decreasing for P1184 under the same conditions (Table 13). This difference was also

responsible for causing the H x P interaction at V8. This indicated the excitation capture efficiency differed, particularly under water exclusion. Across populations and hybrids, the ratios were lower under water exclusion compared to irrigation at V8 and R5 (Table 13) indicating lower excitation capture efficiency.

The W x H x P interaction for Φ_{II} at V8 was a result of the ratio decreasing only for P1184 under water exclusion as population increased (Table 13). At both V8 and V14, P1184 exhibited greater Φ_{II} than P1352 but this difference was not evident at R2 and R5. The ratio decreased for both hybrids at the R5 growth stage as population increased to 124,000 plants ha⁻¹. These results suggested photosynthetic efficiency was affected by water exclusion early in the implementation stage (V8) and late in the season (R5). Additionally, the hybrids varied in their efficiency in the vegetative stages, but this difference was not detected during the reproductive stages. The increased population may have increased plant stress late in the season, resulting in a lower ratio at 124,000 plants ha⁻¹.

Relative chlorophyll content, leaf N concentration, and pigment concentrations were not affected by water exclusion at any stage, but were affected by population and differed among hybrids (Table 14). A H x P interaction for RCC at R2 was attributed to the magnitude of decrease in RCC being greater for P1352 than P1184 in response to increasing plant population. At all growth stages, RCC, leaf N concentration, and concentration of chlorophyll *a*, *b*, and total carotenoids were greater for P1184 compared to P1352, and decreased as the population increased. The pigment concentrations were slightly less than previously reported in maize (Daughtry et al., 2000), but the analyzed

samples in this study homogenized all green leaf tissue and had been dried at 60°C. Dried tissue has been shown to have 5 to 7% lower pigment concentrations compared to fresh tissue (Harriman, 1930). Content of chlorophyll *a*, *b*, and total carotenoids exhibited a Y x H x P interaction, which was driven by a significant decrease in concentration as population increased for P1352 and not P1184 in 2013, and a significant decrease for P1184 and not P1352 in 2014. The concentrations increased slightly with population for P1184 in 2013 (not significant), but in 2014 the concentration decreased for both hybrids. The ratio of chlorophyll *a* to chlorophyll *b* was similar across populations, and was greater in P1352 than P1184 (Table 14). The ratio of total chlorophyll to total carotenoids was lower in P1352 than P1184, and was unchanged as population increased. This suggested a greater level of chlorophyll *b* relative to chlorophyll *a* in P1184 compared to P1352, but the carotenoid content in P1352 was greater relative to chlorophyll content. Carotenoids are important to protect cells from photooxidation and dissipating energy through non-photochemical quenching (Pogson and Rissler, 2000), and P1352 may have greater carotenoid concentrations to help dissipate excess light energy as a result of fewer electrons being available for photosynthesis due to lower water use.

Flowering Synchrony, Grain Yield, and Grain Quality

Watering treatment did not influence the ASI, but water exclusion decreased grain yields across hybrids and populations by 15%. P1352 had a shorter ASI than P1184 by almost an entire day (Table 15), but did not result in a greater yield. Hybrid P1352 grain yield was 8% less than P1184. Increasing plant population from 74,000 to 124,000 plant

ha⁻¹ lengthened ASI by 1.5 days, and reduced yield by 7%. Previous research demonstrated shorter ASI can increase yield (Otegui, 1997; Boomsma et al., 2009), but these results suggest ASI alone may not be an accurate predictor of grain yield. Stalk lodging at harvest was greatest for P1352 under water exclusion at 124,000 plants ha⁻¹ (Table 15), which suggested this hybrid may not be as tolerant of high populations under water-limited conditions.

The grain moisture at harvest was similar regardless of treatment (Table 15). The kernel weight was reduced under water exclusion compared to the irrigated treatment, and was lower for plants grown at 124,000 plants ha⁻¹ compared to those at 74,000 plants ha⁻¹. Similar reductions in kernel weight have been observed with increasing population (Eichenberger et al., 2015). Hybrid P1352 had greater kernel weights than P1184, but produced fewer kernels per row and kernels per plant. Other researchers have demonstrated that plants with fewer kernels have heavier kernels (Borrás and Otegui, 2001). Both hybrids exhibited similar grain quality profiles, and were not influenced by increasing plant density (Table 16). Water exclusion (across hybrids and populations) increased grain protein content from 95.9 to 100.9 g kg⁻¹ and decreased starch content from 703 to 697 g kg⁻¹, but no change in oil content was measured. The effect of water exclusion on grain quality changes can be attributed to the dilution effect of the grain yield increase; previous researchers have documented a decrease in protein and an increase in starch as grain yield increases (Genter et al., 1956; Thomison et al., 2004).

Plant Growth Parameters Correlated to Grain Yield

Significant correlations between grain yield and multiple parameters were observed (Table 17). However, many of the parameters exhibited varying degrees of correlation to one another. The results of the principle component analysis demonstrate that the first five components of the 39 evaluated explained 67% of the variation within the study (Table 18). Based on the greatest eigenvectors, PC1 represented the leaf characteristics with heavy loadings ($>|0.23|$) for RCC at V8, R2, R5, chlorophyll *a* and *b* concentration, LAI and SLA. The second PC could be summarized as early-season photosynthetic and flowering efficiency, with heavy loadings from total carotenoids, $F_v' F_m'^{-1}$ at V8 and V14, stomatal conductance at V8, photosynthesis at R2, and ASI. The third PC was soil moisture and plant stress during grain fill due to heavy loadings for soil moisture at R2 and R5, as well as photosynthesis and $F_v' F_m'^{-1}$ at R5. Both fluorescence ratios at R2 and gas exchange parameters at R5 contributed to PC4, (grain fill productivity). Finally, PC5 loaded heavily for biomass at V8 and R2 as well as soil moisture at V14 and R2 (soil moisture and vegetative productivity prior to grain fill).

Using the PC scores calculated for each plot, only PC1 (leaf characteristics; $r = 0.624$) and PC3 (soil moisture and plant stress during grain fill; $r = 0.506$) correlated to grain yield ($P = 0.001$). Additionally, ordination plots for each PC exhibited clusters for each hybrid, population, and watering treatment (Figure 6a-d). Hybrid P1184 separated from P1352 for PC1 and PC2, and 74,000 plants ha⁻¹ from 124,000 plants ha⁻¹ for PC1, PC2, and PC4. Separation of watering regimes was most evident for PC3.

CONCLUSIONS

Many differences were observed between the drought-tolerant and conventional hybrids, ranging from physiological (i.e., chlorophyll fluorescence, stomatal conductance) to morphological (i.e., LAI, ASI). The drought tolerant hybrid consistently exhibited lower RCC, pigment concentrations and ratios, stomatal conductance, and fluorescence ratios early in the season than the conventional hybrid, but also exhibited greater SLA and LAI than the conventional hybrid. However, the decreased stomatal conductance and greater leaf area did not improve grain yield; grain yield was 8% less in the drought-tolerant hybrid compared to the conventional hybrid. Increasing plant population influenced both hybrids similarly and decreased yield by 7%, but the drought-tolerant hybrid experienced significantly more lodging at 124,000 plants ha⁻¹ as compared to the conventional hybrid. These results suggested the drought-tolerant hybrid in this study may not tolerate high plant populations as well as the conventional hybrid, and the drought tolerance traits may not be advantageous in well-watered conditions.

Differences in soil moisture during the reproductive growth stages were observed between water exclusion treatments, but soil moisture did not fall to or below the permanent wilting point at any stage. While visual symptoms associated with severe drought stress (i.e., leaf rolling, early leaf senescence) were not observed, differences in plant available moisture were recorded and grain yield was reduced by 15% when water was excluded. Additionally, the watering treatment influenced grain quality by decreasing starch content and increasing protein levels under exclusion but differences associated with drought tolerance were not evident. Initiating the mulch earlier in the season may be more effective in reducing spring soil moisture to achieve stress during

reproductive stages, but could influence seedling development due to increased light reflection and soil temperatures. Future research should investigate the timing of stress initiation to further evaluate the method of plastic for water exclusion.

TABLES AND FIGURES

	OM	CEC	pH	P	K	Soil	Field	Permanent	Available
						Bulk	Capacity	Wilting	Water
						Density		Point	Content
	g	cmol(+)		mg kg ⁻¹		g cm ⁻³		g kg ⁻¹	
	kg ⁻¹	kg ⁻¹							
2013	23	13.8	6.5	32	94	1.40	252	99.8	152
2014	24	13.6	6.1	67	183	1.50	283	123	160

Table 9. Initial soil organic matter (OM), cation exchange capacity (CEC), pH, P (Bray P₁), K, and physical properties from 2013 and 2014.

Month	Average Temperature			Total Precipitation (Irrigation) ⁶		
	2013	2014	30-yr average	2013	2014	30-yr average
	-----°C-----			-----mm-----		
May	17.8	17.3	16.0	77.0	147	118
June	21.2	22.0	21.0	111	132	105
July	22.3	20.4	22.7	143 (30.8)	94.0 (48.9)	118
Aug.	21.3	21.9	21.9	50.0 (30.8)	91.4 (80.7)	81.8
Sept.	18.3	17.6	17.9	79.8 (13.2)	34.3	83.8
Oct.	11.9	11.5	11.5	118	68.6	72.6
Season	18.8	18.4	18.5	579 (74.8)	567 (129.6)	581

Table 10. Average temperature, cumulative precipitation, and irrigation events for 2013 and 2014.

⁶ Total precipitation for the month is the first value, and the value in parenthesis is the total irrigation applied within the month. Absence of the parenthetical value denotes no irrigation was applied in that month. One irrigation event per month (18 July, 20 Aug., 5 Sept.) occurred in 2013. In 2014, two events occurred in July (17 and 18 July) and three events in Aug. (6, 11, and 26 Aug.).

Water	H	P	Light Interception			Green	LAI	SLA
			V8	V14	R2	Leaf Number	R2	R2
			10 ³				m ² m ⁻²	cm ² g ⁻¹
			ha ⁻¹					
IRR	P1184	74	0.690	0.887	0.955	12.7	3.78	115
		124	0.720	0.960	0.986	12.8	4.83	120
	P1352	74	0.780	0.929	0.963	13.0	4.07	126
		124	0.853	0.954	0.975	12.6	6.30	143
EX	P1184	74	0.624	0.853	0.940	13.0	3.64	115
		124	0.687	0.933	0.970	12.6	4.91	117
	P1352	74	0.733	0.923	0.965	13.1	4.30	127
		124	0.854	0.961	0.984	12.7	6.39	140
		W	0.129	0.311	0.298	0.605	0.772	0.673
		H	<0.001	0.009	0.045	0.777	<0.001	<0.001
		LSD _{0.05}	0.037	0.024	0.009		0.44	5.4

continued

Table 11. Light interception, green leaf number, leaf area index (LAI), and specific leaf area (SLA) for each hybrid (H) at both populations (P) under irrigation (IRR) and water exclusion (EX).

Table 11. continued

	Light Interception			Green Leaf Number	LAI	SLA
	V8	V14	R2	R2	R2	R2
P	<0.001	<0.001	<0.001	0.102	<0.001	0.003
LSD _{0.05}	0.037	0.024	0.009		0.44	5.4
H x P	0.158	0.059	0.085	0.481	0.028	0.0411
LSD _{0.05}					0.88	7.8
W x H x P	0.836	0.910	0.661	0.400	0.671	0.997

W	H	P	Net Photosynthesis				Stomatal Conductance			
			V8	V14	R2	R5	V8	V14	R2	R5
			10 ³ -----μmol CO ₂ m ⁻² s ⁻¹ -----				-----mmol H ₂ O m ⁻² s ⁻¹ -----			
			ha ⁻¹							
IRR	P1184	74	4.24	3.81	3.90	4.54	403	180	180	143
		124	4.18	4.15	3.88	4.70	423	237	139	123
	P1352	74	4.04	4.66	4.53	4.36	302	176	103	91.2
		124	4.57	4.43	3.91	4.58	338	140	101	94.5
EX	P1184	74	3.87	3.44	4.20	3.90	364	223	145	107
		124	4.54	4.32	4.21	3.80	381	304	183	93.0
	P1352	74	3.97	4.56	3.81	4.01	355	262	102	71.4
		124	5.05	4.41	3.57	4.03	337	137	101	74.9
		W	0.67	0.75	0.758	0.061	0.808	0.205	0.906	0.292
		H	0.37	0.02	0.752	0.957	0.035	0.072	<0.01	<0.01
		LSD _{0.05}		0.49			55		21	17
		P	0.02	0.38	0.458	0.732	0.601	0.850	0.904	0.415
		LSD _{0.05}	0.46							
		H x P	0.27	0.10	0.472	0.839	0.857	0.021	0.994	0.227
		LSD _{0.05}						89		
		W x H x P	0.83	0.62	0.770	0.940	0.625	0.356	0.172	0.839

Table 12. Photosynthetic measurements for each hybrid (H) at each population (P) under irrigation (IRR) and water exclusion (EX) water treatments (W) at V8, V14, R2, and R5.

W	H	P	$F_v' F_m^{-1}$ (Efficiency of open photosystem II centers for excitation capture)				Φ_{II} (Quantum efficiency of photosystem II)			
			10^3	V8	V14	R2	R5	V8	V14	R2
			ha^{-1}							
IRR	P1184	74	0.722	0.717	0.714	0.696	0.704	0.688	0.656	0.636
		124	0.728	0.732	0.718	0.707	0.710	0.681	0.648	0.610
	P1352	74	0.731	0.735	0.723	0.711	0.709	0.667	0.661	0.611
		124	0.728	0.734	0.713	0.716	0.697	0.664	0.619	0.615
EX	P1184	74	0.725	0.722	0.724	0.691	0.714	0.685	0.666	0.617
		124	0.710	0.729	0.729	0.686	0.700	0.679	0.678	0.583
	P1352	74	0.704	0.729	0.723	0.696	0.697	0.674	0.666	0.609
		124	0.723	0.729	0.717	0.691	0.693	0.659	0.636	0.575
		W	0.019	0.656	0.374	0.020	0.267	0.915	0.500	0.125
		LSD _{0.05}	0.008			0.012				
		H	0.804	0.098	0.557	0.023	0.01	0.004	0.136	0.408
		LSD _{0.05}				0.007	0.006	0.011		
		P	0.493	0.190	0.641	0.713	0.077	0.155	0.114	0.046
		LSD _{0.05}								0.022

continued

Table 13. Chlorophyll fluorescence measurements at each stage for each hybrid (H) at each population (P) under irrigation (IRR) or water exclusion (EX) treatment (W).

Table 13. continued

	$F_v' F_m^{-1}$ (Efficiency of open photosystem II centers for excitation capture)				Φ_{II} (Quantum efficiency of photosystem II)			
	V8	V14	R2	R5	V8	V14	R2	R5
H x P	0.025	0.146	0.089	0.680	0.581	0.851	0.081	0.482
LSD _{0.05}	0.008							
W x H x P	<0.01	0.546	0.856	0.697	0.038	0.543	0.851	0.476
LSD _{0.05}	0.011				0.013			

W	H	P	Relative Chlorophyll Content				Leaf N	<i>a</i>	<i>b</i>	<i>c</i>	<i>a:b</i>	<i>t:c</i>
			V8	V14	R2	R5						
		10 ³ ha ⁻¹	-----%-----				g kg ⁻¹	-----μg cm ⁻² -----				
IRR	P1184	74	100	100	100	100	31.8	39.5	12.3	4.67	3.23	11.63
		124	98.2	93.9	96.8	92.5	29.7	36.9	11.4	4.33	3.25	11.34
	P1352	74	94.7	93.5	97.7	91.8	28.4	32.5	9.81	3.75	3.32	11.42
		124	90.0	86.7	87.9	86.0	27.8	26.2	8.08	3.15	3.25	11.19
EX	P1184	74	100	101	101	97.4	31.1	39.7	12.2	4.65	3.26	11.70
		124	96.4	91.4	96.8	87.5	30.4	37.8	11.9	4.38	3.18	11.65
	P1352	74	96.4	96.0	96.1	92.5	28.7	32.2	9.72	3.79	3.32	11.30
		124	90.3	94.8	87.7	79.5	27.7	27.2	8.27	3.34	3.31	10.93

continued

Table 14. Relative chlorophyll content, leaf N concentration, chlorophyll *a* content (*a*), chlorophyll *b* content (*b*), total carotenoid content (*c*), and the ratios of *a* to *b* (*a:b*) and total chlorophyll content (*t*) to *c* for each hybrid (H) at each population (P) under irrigation (IRR) or water exclusion (EX) treatment (W).

Table 14. continued

	Relative Chlorophyll Content				Leaf N	<i>a</i>	<i>b</i>	<i>c</i>	<i>a:b</i>	<i>t:c</i>
	V8	V14	R2	R5		R2				
	-----%-----				g kg ⁻¹	-----µg cm ⁻² -----				
W	0.780	0.456	0.754	0.143	0.849	0.681	0.720	0.579	0.924	0.990
H	<0.001	0.158	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.046	0.051
LSD _{0.05}	1.5		1.7	3.5	0.7	2.0	0.7	0.22	0.06	0.37
P	<0.001	0.034	<0.001	<0.001	0.005	<0.001	0.004	0.001	0.260	0.198
LSD _{0.05}	1.5	5.3	1.7	3.5	0.7	2.0	0.7	0.22		
H x P	0.138	0.482	0.003	0.836	0.394	0.096	0.180	0.312	0.835	0.721
LSD _{0.05}			2.3							
W x H x P	0.708	0.396	0.515	0.483	0.244	0.891	0.814	0.898	0.182	0.601

W	H	P	Stalk Lodging	ASI	Yield	Harvest Moisture	Kernel weight	Kernel rows	Kernels row ⁻¹	Kernels plant ⁻¹	
		10 ³ ha ⁻¹	%	days	Mg ha ⁻¹	g kg ⁻¹	mg	-----count-----			
							kernel ⁻¹				
IRR	P1184	74	0.9	0.97	14.36	225	343	16.1	38.4	617	
		124	3.9	1.76	14.05	217	327	16.2	31.9	517	
	P1352	74	3.6	-0.10	13.11	221	357	16.4	32.6	535	
		124	9.1	-0.33	13.25	216	337	16.0	27.8	442	
	EX	P1184	74	5.2	0.45	12.50	208	320	16.5	34.9	576
			124	9.0	2.75	11.80	200	300	16.1	27.2	447
		P1352	74	5.9	-1.32	12.42	206	330	16.7	34.7	578
			124	31.5	2.04	9.60	204	330	16.3	25.1	406

continued

Table 15. Stalk lodging at harvest, anthesis-silking interval (ASI), grain yield, harvest moisture, and yield components for each hybrid (H) at each population (P) under irrigation (IRR) or water exclusion (EX) water treatment (W).

Table 15. continued

	Stalk Lodging	ASI	Yield	Harvest	Kernel	Kernel	Kernels	Kernels
	%	days	Mg ha ⁻¹	g kg ⁻¹	mg kernel ⁻¹	rows	row ⁻¹	plant ⁻¹
						-----count-----		
W	0.23	0.59	0.015	0.063	0.046	0.280	0.105	0.188
LSD _{0.05}			1.34		20			
H	0.01	0.02	0.020	0.900	0.021	0.519	0.002	0.005
LSD _{0.05}	5.6	1.15	0.89		14		1.9	32
P	0.002	0.01	0.042	0.280	0.027	0.117	<0.001	<0.001
LSD _{0.05}	5.6	1.15	0.89		14		1.9	32
H x P	0.036	0.99	0.334	0.661	0.449	0.467	0.817	0.573
LSD _{0.05}	7.9							
W x H x P	0.09	0.36	0.143	0.875	0.357	0.422	0.266	0.439

Water	H	P	Grain Protein	Grain Oil	Grain Starch
		10^3 ha^{-1}	-----g kg ⁻¹ -----		
IRR	P1184	74	97.6	37.5	702
		124	96.3	37.6	704
	P1352	74	95.5	37.2	704
		124	94.1	37.4	704
EX	P1184	74	99.3	37.9	698
		124	102.6	37.6	697
	P1352	74	100.0	38.5	698
		124	101.9	38.2	695
		W	0.011	0.187	0.028
		LSD _{0.05}	2.8		5
		H	0.235	0.564	0.599
		P	0.494	0.945	0.521
		H x P	0.674	0.980	0.502
		W x H x P	0.711	0.998	0.893

Table 16. Grain quality measurements for each hybrid (H) at each population (P) under irrigation (IRR) or water exclusion (EX).

Parameter	Stage	r, P -value	Parameter	Stage	r, P -value
Dry Biomass	V8	0.230, 0.153	Photosynthesis	V8	-0.061, 0.851
	V14	0.519, <0.001		V14	-0.295, 0.064
	R2	-0.010, 0.953		R2	0.473, 0.002
		R5		0.327, 0.039	
Percent Light Interception	V8	-0.130, 0.425	Stomatal Conductance	V8	-0.180, 0.265
	V14	-0.192, 0.236		V14	0.109, 0.505
	R2	-0.221, 0.170		R2	0.256, 0.111
		R5		0.605, <0.001	
LAI	R2	-0.432, 0.005	$F_v' F_m^{-1}$	V8	-0.123, 0.448
SLA	R2	-0.435, 0.005		V14	0.223, 0.166
				R2	0.405, 0.010
Chlorophyll <i>a</i>	R2	0.359, 0.023	R5	0.286, 0.073	
Chlorophyll <i>b</i>	R2	0.289, 0.070	Φ_{II} (Quantum efficiency of photosystem II)	V8	0.158, 0.331
Total Carotenoid	R2	-0.009, 0.958		V14	0.442, 0.004
Leaf N Concentration	R2	0.182, 0.262		R2	0.351, 0.026
				R5	0.261, 0.103

continued

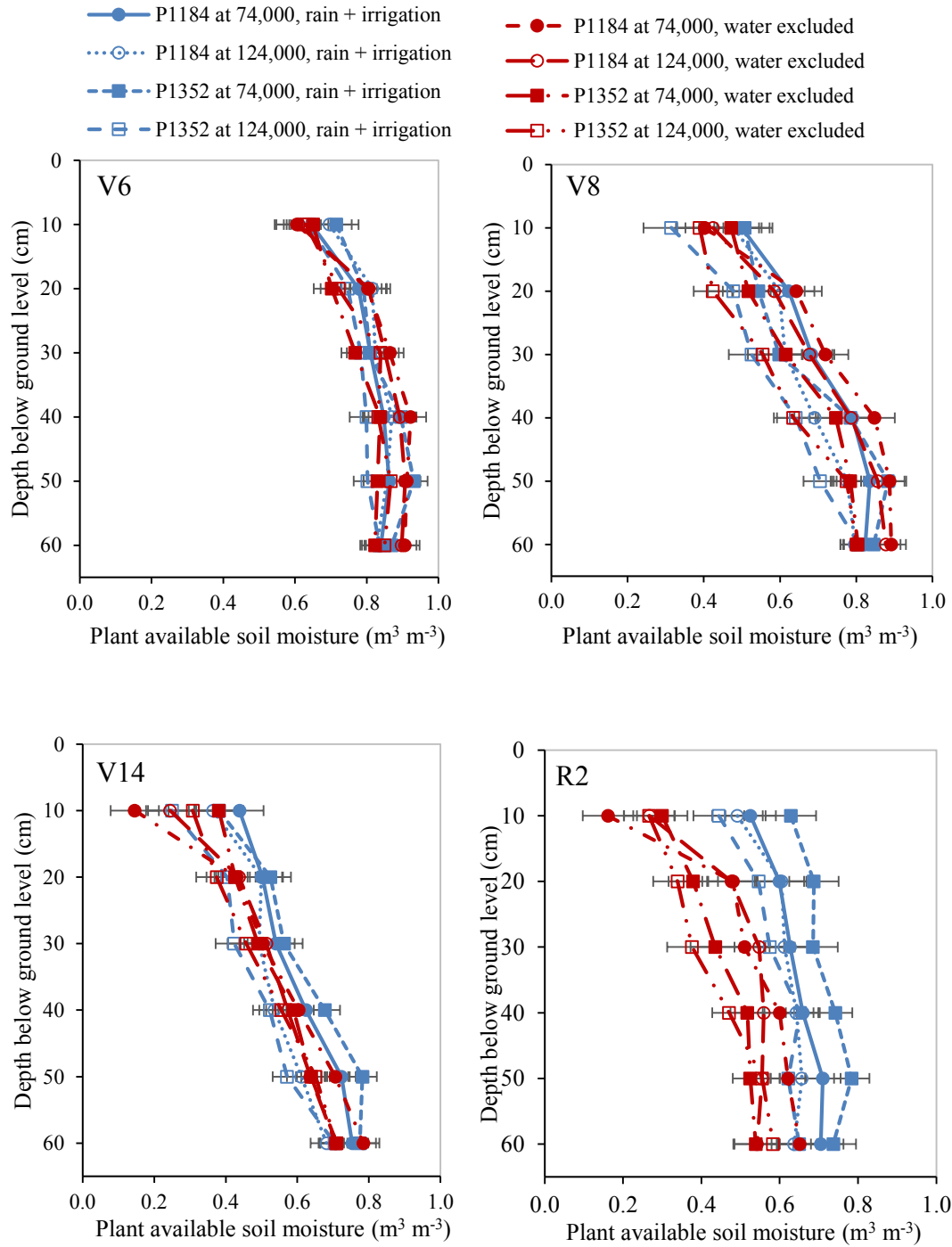
Table 17. Pearson correlation coefficients (r) between different growth parameters and grain yield.

Table 17. continued

Parameter	Stage	<i>r</i> , <i>P</i> -value	Parameter	Stage	<i>r</i> , <i>P</i> -value
Relative Chlorophyll Content	V8	0.412, 0.008	Anthesis	VT	-0.470, 0.003
			Silking	to R1	
			Interval		
	V14	-0.014, 0.931	Soil Moisture	V14	0.376, 0.017
	R2	0.401, 0.010		R2	0.497, 0.001
	R5	0.590, <0.001		R5	0.263, 0.102

	Eigenvalue	Proportion of Variation Explained	Cumulative Variation Explained	Factor Represented
1	10.6088	0.2720	0.2720	Leaf characteristics
2	7.1932	0.1844	0.4565	Early-season photosynthetic and flowering efficiency
3	3.5872	0.0920	0.5484	Soil moisture and plant stress during grain fill
4	2.8446	0.0729	0.6214	Grain fill productivity
5	2.0724	0.0531	0.6745	Soil moisture and vegetative productivity prior to grain fill

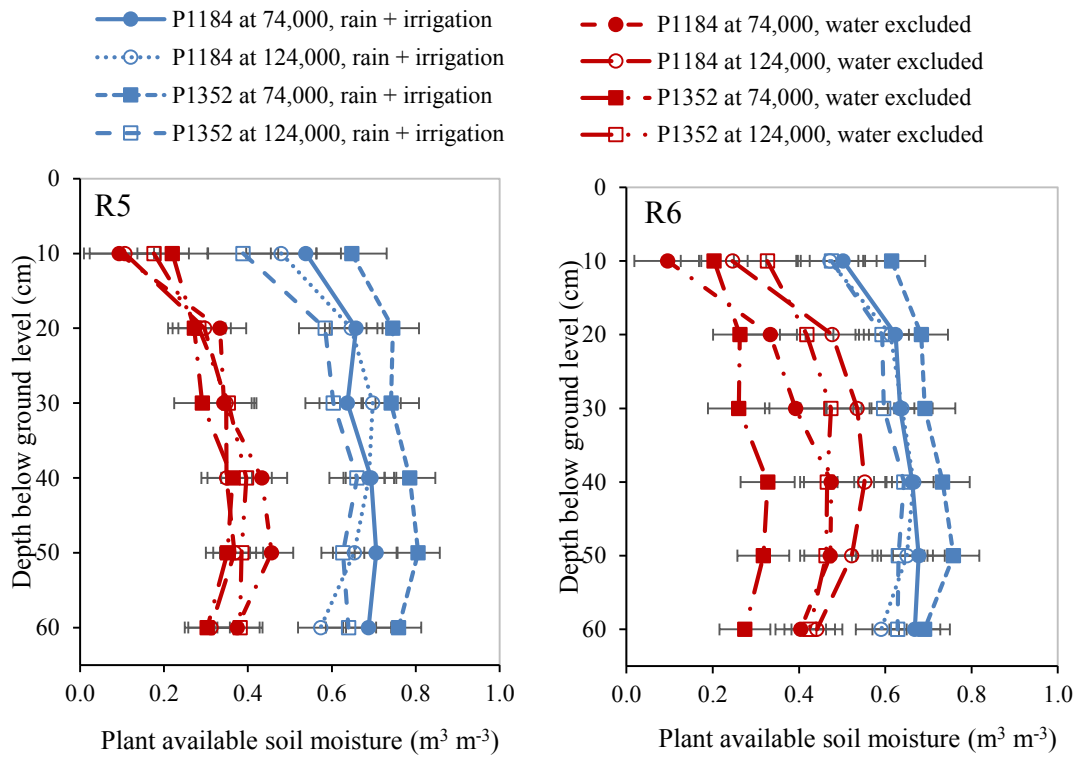
Table 18. The first five Eigenvalues out of 39 evaluated after conducting a principle component analysis of all growth parameters.

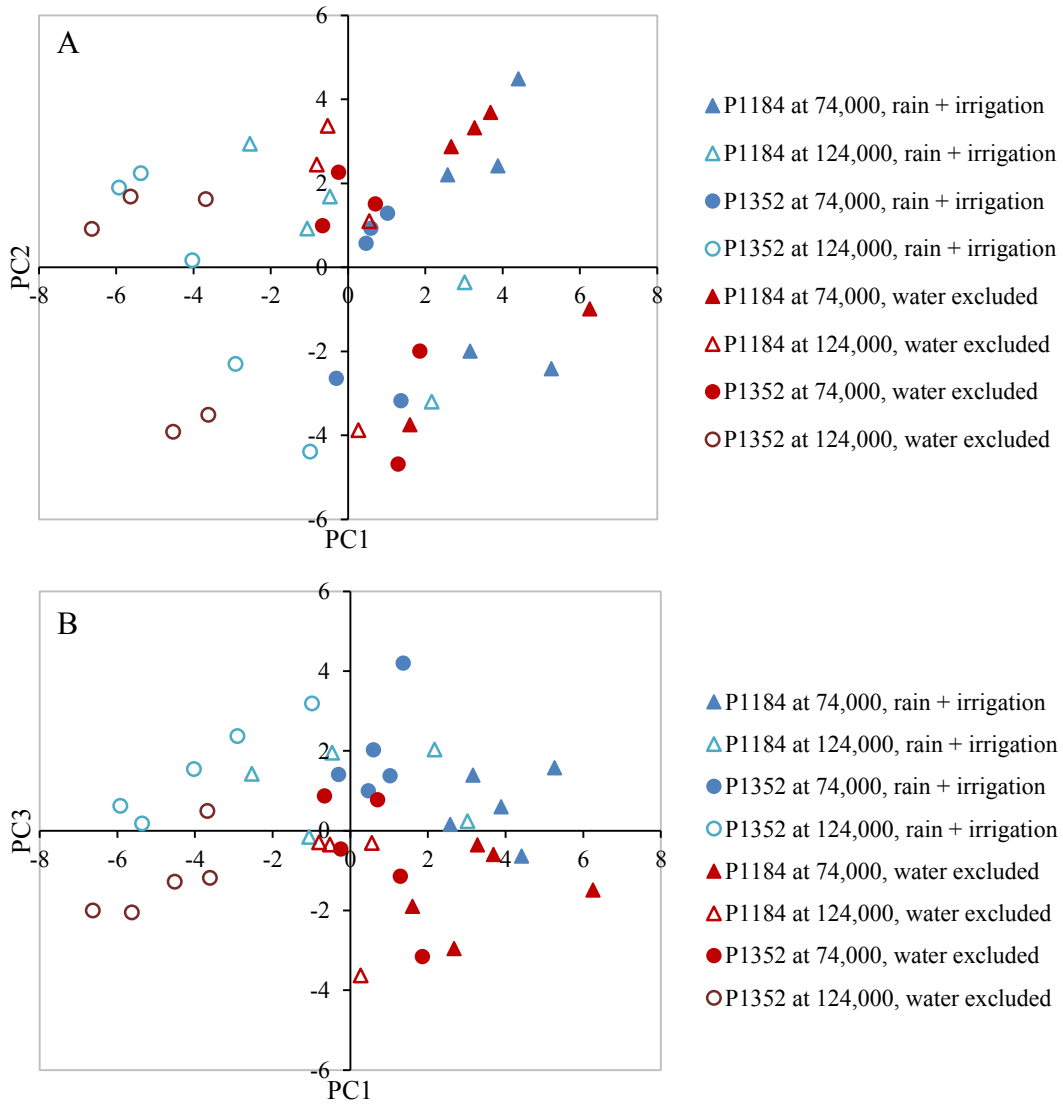


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Figure 5. Plant available soil moisture for each treatment at V6, V8, V14, R2, R5, and R6 growth stages.

Figure 5. continued

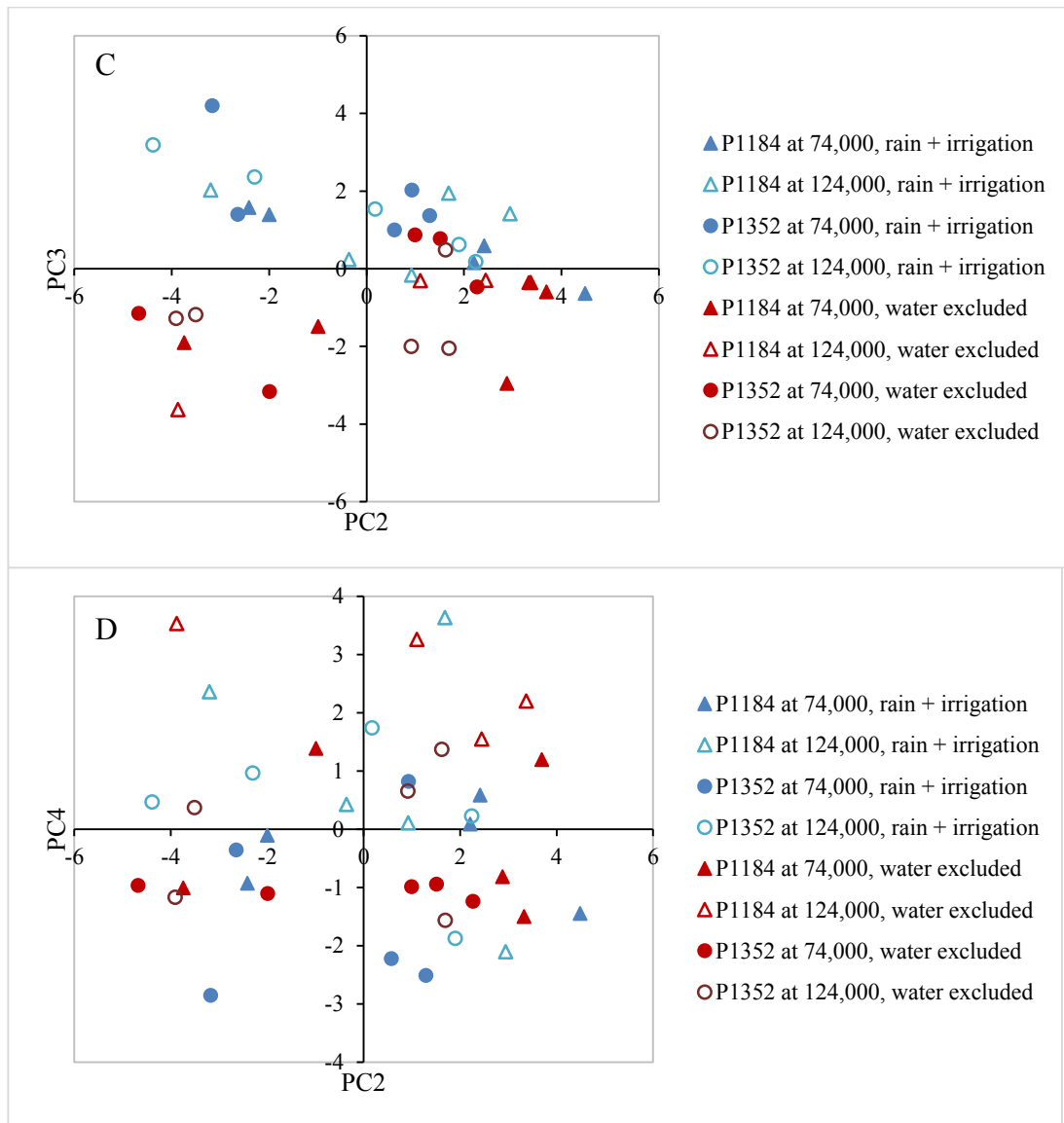




continued

Figure 6. A: Ordination plot of the leaf characteristics component (PC1) against the early-season photosynthetic and flowering efficiency component (PC2). B: Ordination plot of the leaf characteristics component (PC1) against the soil moisture and plant stress during grain fill (PC3). C: Ordination plot of the early-season photosynthetic and flowering efficiency component (PC2) against the soil moisture and plant stress during grain fill (PC3). D: Ordination plot of the early-season photosynthetic and flowering efficiency component (PC2) against the grain fill productivity (PC4).

Figure 6. continued



CHAPTER 4: DROUGHT-TOLERANT MAIZE (*ZEA MAYS* L.) HYBRID AND RELATIVE MATURITY YIELD RESPONSE TO PLANT POPULATION AND PLANTING DATE

INTRODUCTION

Several climatic models have predicted precipitation events will become more erratic with increasing temperatures (Lobell et al., 2008), and the second half of the 21st century is predicted to be drier than any period on geologic record in the U.S. Central Plains and Southwest (Cook et al., 2015). The concerns regarding water availability has led to the development and release of new drought-tolerant maize hybrids (Cooper et al., 2014). Drought tolerance can be defined as the ability of a hybrid to minimize the yield reduction due to water stress compared to non-stressed conditions (Clarke et al., 1992). Initially marketed for use in the U.S. Central Plains, the hybrids have increasingly been marketed in the Eastern U.S. Corn Belt. Research from Kansas suggested drought-tolerant hybrids provided a yield advantage over conventional hybrids when the yield potential was below 8.5 Mg ha⁻¹ (Ciampitti et al., 2015). Cooper et al. (2014) demonstrated an AQ hybrid produced 1.0 to 3.0 Mg ha⁻¹ greater yield compared to a conventional hybrid during drought, but was 0.3 to 1.0 Mg ha⁻¹ lower yielding than the conventional hybrid under favorable conditions across multiple US locations. Across 2,006 locations experiencing drought

conditions, Optimum® AQUAmax® (AQ) hybrids, a non-transgenic drought-tolerant hybrid type from Pioneer®, produced greater grain yield than their conventional hybrid counterparts by 6.5% (Gaffney et al., 2015). Additionally, yield was 1.9% greater for AQ hybrids under favorable conditions (8,725 locations) compared to conventional hybrids. However, limited agronomic research has been conducted on these hybrids in Eastern environments regarding adequate plant populations, planting date, and how comparative relative maturity (CRM) will influence agronomic management (Roth et al., 2013; Gaffney et al., 2015).

A change in frequency or distribution of precipitation throughout the growing season could drastically impact crop production. Increased rain during April and May could delay maize planting into June, which has been shown to decrease yields by 15% (Van Roekel and Coulter, 2011) and delay flowering (Nielsen et al., 2002). Elevated temperatures can delay male flower emergence, resulting in a decrease in pollination and yield reduction (Edriera et al., 2011). If flowering efficiency is improved with drought-tolerant hybrids, they may tolerate late planted conditions better than conventional hybrids. Delayed planting has been shown to decrease yield of full-season hybrids more drastically than short-season hybrids (Kratochvil et al., 2005; Tsimba et al., 2013), so evaluating multiple maturities of drought-tolerant hybrids should be conducted.

The plant population necessary to maximize maize grain yield per unit area has increased over time, with 43% of Ohio maize fields having a final plant population of greater than 74,000 plants ha⁻¹ (USDA-NASS, 2014). Research in

Indiana observed an increase in grain yield for populations ranging from 54,000 to 104,000 plants ha⁻¹ (Ciampitti and Vyn, 2011), with a second study only exhibiting a population response in one of three years where yield was maximized at 81,000 plants ha⁻¹ (Robles et al., 2012). Maximum yield was achieved at 88,000 plants ha⁻¹ in one of two years, with no response to increasing population in the second year in New York (Cox and Cherney, 2012). The response to plant population may differ for drought-tolerant hybrids as compared to conventional hybrids. Cooper et al. (2014) observed that an AQ hybrid exhibited a positive yield response when population increased from 30,000 to 80,000 plants ha⁻¹ under both favorable and stress conditions, whereas a conventional hybrid only had a positive yield response under favorable conditions. A similar evaluation observed greater yield for AQ hybrids compared to conventional hybrids for populations ranging from 19,768 to 69,188 plants ha⁻¹ (Gaffney et al., 2015). Two AQ hybrids (111 and 114-d CRM) in Indiana exhibited a similar yield response as two conventional hybrids of similar CRM when population increased from 79,000 to 104,000 or 109,000 in both favorable and stress conditions (Roth et al., 2013).

There is also limited research on the yield response of AQ hybrids to increasing population in late-planted conditions. Research in Minnesota observed similar responses to population when planting was delayed (Van Roekel and Coulter, 2011), but research from Ohio suggests in some regions optimum yield was achieved at lower plant populations (Lindsey et al., 2015). Grain protein, oil, and starch content of drought-tolerant hybrids may also differ due to differences in metabolism

associated with drought tolerance, and also exhibit a differential response to increasing plant population. Therefore, the objectives of this study were to: 1) investigate any yield advantage or disadvantage associated with drought-tolerant hybrids under May and June planting dates; 2) evaluate drought-tolerant hybrid response to plant population, and determine if the optimum population varies with planting date; and 3) determine the effect of maturity group on optimizing plant population under May and June planting dates.

MATERIALS AND METHODS

A field experiment was conducted in 2012, 2013, and 2014 at the Northwest Agricultural Research Station in Hoytville, OH (NWARS; 41°13'N, 83°45' W; 212 m elevation), the Western Agricultural Research Station in South Charleston, OH (WARS; 39°51'N, 83°40' W; 333 m elevation), and the Ohio Agricultural Research and Development Center in Wooster, OH (WST; 40°47'N, 81°50' W; 368 m elevation). The soil at NWARS was a Hoytville silty clay loam (fine, illitic, mesic Mollic Epiaqualfs) in 2012 and 2014 and a Hoytville clay loam in 2013. The soil was a Kokomo silty clay loam (fine, mixed, superactive, mesic Typic Argiaquolls) in all years at WARS, and at WST the soil was a Canfield silt loam (fine-loamy, mixed, active, mesic Aquic Fragiudalfs) all years. The soil chemical properties are listed in Table 19. Tillage was conducted as needed to prepare the seedbed for planting, and insects and weeds were controlled as needed to minimize interference.

The treatment design within each planting date was a two-way factorial of plant population and hybrid. The planting date factor, May or June planting, was replicated over time with year serving as the replicate. Within each planting date, the experimental design was a split-plot randomized complete block with four replications of the whole-plot factor within each plant date. The whole-plot factor was plant population with five levels (59,000, 74,000, 89,000, 104,000, and 124,000 plants ha⁻¹). Four Pioneer brand hybrids were randomized within each plant population as the sub-plot factor, and are listed in Table 20. Two hybrids had early CRMs for Ohio environments [considered short-season (Short)], and two had late CRMs [termed full-season (Full)]. One of the hybrids within each pair was marketed as a non-transgenic drought-tolerant AQ hybrid [hereafter referred to as drought-tolerant (Tol)], and had a greater drought tolerance rating (9 to 1 scale, 9=high drought tolerance) in company literature as compared to the non-AQ counterpart [referred to as conventional (Con)]. In 2012, the genetics of the refuge included in the bag for the traits with an “AM” designation were the same as the main hybrid aside from the resistance traits. Each sub-plot (7.6 x 3.1 m) consisted of four maize rows (76-cm spacing).

Planting, flowering, and harvest dates are indicated in Table 21. Plots were fertilized with 45 kg N, 20 kg P, and 37 kg K ha⁻¹ at planting using starter fertilizer in 2012 and 2013, and 30 kg N, 27 kg P, and 0 kg K ha⁻¹ in 2014. Plots at WARS received a preplant anhydrous ammonia (82N-0P-0K) N application of 202 kg N ha⁻¹. At NWARS and WST, plots were sidedressed at V4-V6 with 180 to 190 kg N ha⁻¹

using 28% urea-ammonium nitrate (28N-0P-0K). Stand counts were collected at V5-7 based on the method described by Abendroth et al. (2011) on the center two rows and were thinned if necessary to achieve desired plant populations. Ten SPAD 502c Meter (Konica Minolta, Chiyoda, Japan) readings per plot were collected at R5 in 2012 and at V10, R2, and R5 in 2013 and 2014. Relative chlorophyll content (RCC) within each site was determined by dividing all SPAD values by the treatment with the greatest SPAD value, which in all cases was for the conventional hybrids grown at 59,000 plants ha⁻¹. The uppermost collared leaf was measured at V10, and the uppermost ear-leaf was measured at R2 and R5. Visual ratings of drought stress were conducted when symptoms were present using a 9.0 to 1.0 scale, where 9.0 as non-stressed and 1.0 as severe rolling, senescence, and irreversible damage lower leaf senescence (J. Schussler, personal communication, 2012).

The anthesis-silking interval (ASI) of each plot was measured from VT-R1. Anthesis was evaluated by counting the number plants in the center two rows exhibiting tassels, and silking was evaluated by counting the number of plants in the center two rows with silks emerged. Each plot was counted at least three times for tassel and silk emergence. The data from each plot was used to develop a linear regression for tassel emergence and silk emergence, and the slope of these equations were used to compute the calendar date when 50% tassels had emerged and when 50% of the silks had emerged. The difference between these calendar dates was considered the ASI for the plot. Cumulative precipitation and growing degree units

(GDU; 30°C upper limit and 10°C lower limit) were calculated for each location each year (Table 22).

After R6 (physiological maturity), all plots were rated for stalk lodging (stalk breakage below the ear) at harvest. Grain yield was collected from the center two rows of each plot using a plot combine, and grain moisture at harvest was also collect. Reported yields have been adjusted to 155 g kg⁻¹ moisture. Samples of the grain from each plot were evaluated for protein, oil, and starch content using an Infratec NIR grain analyzer (Foss Tecator, Höganäs, Sweden) and for kernel weight.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). Data were standardized prior to analysis by dividing each point by the grand mean within each environment (E, each site-year) to fully determine treatment effects. An ANOVA was conducted using the MIXED model, where E and replication (Rep) nested within E x plant date (PD) were set as random factors, with E x PD set as the error term for PD, and population (P) x Rep (E x PD) as the error term for P. Fixed factors within the model were PD, P, and either drought tolerance classification (D) or maturity group (M). When analysis was conducted for each site, year was used within the model instead of E. When the Global *F*-test was significant ($\alpha = 0.05$), means were separated using Fisher's protected least significant difference (LSD).

To measure the agronomic optimum plant population (AOPP) and maximum yield at AOPP (AOMY), a quadratic model was used and the first order derivative

was used to calculate optimum population using the NLIN procedure. Prior to analysis, yields were standardized within each PD for each environment. The adjustment of AOPP and AOMY for the economic optimum plant population (EOPP) and maximum yield at EOPP (EOMY) was made by subtracting the ratio of seed cost (assumed as \$300 80,000 kernels⁻¹) to grain price (\$157 Mg⁻¹) from the first order constant. Grain quality components were analyzed for linear correlation using the CORR procedure.

RESULTS AND DISCUSSION

Weather Conditions

Cumulative precipitation was above average for all planting dates and sites in 2013 and the May planting at NWARS in 2012, but was below average for all other sites and planting dates (Table 22). The distribution of rainfall within the season varied, and was never above average during both the V10-R2 and R2-R5 growth periods (Table 22). Only at WST in 2012 was the May planting below average during both stages. Visually, drought symptoms (i.e., change in leaf color, leaf rolling) only occurred in 2012 and at V10 in the NWARS June 2014 planting. The drought tolerant hybrids received lower (0.2) or equivalent (within 0.1) ratings at V10 and V14 compared to the conventional hybrids in when planted in May in 2012 (Table 23). The drought-tolerant hybrids received ratings 0.2-0.8 higher than the conventional hybrids at the R2 stage when planted in May and in all stages when planted in June (Table 23). The ratings for all hybrids at V10 and V14 at NWARS

decreased by 0.9 to 1.3 when population increased from 59,000 to 124,000 plants ha⁻¹, but increased by 0.3 at R2 for the 2012 planting at WARS. Aside from the sites previously discussed, plants appeared to be minimally stressed throughout the growing season.

The May planting in 2012 at all sites accumulated more GDUs than the 30-yr average (Table 22). The lack of symptoms in 2013 and most sites in 2014 may have been from the average or below-average GDU accumulation during the growing seasons. Aside from the May planting at NWARS and WST in 2013, which were slightly above average, the other plantings accumulated below average GDUs. Most of the June plantings failed to accumulate the number of GDUs as required to reach maturity in the company literature, but Nielsen et al. (2002) demonstrated that many hybrids require fewer GDUs to achieve maturity when planting is delayed beyond early May. GDU accumulation during the late-vegetative stages through flowering were below average except for the May planting at NWARS and WARS in 2012. GDU accumulation during the grain-fill period was above average in the May planting at WST in 2013 and both WST and NWARS in 2014. The June planting at WST in 2012 also accumulated above average GDUs.

Plant Growth Measurements

Across sites for all hybrids in all planting dates, as plant population increased RCC decreased from 0.99 at 59,000 plants ha⁻¹ to 0.89 at 124,000 plants ha⁻¹ (data not shown). The response of RCC to increasing population was a linear response,

with every 10,000 plants ha⁻¹ increase decreasing RCC by 0.010 at V10 ($P < 0.001$) and 0.014 at R2 and R5 ($P < 0.001$). At all stages, across planting dates and populations the drought-tolerant hybrids had 2 to 3% lower RCC values than the conventional hybrids (0.92-0.93 vs. 0.94-0.95, respectively). The full-season hybrids also exhibited 3% lower RCC than the short-season hybrids at V10, and 1% lower RCC at R2 and R5 (data not shown). When a PD x D or D x P interaction occurred, it was caused by a difference in the magnitude of the response of each hybrid class to planting date or population. However, the trends discussed were consistent for all sites so only the combined results are discussed.

The ASI response to planting date and population was similar at each site, so the results from across sites are presented. The drought-tolerant hybrids exhibited ASIs that were 0.78 days shorter than the conventional hybrids across planting dates and populations (Table 24). Additionally, the short-season hybrids had ASIs 0.63 days shorter than the full-season hybrids. All hybrids responded similarly to population, with ASI increasing from -0.38 to 0.22 days as population increased from 59,000 to 124,000 plants ha⁻¹. The lack of a significant PD effect or PD x D or PD x M interactions implies that delaying planting until June did not impact the flowering interval for the hybrids.

As population increased, lodging steadily increased for the drought-tolerant hybrids. The drought-tolerant hybrids were more susceptible to stalk lodging in May plantings compared to the conventional hybrids, but lodging was similar when planted in June. The D x P interaction was caused by the conventional hybrids at

104,000 plants ha⁻¹ lodging less than those at 89,000 plants ha⁻¹ whereas the lodging was greater at 104,000 plants ha⁻¹ for the drought-tolerant hybrids (Table 24). Both maturity groups exhibited increased lodging as population increased. The PD x M interaction was a result of the short-season hybrids lodging less than the full-season hybrids in the first planting date, but the maturity groups exhibited similar levels of stalk lodging in the June planting date.

Grain Yield and Optimum Population

Table 25 displays the ANOVA table for grain yield as influenced by PD, D, M, P, and their interactions. No significant no three-way interactions were observed. The two-way interactions between M x P and D x P, as well as PD x M imply the response to population and planting date differed between each hybrid class and maturity group. Previous research conducted with drought-tolerant hybrids observed no relationship between drought tolerance and plant population, but only two site-years were evaluated with two levels of plant population (79,000 and 104,000 or 109,000 plants ha⁻¹) each year (Roth et al., 2013). To allow for regionally-specific recommendations regarding AOPP and EOPP, the regression analysis was conducted for each site in addition to across sites.

The drought-tolerant hybrids consistently produced greater or equivalent AOMY at lower AOPP than the conventional hybrids when planted in May at each site (Table 26). Across sites, when planted in May the AOMY was 0.2 Mg ha⁻¹ greater and the AOPP was 3,600 plants ha⁻¹ less for the drought-tolerant hybrids

compared to the conventional hybrids. This result differed from the hypothesis that drought-tolerant hybrids would maximize yield at greater populations due to improved tolerance to stress. The conventional hybrids at WST planted in May did not exhibit a quadratic yield response to population, but across populations the yield was 0.6 Mg ha^{-1} less than the AOMY of the drought-tolerant hybrids. The kernel weight was greater for drought-tolerant hybrids in the May planting, and grain moisture was similar for the drought tolerant and conventional hybrids (Table 27). Both planting dates decreased kernel weight with increasing population, which has been documented by previous researchers (Eichenberger et al., 2015). When planted in June at WARS and WST, the drought-tolerant hybrids had lower AOMY than the conventional hybrids but maintained the lower AOPP. In June, the AOMY was 0.1 Mg ha^{-1} less but the AOPP was $16,400 \text{ plants ha}^{-1}$ less for the drought-tolerant hybrids. The drought-tolerant hybrids at NWARS planted in June did not have a significant yield response to plant population, but grain yield across populations was 0.2 Mg ha^{-1} greater than the AOMY of the conventional hybrids. Conversely, across populations the drought-tolerant hybrids at WST had 0.7 Mg ha^{-1} lower grain yield than the AOMY for the conventional hybrids. Within the June plant date, the harvest moisture was 10 g kg^{-1} less and the kernel weights were similar for the drought-tolerant hybrids compared to the conventional hybrids (Table 27).

To help visualize any yield advantage from using a drought-tolerant hybrid over a conventional hybrid, the yield of the conventional hybrid (x-axis) was plotted against the yield of the comparable maturity drought-tolerant hybrid (y-axis) in

Figure 7. If the yields of each hybrid under identical growing conditions were similar, a 1:1 relationship would be expected. The regression analysis of the data reveals a linear relationship with a slope that differs from 1.0 (1.0 would be expected if the yields were similar). When the yield of the conventional hybrid was subtracted from the drought-tolerant hybrid yield, the yield advantage of the drought-tolerant hybrid is evident when plotted against the conventional yield (Fig. 8). In environments with yields below 12.2 Mg ha⁻¹, the drought-tolerant hybrids produced greater yields than their conventional counterparts. However, in yield environments above 12.2 Mg ha⁻¹, the conventional hybrids produced greater yields than the tolerant hybrids and a yield disadvantage was observed from the drought-tolerant hybrids. Ciampitti et al. (2015) observed similar relationships between drought-tolerant hybrids and conventional hybrids of similar maturities in Kansas, but observed that above 8.5 Mg ha⁻¹ there was no yield advantage or disadvantage.

The PD x M interaction observed at NWARS in Table 25 can be attributed to the magnitude of difference in AOMY being smaller for the May planting compared to the June planting (Table 26). The quadratic model did not significantly fit the full-season hybrid yield response to population, and the full-season hybrids across populations had a grain yield equivalent to the AOMY of the short-season hybrids. At WARS and WST, the PD x M interaction from Table 25 was because the difference between the AOMY of short-season hybrids and full-season hybrids was greater in May than June. The short-season hybrid average yield at WARS in June was 0.6 Mg ha⁻¹ less than the AOMY for the full-season hybrids, and the May

planted full-season hybrids at WST had 0.6 Mg ha⁻¹ greater yield across populations compared to the short-season hybrids. The grain moisture at harvest was similar for both maturity groups when planted in May, but was 38 g kg⁻¹ greater for the full-season hybrids when planted in June (Table 27). The M x P suggests the short-season hybrids need to be grown at plant populations 5,000 to 15,000 plants ha⁻¹ greater to maximize yields compared the full-season hybrids. Additionally, AOMY for the short-season hybrids were still 300-800 kg ha⁻¹ less at the AOPP regardless of site.

If the cost of seed and harvested grain is included in the analysis (Table 28), the same trends are observed for the EOPP and EOMY as compared to the AOPP and AOMY. The EOPP was approximately 10 to 12% lower than the AOPP. However, this population decrease only resulted in a yield reduction of less than 1%. The EOPP are similar to currently recommended seeding rates for maize production in Ohio (Thomison et al., 2005). Similar to Lindsey et al. (2015), AOPP and EOPP for each planting date differed by location. At NWARS, delaying planting from May to June increased the AOPP across hybrids by 6,700 plants ha⁻¹, but the EOPP decreased (1,300 plants ha⁻¹) across hybrids. Delayed planting at WST increased both the AOPP (11,900 plants ha⁻¹) and the EOPP (9,000 plants ha⁻¹). WARS was the only location that resulted in a lower AOPP and EOPP when planting was delayed from May to June, which is similar to the observations made by Lindsey et al. (2015). Across all locations, delaying planting resulted in an AOPP increase of 1,300 plants ha⁻¹, but decreased EOPP by 3,100 plants ha⁻¹.

Grain Quality

The drought-tolerant hybrids had lower protein levels (88-95 g kg⁻¹) than the conventional hybrids (91-96 g kg⁻¹) (Table 29). The protein level in the May planted conventional hybrids was greater than the June planted drought-tolerant hybrids, resulting in the PD x D interaction. The decrease in grain protein when population increased was greater in the May planting than the June planting, but was similar regardless of hybrid type. Grain oil content was greater in the drought-tolerant hybrids (37.5-40.5 g kg⁻¹) compared to the conventional hybrids (36.4-38.6 g kg⁻¹), and when planting was delayed the grain oil increased more for the drought-tolerant hybrids compared to the conventional hybrids (Table 29). Similarly, Ali et al. (2010) observed one drought-tolerant maize line exhibited lower protein and greater oil concentration when compared to a conventional maize line. Oil content was unchanged with population in the June planting, but decreased with population in the May planting. Starch content was greater in the drought-tolerant hybrids (690-695 g kg⁻¹) compared to the conventional hybrids (691-694 g kg⁻¹), and the increase in starch with increasing population was more evident when planted in May than when planted in June (Table 29).

For both the drought-tolerant and conventional hybrids, a strong negative correlation existed between protein and starch ($r > |-0.53|$, $P < 0.001$) as has been described by multiple researchers (Miller and Brimhall, 1951; Cook et al., 2012). The conventional hybrids exhibited a positive correlation ($r = 0.21$, $P < 0.001$) between oil and protein, as has been documented in previous research (Miller and

Brimhall, 1951; Cook et al., 2012), the drought-tolerant hybrids exhibited the opposite trend ($r = -0.24$, $P < 0.001$). Similarly, Thomison et al. (2004) observed a small negative correlation between oil and protein in hybrids with enhanced grain quality traits. A negative relationship between oil and starch was observed in both the drought-tolerant ($r = -0.19$) and conventional hybrids ($r = -0.27$), which is common of multiple maize genotypes (Cook et al., 2012).

Grain protein levels were greater in the full-season hybrids compared to the short-season hybrids (Table 29). Additionally, the full-season hybrids experienced a greater decline in protein levels as population increased as compared to the short-season hybrids. Grain oil content was slightly greater in the full-season hybrids than the short-season hybrids, and increased to a greater degree with delayed planting (Table 29). Starch content was greater in the short-season hybrids than the full-season hybrids (Table 29). Short-season hybrid starch content increased to a lesser degree than full-season hybrids with population, but increasing population increased starch content for both maturity groups. Regardless of maturity group, negative correlations were observed between all quality components ($P < 0.001$), with a strong negative correlation between protein and starch ($r > |-0.34|$). Short-season hybrids exhibited a strong negative correlation between protein and oil ($r = -0.39$), whereas the full-season hybrids relationship was less strong ($r = -0.19$). There was also a negative relationship between oil and starch for both maturity groups ($r = -0.13$ to -0.18). However, all variation was within 3.0 g kg^{-1} , which may not be biologically significant.

CONCLUSIONS

Drought-tolerant hybrids exhibited shorter ASIs as compared to conventional hybrids, but this did not always translate into improved grain yield. The drought-tolerant hybrids were able to produce AOMY and EOMY at lower AOPP and EOPP as compared to conventional hybrids when planted in May. However, when planting was delayed until June, the conventional hybrids produced greater yields than the drought-tolerant hybrids but AOMY and EOMY was achieved at lower plant populations for the drought-tolerant hybrids. The reduced yield from the June plantings for the drought-tolerant hybrids suggests that the shorter ASI may provide a limited yield benefit, and some other factor is limiting yield (possibly RCC). The drought-tolerant hybrids were also more susceptible to stalk lodging as populations increased as compared to the conventional hybrids, which suggests the drought-tolerant hybrids may not tolerate elevated plant populations. Across all locations, the drought-tolerant hybrid yield was greater than the conventional hybrid yield when the yield potential was below 12.2 Mg ha⁻¹. However, above 12.2 Mg ha⁻¹, the conventional hybrids produced a greater yield than the drought-tolerant hybrids.

The AOPP and EOPP of short-season hybrids were greater than full-season hybrids for all planting dates, but the AOMY and EOMY were greater for the full-season hybrids. As planting date was delayed until June, WST exhibited an increase in AOPP and EOPP, NWARS increased AOPP but decreased EOPP, and WARS decreased both AOPP and EOPP. Across locations, the AOPP needed to increase by

1,300 plants ha⁻¹ across all locations when planting was delayed, but the EOPP decreased by 3,100 plants ha⁻¹ to achieve EOMY. Delaying planting increased harvest moisture, but the difference in moisture between the short-season and full-season hybrids was only 10 g kg⁻¹ greater when planted in June compared to May planting.

In conclusion, drought-tolerant hybrids may provide growers a viable alternative in environments with known yield potential below 12.2 Mg ha⁻¹. Additionally, when planted in May these hybrids would provide a yield advantage with a lower optimum plant population as compared to their conventional counterparts. However, stalk lodging increased when populations were elevated, and the AOMY and EOMY was less with these hybrids when planting was delayed until June. If concerned about hybrid maturity, these results suggest a full-season hybrid will produce greater grain yield at a lower optimum population as compared to a short-season hybrid regardless of planting date.

TABLES AND FIGURES

Site	Year	Previous Crop	OM	CEC	pH	P	K
			g kg ⁻¹	cmol(+) kg ⁻¹	mg kg ⁻¹		
NWARS	2012	soybeans	47	17.3	5.8	52	218
	2013	wheat	30	22.2	6.2	23	185
	2014	soybeans	32	22.4	6.6	20	176
WARS	2012	soybeans	31	16.4	6.0	32	108
	2013	soybeans	31	17.4	6.6	15	133
	2014	soybeans	34	23.7	5.3	37	165
WST	2012	maize	20	8.0	6.1	60	123
	2013	soybeans	13	7.0	5.5	46	145
	2014	soybeans	25	6.5	6.4	54	131

Table 19. Organic matter (OM), cation exchange capacity (CEC), pH, Bray P₁, and K soil levels in 2012-2014 for the Northwest Agricultural Research Station (NWARS), Western Agricultural Research Station (WARS), and the Ohio Agricultural Research and Development Center in Wooster (WST).

Characteristic	Hybrid			
	P0210	P0448	P1184	P1352
GDU to silk (°C)	730	705	770	740
GDU to maturity (°C)	1405	1390	1470	1430
CRM	102	104	111	113
Drought Tolerance	9	7	7	9
Drought Tolerance Classification	Tolerant (Tol)	Conventional (Con)	Conventional (Con)	Tolerant (Tol)
Maturity Group	Short	Short	Full	Full
Traits ⁷	AM-R, YXR, YXR	AMX-R, YXR, YXR	YXR, XR, XR	AMX-R, YXR, CYXR

Table 20. Growing degree units (GDUs) to silk, maturity, and comparative relative maturity (CRM) for each evaluated hybrid. Drought tolerance ratings are listed as determined by the company, with 9 being most tolerant and 1 being most susceptible. Insect and herbicide resistance traits are listed for 2012 to 2014, with commas separating the trait from each year.

⁷ Abbreviations: AM-R, YieldGard® Corn Borer (YGCB), Herculex® 1 (HX1), and Roundup Ready® Corn 2 (RR2); AMX-R, YGCB, HX1, Herculex® XTRA (HXX), and RR2; CYXR, YGCB, Agrisure® RW, HXX, LibertyLink® (LL), and RR2; XR: HXX, LL, and RR2; YXR, YGCB, HXX, LL, and RR2.

Site	Year	PD	Planting Date	Flowering Dates	Harvest Date
NWARS	2012	May	3 May	16-23 July	5 Nov.
		June	7 June	1-9 Aug.	7 Nov.
	2013	May	7 May	9-19 July	22 Oct.
		June	7 June	30 July-9 Aug.	5 Nov.
	2014	May	20 May	16-25 July	29 Oct.
		June	14 June	10-17 Aug.	19 Nov.
WARS	2012	May	14 May	10-19 July	5 Oct.
		June	8 June	30 July-7 Aug.	8 Nov.
	2013	May	20 May	15-22 July	29 Oct.
		June	12 June	1-12 Aug.	25 Nov.
	2014	May	30 May	24-31 July	22 Oct.
		June	16 June	9-15 Aug.	10 Nov.
WST	2012	May	7 May	13-20 July	26 Oct.
		June	6 June	27 July-6 Aug.	11 Nov.
	2013	May	16 May	18-28 July	15 Nov.
		June	15 June	12-19 Aug.	15 Nov.
	2014	May	25 May	26 July-2 Aug.	12 Nov.
		June	13 June	17-23 Aug.	5 Dec.

Table 21. Date of planting, flowering dates, and harvest date for each planting date treatment (PD) in each site-year.

Site	Year	PD	Cumulative Precipitation			Cumulative GDU		
			V10- R2	R2-R5	Season Total	V10- R2	R2- R5	Season Total
			-----mm-----			-----°C-----		
NWARS	2012	May	56.6	139.4	479.3	462	310	1753
		June	161.3	94.2	413.3	382	314	1436
	2013	May	225.6	38.1	571.0	377	335	1615
		June	107.7	19.3	550.2	284	343	1368
	2014	May	48.8	101.6	345.7	376	453	1473
		June	39.9	99.6	317.5	358	338	1206
	30-yr average		106.4	95.7	463.7	464	404	1585
WARS	2012	May	122.7	33.5	354.6	509	230	1661
		June	120.1	8.4	390.9	438	308	1450
	2013	May	131.6	37.3	486.9	340	367	1535
		June	67.6	130.3	495.3	332	405	1338
	2014	May	74.7	90.9	377.2	335	403	1363
		June	34.3	108.0	344.7	299	342	1205
	30-yr average		126.0	77.5	453.7	468	420	1561

continued

Table 22. Cumulative precipitation and growing degree unit (GDU) accumulation for each planting date (PD) in each site-year compared to 30-year average.

Table 22. continued

Site	Year	PD	Cumulative Precipitation			Cumulative GDU		
			V10- R2	R2-R5	Season Total	V10- R2	R2- R5	Season Total
			-----mm-----			-----°C-----		
WST	2012	May	57.4	47.0	369.3	433	269	1580
		June	56.4	119.9	397.3	323	422	1380
	2013	May	165.1	51.1	562.6	347	419	1479
		June	43.4	91.7	476.5	295	315	1222
	2014	May	64.0	111.0	417.1	323	410	1349
		June	136.7	25.9	393.4	351	287	1173
	30-yr average		105.6	97.8	443.6	446	393	1427

PD	D	P	2012						2014		
			NWARS			WARS		WST	NWARS		
			V10	V14	R2	R2	V10	V14	R2	V10	
			10^3								
			ha^{-1}								
May	Tol		3.1	2.5	6.4	7.6	7.3	7.9	7.7	--	
	Con		3.3	2.6	6.3	7.4	7.1	7.9	7.3	--	
	D		0.02	0.72	0.35	0.05	0.18	0.87	<0.01	--	
		59	3.7	3.1	7.0	7.5	7.6	7.8	7.8	--	
		74	3.3	2.8	6.4	7.6	7.6	8.0	7.6	--	
		89	3.2	2.3	6.6	7.5	7.2	7.9	7.1	--	
		104	3.0	2.5	6.1	7.5	6.9	7.9	7.4	--	
		124	2.8	2.0	5.8	7.4	6.6	7.8	7.5	--	
		P		<0.01	0.01	<0.01	0.73	0.21	0.56	0.37	--
	June	Tol		--	7.1	7.6	7.7	6.7	7.8	6.9	7.1
Con			--	6.4	7.0	7.6	6.4	7.6	6.8	7.1	
D			--	<0.01	<0.01	0.26	0.06	0.04	0.55	0.78	
		59	--	7.3	7.4	7.5	6.7	7.8	6.9	7.8	
		74	--	7.1	7.5	7.7	6.6	7.8	6.8	7.3	
	89	--	6.7	7.3	7.4	6.2	7.7	6.9	7.1		

continued

Table 23. Visual drought ratings for the drought-tolerant (Tol) and conventional (Con) hybrids and for each plant population (P) for each rated planting date (PD).

Table 23. continued

PD	H	P	NWARS			2012 WARS		WST			2014 NWARS
			V10	V14	R2	R2	V10	V14	R2	V10	
June		104	--	6.5	7.3	7.8	6.4	7.6	6.5	6.9	
		124	--	6.1	7.2	7.8	6.7	7.6	7.2	6.5	
	P		0.015	0.587	0.028	0.794	0.898	0.521	0.001		

PD	P	ASI		Stalk Lodging		ASI		Stalk Lodging	
		Tol	Con	Tol	Con	Short	Full	Short	Full
	10 ³ ha ⁻¹	days		%		days		%	
May	59	-0.75	0.03	2.2	1.1	-0.76	0.03	2.3	1.0
	74	-0.42	0.36	4.6	1.3	-0.38	0.31	4.1	1.9
	89	-0.14	0.51	7.1	1.8	-0.22	0.59	6.0	2.9
	104	-0.26	0.66	6.4	2.2	-0.11	0.51	5.7	3.0
	124	-0.06	0.71	12.3	3.5	0.09	0.60	10.4	5.3
June	59	-0.86	0.05	6.4	5.7	-0.69	-0.10	5.8	6.3
	74	-0.84	0.23	12.6	12.0	-0.51	-0.07	10.3	14.3
	89	-0.75	0.10	11.7	13.4	-0.48	-0.14	12.8	12.4
	104	-0.13	0.22	14.4	9.7	-0.23	0.35	12.6	11.5
	124	-0.15	0.38	21.1	17.4	-0.35	0.61	18.4	20.2

continued

Table 24. Anthesis-silking interval (ASI) and percent stalk lodging at harvest for the drought-tolerant (Tol), conventional (Con), short-season (Short), and full-season (Full) hybrids across sites for each planting date (PD) and population (P).

Table 24. continued

	ASI		Stalk Lodging			ASI		Stalk Lodging	
	Tol	Con	Tol	Con		Short	Full	Short	Full
PD	0.519		0.212		PD	0.534		0.211	
D	<0.001		<0.001		M	<0.001		0.078	
LSD _{0.05}	0.16		1.0		LSD _{0.05}	0.16			
P	<0.001		<0.001		P	<0.001		<0.001	
LSD _{0.05}	0.27		2.4		LSD _{0.05}	0.27		2.4	
PD x D	0.811		0.006		PD x M	0.518		<0.001	
LSD _{0.05}			1.4		LSD _{0.05}			1.5	
PD x P	0.531		0.056		PD x P	0.550		0.055	
D x P	0.753		0.008		M x P	0.953		0.402	
LSD _{0.05}			2.8						
PD x D	0.419		0.150		PD x M	0.462		0.352	
x P					x P				

Effect	<i>P</i> -value			
	Combined	NWARS	WARS	WST
Planting Date (PD)	0.047	0.982	0.031	0.193
Drought Tolerance Classification (D)	0.252	0.002	0.145	0.730
Maturity Group (M)	<0.001	<0.001	<0.001	<0.001
Population (P)	<0.001	<0.001	<0.001	0.004
PD x D	0.301	0.193	0.378	0.014
PD x M	0.100	0.014	0.004	0.021
PD x P	0.668	0.430	0.092	0.714
D x P	0.012	0.284	0.431	0.115
M x P	0.039	0.002	0.018	0.679
PD x D x P	0.127	0.894	0.854	0.050
PD x M x P	0.660	0.185	0.887	0.314

Table 25. Results of the analysis of variance tests for planting date (PD), drought tolerance classification (D), maturity group (M), population (P), and their interactions on yield both across locations and for each location separately.

Region	PD	D	AOPP	AOMY ⁸	M	AOPP	AOMY
			10 ³ ha ⁻¹	Mg ha ⁻¹		10 ³ ha ⁻¹	Mg ha ⁻¹
Combined	May	Tol	96.5	13.35	Short	100.1	12.93
		Con	100.1	13.18	Full	96.0	13.62
		Avg.	98.1	13.27			
	June	Tol	91.5	11.53	Short	104.1	11.28
		Con	107.9	11.63	Full	96.1	11.83
		Avg.	99.4	11.55			
	Avg.	Tol	94.6	12.43	Short	101.4	12.10
		Con	103.3	12.40	Full	96.1	12.72
		Avg.	98.6	12.40			
NWARS	May	Tol	88.8	11.64	Short	93.0	11.48
		Con	92.9	11.52	Full	NS	11.57
		Avg.	90.9	11.58			
	June	Tol	NS	11.50	Short	102.0	11.12
		Con	105.4	11.33	Full	NS	11.75
		Avg.	97.6	11.48			
	Avg.	Tol	89.6	11.65	Short	95.8	11.28

continued

Table 26. Agronomic optimum plant population (AOPP) and maximum hybrid yield (AOMY) by plant date (PD) for tolerant (Tol), conventional (Con), short-season (Short), and full-season (Full) hybrids across sites and within each site.

⁸ When the AOPP model was not significant (NS), the reported AOMY is the average yield across populations as reported from the ANOVA analysis.

Table 26. continued

Region	PD	D	AOPP	AOMY	M	AOPP	AOMY
			10^3 ha^{-1}	Mg ha^{-1}		10^3 ha^{-1}	Mg ha^{-1}
NWARS	Avg.	Con	96.9	11.41	Full	NS	11.64
		Avg.	93.3	11.52			
WARS	May	Tol	101.5	14.83	Short	116.6	14.41
		Con	104.7	14.91	Full	97.8	15.48
		Avg.	103.0	14.87			
	June	Tol	90.0	11.83	Short	NS	11.53
		Con	100.9	12.11	Full	93.3	12.17
		Avg.	95.2	11.96			
	Avg.	Tol	96.9	12.97	Short	109.2	12.74
		Con	103.1	13.18	Full	96.1	13.46
		Avg.	99.9	13.07			
WST	May	Tol	95.8	14.14	Short	97.4	13.52
		Con	NS	13.50	Full	NS	14.11
		Avg.	97.8	13.90			
	June	Tol	NS	11.08	Short	117.7	11.20
		Con	125.9	11.75	Full	105.0	11.67
		Avg.	109.7	11.42			
Avg.	Tol	95.7	12.68	Short	103.0	12.34	

continued

Table 26. continued

Region	PD	D	AOPP	AOMY	M	AOPP	AOMY
			10^3 ha^{-1}	Mg ha^{-1}		10^3 ha^{-1}	Mg ha^{-1}
WST	Avg.	Con	118.1	12.71	Full	102.5	12.97
		Avg.	103.1	12.65			

PD	P	Harvest		Kernel		Harvest		Kernel weight	
		Moisture		weight		Moisture			
		Tol	Con	Tol	Con	Short	Full	Short	Full
	10^3 ha^{-1}	---g kg ⁻¹ ---		mg kernel ⁻¹		-----g kg ⁻¹ -----		mg kernel ⁻¹	
May	59	214	212	373	358	197	230	358	373
	74	212	212	357	345	197	227	343	359
	89	211	211	340	337	194	227	328	349
	104	211	215	333	328	199	227	323	338
	124	211	216	322	321	198	229	314	330
June	59	261	264	339	330	244	282	326	343
	74	261	268	327	322	246	283	317	333
	89	252	269	310	315	241	280	302	323
	104	261	271	307	310	247	284	299	318
	124	260	273	298	303	246	286	289	311

continued

Table 27. Grain moisture and kernel weight for each drought tolerance classification and maturity group across sites for each planting date (PD) and population (P).

Table 27. continued

	Harvest		Kernel		Harvest		Kernel	
	Moisture		weight		Moisture		weight	
	Tol	Con	Tol	Con	Short	Full	Short	Full
PD	0.008		0.029		PD	0.008		0.029
LSD _{0.05}	34		22		LSD _{0.05}	34		22
D	<0.001		<0.001		M	<0.001		<0.001
LSD _{0.05}	3		2.1		LSD _{0.05}	2		1.9
P	0.358		<0.001		P	0.205		<0.001
LSD _{0.05}			3.3		LSD _{0.05}			3
PD x D	0.004		0.002		PD x M	0.003		0.238
LSD _{0.05}	4		19		LSD _{0.05}	3		
PD x P	0.882		0.036		PD x P	0.875		0.017
LSD _{0.05}			4.7		LSD _{0.05}			19
D x P	0.332		<0.001		M x P	0.935		0.414
LSD _{0.05}			4.6					
PD x D x P	0.744		0.992		PD x M x P	0.981		0.831

Region	PD	D	EOPP	EOMY	M	EOPP	EOMY
			10^3 ha^{-1}	Mg ha^{-1}		10^3 ha^{-1}	Mg ha^{-1}
Combined	May	Tol	88.6	13.30	Short	91.6	12.87
		Con	90.1	13.12	Full	86.9	13.56
		Avg.	89.2	13.21			
	June	Tol	78.7	11.46	Short	87.5	11.19
		Con	94.1	11.56	Full	85.2	11.77
		Avg.	86.1	11.48			
	Avg.	Tol	84.8	12.37	Short	90.2	12.04
		Con	91.8	12.33	Full	86.1	12.66
		Avg.	88.0	12.34			
NWARS	May	Tol	79.1	11.59	Short	87.2	11.44
		Con	84.6	11.47	Full	NS	NS
		Avg.	82.0	11.53			
	June	Tol	NS	NS	Short	88.7	11.04
		Con	87.5	11.23	Full	NS	NS
		Avg.	80.7	11.39			
	Avg.	Tol	77.4	11.59	Short	87.7	11.24

continued

Table 28. Economic optimum plant population (EOPP) and maximum yield at EOPP (EOMY) across all regions and within each region for each planting date (PD) for drought-tolerant (Tol), conventional (Con), short-season (Short), and full-season (Full) hybrids using a seed cost of \$300 80,000 kernels⁻¹ and \$157 Mg⁻¹ grain price.

Table 28. continued

Region	PD	D	EOPP	EOMY	M	EOPP	EOMY
			10^3 ha^{-1}	Mg ha^{-1}		10^3 ha^{-1}	Mg ha^{-1}
NWARS	Avg.	Con	85.6	11.35	Full	NS	NS
		Avg.	81.6	11.46			
WARS	May	Tol	95.4	14.79	Short	105.0	14.34
		Con	98.0	14.87	Full	93.4	15.45
		Avg.	96.6	14.83			
	June	Tol	81.2	11.79	Short	NS	NS
		Con	91.5	12.06	Full	86.9	12.14
		Avg.	86.1	11.91			
	Avg.	Tol	89.8	12.93	Short	96.3	12.66
		Con	95.4	13.13	Full	90.9	13.43
		Avg.	92.5	13.02			
	WST	May	Tol	86.2	14.07	Short	86.5
Con			NS	NS	Full	NS	NS
Avg.			82.8	13.80			
June		Tol	NS	NS	Short	90.8	11.06
		Con	107.1	11.65	Full	92.1	11.60
		Avg.	91.8	11.33			
Avg.	Tol	83.4	12.60	Short	87.7	12.25	

continued

Table 28. continued

Region	PD	D	EOPP	EOMY	M	EOPP	EOMY
			10 ³ ha ⁻¹	Mg ha ⁻¹		10 ³ ha ⁻¹	Mg ha ⁻¹
WST	Avg.	Con	93.9	12.56	Full	86.0	12.87
		Avg.	86.9	12.56			

PD	P	Protein		Oil		Starch	
		Tol	Con	Tol	Con	Tol	Con
	10 ³ ha ⁻¹	-----g kg ⁻¹ -----					
May	59	94.9	96.2	38.5	37.2	690	691
	74	93.1	94.7	37.9	36.8	692	692
	89	91.1	94.4	38.0	37.0	693	692
	104	90.6	93.6	37.8	36.5	694	693
	124	90.9	94.2	37.5	36.4	694	693
June	59	89.1	91.9	40.4	38.5	693	692
	74	88.0	91.3	40.2	38.6	694	694
	89	87.3	90.8	40.3	38.1	695	694
	104	87.1	90.6	40.2	38.6	695	694
	124	87.6	91.4	40.5	38.4	694	693

continued

Table 29. Grain quality for drought-tolerant (Tol), conventional (Con), short-season (Short), and full-season (Full) hybrids across sites at each population (P) and planting date (PD).

Table 29. continued

PD	P	Protein		Oil		Starch	
		Tol	Con	Tol	Con	Tol	Con
	PD	0.109		0.051		0.680	
	LSD _{0.05}			2.0			
	D	<0.001		<0.001		0.003	
	LSD _{0.05}	0.4		0.2		0.4	
	P	<0.001		0.008		<0.001	
	LSD _{0.05}	0.6		0.3		0.7	
	PD x D	0.036		<0.001		0.336	
	LSD _{0.05}	4.3		0.2			
	PD x P	0.015		0.010		0.006	
	LSD _{0.05}	4.3		0.4		1.0	
	D x P	0.076		0.898		0.136	
	PD x D x P	0.663		0.646		0.857	
		Short	Full	Short	Full	Short	Full
May	59	92.6	98.5	38.3	37.4	692	689
	74	91.2	96.6	37.5	37.2	694	691
	89	90.1	95.5	37.7	37.3	694	692
	104	89.7	94.5	37.4	36.9	695	693
	124	90.4	94.7	37.2	36.8	694	693

continued

Table 29. continued

PD	P	Protein		Oil		Starch	
		Short	Full	Short	Full	Short	Full
June	59	87.7	93.4	38.8	40.2	696	690
	74	87.3	92.1	38.7	40.1	697	692
	89	86.7	91.5	38.8	39.5	696	693
	104	86.7	91.0	38.8	40.0	696	692
	124	87.4	91.5	38.7	40.2	696	691
	PD	0.108		0.051		0.680	
	LSD _{0.05}			2.0			
	M	<0.001		<0.001		<0.001	
	LSD _{0.05}	0.3		0.2		0.4	
	P	<0.001		0.018		<0.001	
	LSD _{0.05}	0.5		0.3		0.5	
	PD x M	0.214		<0.001		<0.001	
	LSD _{0.05}			0.3		0.3	
	PD x P	0.003		0.020		0.001	
	LSD _{0.05}	4.3		0.4		0.8	
	M x P	0.034		0.574		0.027	
	LSD _{0.05}	0.7				0.8	
	PD x M x P	0.092		0.384		0.350	

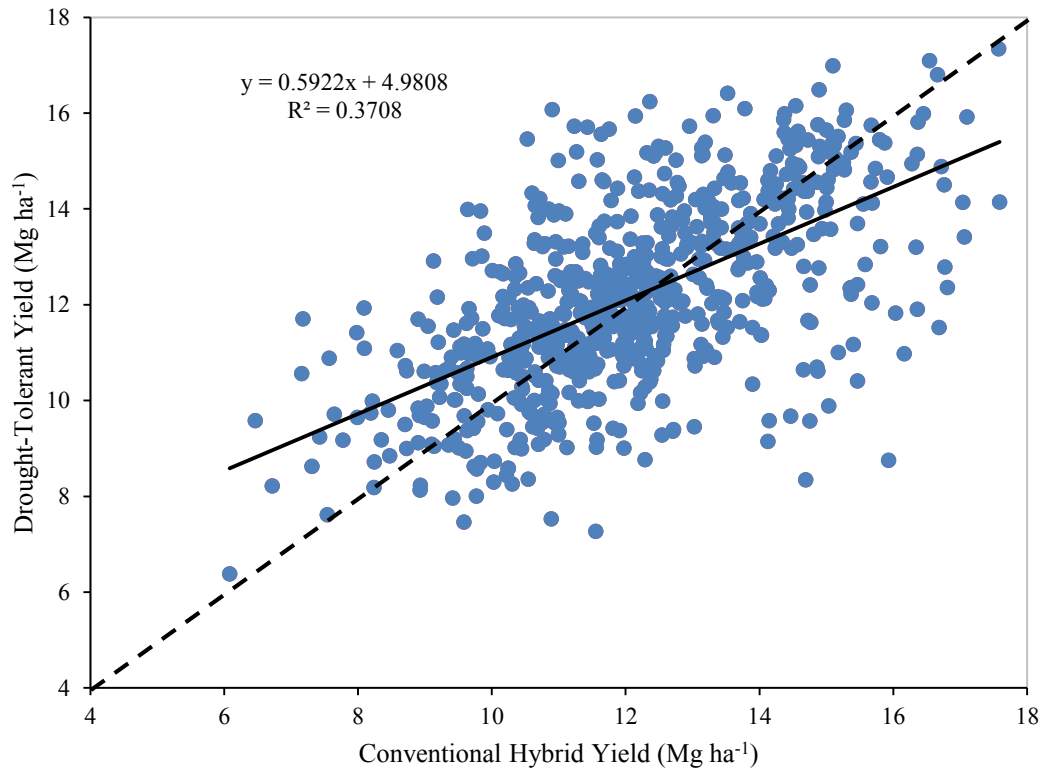


Figure 7. Yield of the conventional hybrid plotted against the yield of the drought-tolerant hybrid of similar maturity under identical plant populations and planting dates. The dashed line represents a line with a slope of 1, which is the expected relationship if yields were equivalent. The solid line is the linear relationship observed for the dataset.

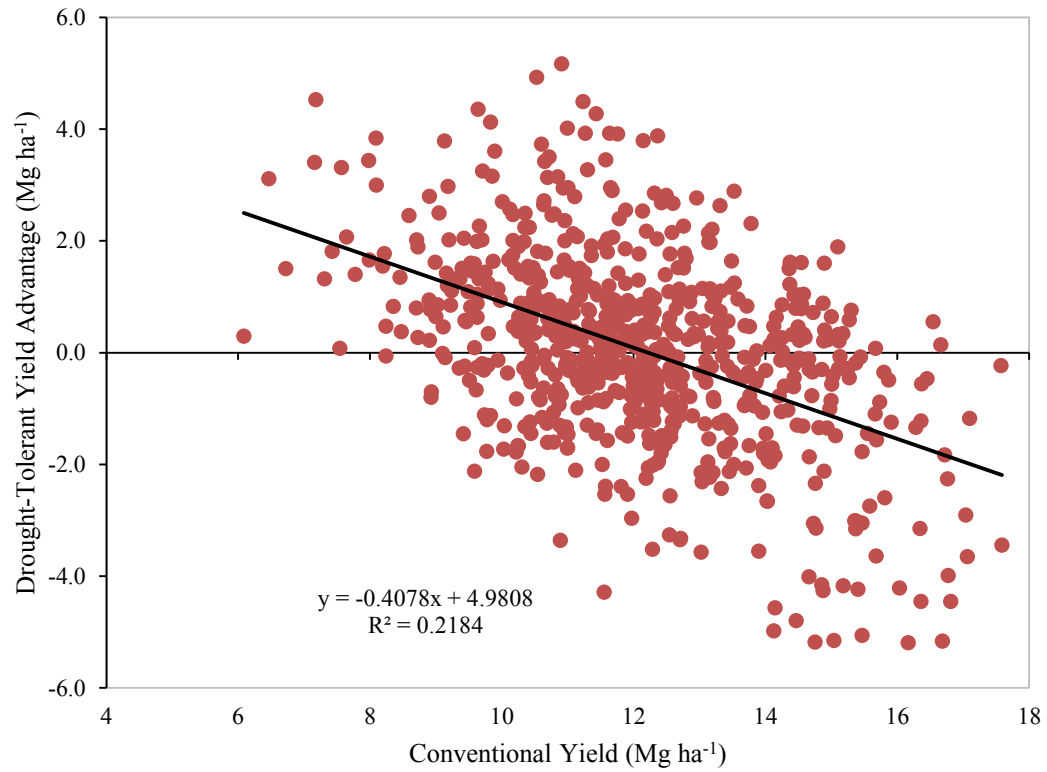


Figure 8. Yield of the conventional hybrid plotted against the yield advantage of the drought-tolerant hybrid of similar maturity under identical plant populations and planting dates. The solid line represents the linear relationship between conventional hybrid yield and the yield advantage from the equivalent drought-tolerant hybrid under identical management conditions.

CHAPTER 5: DROUGHT-TOLERANT MAIZE (*ZEA MAYS* L.) HYBRID
MORPHO-PHYSIOLOGICAL RESPONSE TO PLANT POPULATION AND
PLANTING DATE

INTRODUCTION

Recent research has predicted that the second half of the 21st century will be drier than any period on geologic record in parts of the central and southwestern United States (Cook et al., 2015), which ultimately could impact weather patterns in the Eastern U.S. Corn Belt. Increased incidence of drought could result in more frequent yield losses, which can range from 40 to 65% of the potential yield (Bray et al., 2000). Increasing the drought tolerance of crops has become a top priority of major seed companies. Drought tolerance has been defined previously as enduring drought periods with low tissue water levels (Levitt, 1972), and has been bred for using yield stability in both water stressed and non-stressed conditions (Finlay and Wilkinson, 1963; Clarke et al., 1992). Through advances in selection techniques, several companies have released new non-transgenic drought-tolerant maize hybrids that may have improved water use efficiency (producing equivalent or greater biomass and yield while using less water) over their conventional counterparts (Cooper et al., 2014).

One class of drought-tolerant hybrids, the Optimum® AQUAmax® (AQ) hybrids from Pioneer®, has been shown to provide a grain yield advantage over conventional hybrids under drought conditions (Cooper et al., 2014; Gaffney et al., 2015) or in environments with yields less than 8.5 Mg ha⁻¹ (Ciampitti et al., 2015), but hybrids with the AQ designation have limited research conducted on their physiology. In Indiana, two hybrid pairs containing one AQ hybrid and one conventional hybrid did not differ in cumulative photosynthesis from V10 to R5, but cumulative transpiration over the same period was lower for the drought-tolerant hybrids when compared to the conventional hybrids (Roth et al., 2013). However, there was little variation in photosynthesis and transpiration rates at the time of sampling. Additionally, they did not examine chlorophyll fluorescence ratios at the time of the gas exchange measurements.

The efficiency of open photosystem II reaction centers for excitation capture ($F_v' F_m'^{-1}$) can be measured on light-adapted leaves by measuring the maximum variable fluorescence (F_v') and dividing by the maximal fluorescence (F_m') (Lu et al., 2003). The F_v' is calculated by subtracting the fluorescence intensity with all open photosystem II centers, or minimal fluorescence (F_o'), from the fluorescence intensity with all closed photosystem II centers, or F_m' (van Kooten and Snel, 1990). Higher values of this ratio can indicate lower levels of stress given an environmental stress, such as increased temperature (Lu et al., 2003). Another measure of plant stress using fluorescence is the quantum efficiency of photosystem II (Φ_{II}). This can be measured under light adapted conditions by subtracting the steady-state

fluorescence level (F_s) from F_m' and dividing by F_m' and has been related to leaf photosynthetic capacity (Earl and Tollenaar, 1999). Plants can reduce their photosynthetic capacity to maximize both dark and light reactions under drought conditions (Lambers et al., 2008b), so evaluating fluorescence parameters may detect physiological responses to environmental stress prior to visual manifestation. Evaluating differences between hybrids may provide insight into drought tolerance, but the plasticity of the traits in response to agronomic management needs to be evaluated.

Across hybrids, increasing population has been shown to decrease relative chlorophyll content (RCC) and decrease per plant leaf area (Boomsma et al., 2009). Changes in leaf area could affect specific leaf area (SLA), or $\text{cm}^2 \text{g}^{-1}$ leaf tissue, which if lowered could increase drought tolerance (Poorter and Garnier, 2007). Previous research has also identified traits such as anthesis-silking interval (ASI), plant height, and leaf number can all contribute to improved stress tolerance (Bolaños and Edmeades, 1996; Edreira and Otegui, 2012; Escobar-Gutiérrez and Combe, 2012). Grain quality parameters, such as protein, oil and starch content, may also differ with drought-tolerant hybrids as has been observed with enhanced traits (Thomison et al., 2004) or other drought-tolerant maize lines (Ali et al., 2010).

Roth et al. (2013) observed that cumulative transpiration was more stable for the drought-tolerant hybrids with a population increase from 79,000 plants ha^{-1} to 104-109,000 plants ha^{-1} as compared to the conventional hybrids. However, the drought-tolerant hybrid exhibited lower grain yields and a greater yield decrease than

the conventional hybrid when plant population increased. Previous research has demonstrated that AQ hybrids exhibited a positive yield response when population increased from 20,000 to 80,000 plants ha⁻¹ (Cooper et al., 2014; Gaffney et al., 2015). However, these tested populations are below those currently used in Ohio; 43% of Ohio maize fields have a final plant population of greater than 74,000 plants ha⁻¹ (USDA-NASS, 2014), and plant population recommendations range from 81,000 to 89,000 plants ha⁻¹ (Butzen and Jeschke, 2014).

There is also limited research on the yield response to increasing population in late-planted conditions. Research from Minnesota observed similar responses to population when planting was delayed (Van Roekel and Coulter, 2011), but research from Ohio suggests in some regions optimum yield was achieved at lower plant populations (Lindsey et al., 2015). Increases in rain during April and May could delay maize planting into June, which has been shown to decrease yields (Van Roekel and Coulter, 2011). Delayed planting could increase temperatures at the time of flowering, which could also decrease pollination and result in a grain yield reduction (Nielsen et al., 2002; Edriera et al., 2011). If drought-tolerant hybrids exhibit improved flowering synchrony, this may contribute to the drought-tolerance and may be better able to maintain yields when planted in June conditions when compared to conventional hybrids. Therefore, the objectives of this study were: 1) investigate physiological differences between a drought-tolerant and a conventional hybrid under May and June planting dates; 2) compare the drought-tolerant hybrid response to plant population to that of a conventional hybrid, and determine if the

response changes with planting date; and 3) correlate the physiological traits to differences in grain yield.

MATERIALS AND METHODS

A field experiment was conducted in 2012, 2013, and 2014 at three Ohio locations. The Northwest Agricultural Research Station in Hoytville, OH (NWARS; 41°13'N, 83°45' W; 212 m elevation) was a Hoytville silty clay loam soil (fine, illitic, mesic Mollic Epiaqualfs) in 2012 and 2014 and a Hoytville clay loam soil in 2013. The Western Agricultural Research Station in South Charleston, OH (WARS; 39°51'N, 83°40' W; 333 m elevation) was a Kokomo silty clay loam soil (fine, mixed, superactive, mesic Typic Argiaquolls) in all years, and the Ohio Agricultural Research and Development Center in Wooster, OH (WST; 40°47'N, 81°50' W; 368 m elevation) was a Canfield silt loam soil (fine-loamy, mixed, active, mesic Aquic Fragiudalfs) all years. The soil chemical properties are listed in Table 30. Tillage was conducted as needed to prepare the seedbed for planting, and insects and weeds were controlled to minimize interference.

The treatment design within each planting date was a two-way factorial of plant population and hybrid. Replication of the planting date (two levels, May planting and June planting) was achieved through replication over time. Within each planting date, the experimental design was a split-plot randomized complete block with four replications of the whole-plot factor within each plant date. The whole-plot factor was plant population with five levels (59,000, 74,000, 89,000, 104,000, and

124,000 plants ha⁻¹), but data were only collected at 59,000 and 104,000 plants ha⁻¹ for intensive plant morphological and physiological measurements due to equipment and sampling time limitations. Four Pioneer brand hybrids were selected as the sub-plot factor because they encompassed two relative maturity groups, and one of the hybrids within each pair was marketed as a non-transgenic drought-tolerant AQ hybrid. Measurements in this study focused on the late-maturing hybrid pair described in Table 31. Hybrid P1352 had a greater drought tolerance rating from Pioneer as compared to the non-AQ counterpart (9 to 1 scale, 9=high drought tolerance). In 2012, the refuge included in the bag for P1352 consisted of the same hybrid number without the insect resistance traits. Each sub-plot (7.6 x 3.1 m) consisted of four maize rows (76-cm spacing), with each whole plot being 30.4 x 3.1 m (four rows, 76-cm spacing).

The dates of various field activities are presented in Table 32. Plots were fertilized with 45 kg N, 20 kg P, and 37 kg K ha⁻¹ at planting using starter fertilizer in 2012 and 2013, and 30 kg N, 27 kg P, and 0 kg K ha⁻¹ in 2014. A preplant anhydrous ammonia (82N-0P-0K) N application of 202 kg N ha⁻¹ was made at WARS, and at NWARS and WST plots were sidedressed at V4-V6 with 180 to 190 kg N ha⁻¹ using 28% urea-ammonium nitrate (28N-0P-0K). Stand counts were collected at V5-7 (Abendroth et al., 2011) on the center two rows and were thinned if necessary to achieve desired plant populations.

Aboveground measurements were collected at V8, V14, R2, and R5 in 2012 and V10, R2, and R5 in 2013 and 2014. Plant height was measured to the tallest

extended leaf tip at V8 or V10, and V14, and to the uppermost leaf collar at R2. Plant biomass was measured on two plants per plot collected at V8 or V10 and at R2. Leaf area index (LAI) was determined on the harvested plants at R2 by measuring fresh leaf area using an LI-3000 (Li-Cor Biosciences, Omaha, NE), and dividing by area of ground (Westgate et al., 1997). Green leaf number was determined using the same leaf material each year. Stalk diameter was measured with calipers (147, General Tools and Instruments, New York, NY) on the internode above the brace roots, and dry biomass partitioning was determined on the R2 sampled plants. Samples were dried for 7-14 days at 60°C prior to measuring biomass. Leaf area was divided by dry leaf biomass to determine SLA (Poorter and Garnier, 2007).

Percent total light intercepted (or interception efficiency) was measured at V10 and R2 using a 1-m long Line Quantum Sensor (Li-Cor Biosciences, Omaha, NE), and was calculated by averaging three readings at ground level (light transmission), dividing by the ambient reading collected above the canopy, and subtracting this value from one. The sensor was oriented diagonally between the center rows to obtain interception from between two rows. Ten SPAD 502c Meter (Konica Minolta, Chiyoda, Japan) readings per plot were collected at V10, R2, and R5 except in 2012 at WARS in both plantings and NWARS in the June planting where readings were collected at V8 instead of V10. The RCC within each site-year was determined by dividing all SPAD values by the treatment with the greatest SPAD value (conventional hybrid grown at 59,000 plants ha⁻¹). The uppermost collared leaf was measured at V8, V10, and V14, and the uppermost ear-leaf was

measured at R2 and R5. Visual ratings of drought stress were conducted when symptoms were present on a 9.0 to 1.0 scale (J. Schussler, personal communication, 2012), using 9.0 as non-stressed and 1.0 as severe rolling, senescence, and irreversible damage lower leaf senescence.

Plant physiological activity was measured in 2013 and 2014 at V10, R2, and R5 using a Li-Cor 6400XTF (Li-Cor Biosciences, Omaha, NE) to measure gas exchange and light-adapted chlorophyll fluorescence ratios on three plants per plot at each growth stage. The youngest collared leaf was measured at V10, and the uppermost ear-leaf was measured at R2 and R5 similar to Roth et al. (2013). A light intensity of $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was used to mimic ambient conditions at the ear-leaf within the canopy, minimize acclimation time, and reduce neighboring plant destruction rather than implement an isolation technique. The sample chamber CO_2 was held constant at $400 \mu\text{mol CO}_2 \text{ mol}^{-1}$ air, flow was maintained at $500 \mu\text{mol air s}^{-1}$, and relative humidity of the sample chamber was maintained between 55-65%. The multiphase flash method (Loriaux et al., 2013) was used to measure light-adapted chlorophyll fluorescence ratios of $F_v' F_m'^{-1}$ and Φ_{II} .

Anthesis was measured by counting the total number of plants in the center two rows with tassels emerged, and silking was measured by counting each plant in the center two rows with silks emerged. Each plot was counted at least three times for tassel and silk emergence. A linear regression was conducted for both tassel emergence and silk emergence, and the equations were used to compute the calendar date when 50% tassels had emerged and when 50% of the silks had emerged. The

difference between these calendar dates was considered the ASI for the plot.

Cumulative precipitation and growing degree units (GDU; 30°C upper limit and 10°C lower limit) were calculated for each location each year (Table 33).

After R6 (physiological maturity), all plots were rated for stalk lodging at harvest. Grain yield was collected from the center two rows of each plot using a plot combine, and grain moisture at harvest was also collected. Reported yields have been adjusted to 155 g kg⁻¹ moisture. Samples of the grain from each plot were evaluated for protein, oil, and starch content using an Infratec NIR grain analyzer (Foss Tecator, Höganäs, Sweden) and for kernel weight. Six consecutive ears from one non-harvest row were evaluated for ear yield components, including ear length, number of kernel rows, number of kernels per row, and were visually rated for abnormalities such as missing or aborted kernel rows and poor ear tip and basal kernel fill.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC). All data were standardized within each environment (E, site-year) prior to analysis to determine treatment effects by dividing each mean by the grand mean for the site-year. An ANOVA was conducted using the MIXED model, where plant date (PD), population (P), and hybrid (H) were set as fixed factors, and E and replication (Rep) nested within E x PD were set as random factors. The E x PD term was used as the error term for PD, and P x Rep (E x PD) was the error term for P. When the Global *F*-test

was significant ($\alpha = 0.05$), means were separated using Fisher's protected least significant difference (LSD). To correlate each measured parameter to yield, Pearson's correlation coefficients (r) were determined using the CORR procedure.

RESULTS AND DISCUSSION

Weather Conditions

May planted maize at NWARS in 2012 and each planting at each site in 2013 experienced above average cumulative precipitation (Table 33). Every other planting experienced below average total precipitation, but the distribution of rainfall within the season differed between each site and planting date. Only WST in 2012 had below average precipitation during both the V10-R2 and R2-R5 growth periods in the May planting. At all other sites, the distribution of rainfall was never above average for both the critical growth periods. Visually, drought symptoms (i.e., change in leaf color, leaf rolling) only occurred in 2012 and at V10 in the NWARS June 2014 planting. Hybrid P1352 received similar ratings at V10 and V14 compared to P1184 in when planted in May in 2012 (Table 34). However, when planted in June P1352 exhibited ratings of 0.3-0.8 greater than P1184 at V10 and V14 except at NWARS in 2014 when the ratings were 0.2 lower. Hybrid P1352 received ratings 0.2-0.5 greater than P1184 at the R2 stage when planted in May, but were not different when planted in June (Table 34). Increasing population from 59,000 to 104,000 plants ha⁻¹ lowered the drought ratings for both hybrids similarly at

NWARS in 2012 by 0.7-0.9. Aside from the sites previously discussed, plants appeared to be minimally stressed throughout the growing season.

All sites in 2012 planted in May accumulated more GDUs than the 30-year average (Table 33). The May planting at NWARS and WST in 2013 was also similar to or slightly above the 30-year average. All other plantings accumulated below average GDUs, which may help explain the lack of drought injury symptoms in 2013 and most sites in 2014. Total accumulated GDUs were lower than the GDUs listed by company literature to achieve physiological maturity in most of the June plantings, but Nielsen et al. (2002) demonstrated that hybrids require fewer GDUs to reach physiological maturity when planting is delayed until June. Accumulation of GDUs from V10 to R2 was similar or above average at NWARS and WARS in 2012 planted in May. The GDU accumulation during the grain-fill period (R2-R5) never exceeded the 30-year average at WARS, and was only above average at NWARS when planted in May in 2014. Accumulation during R2-R5 was above average at WST when planted in June in 2012, and May in 2013 and 2014.

Net Photosynthesis, Stomatal Conductance, and Chlorophyll Fluorescence

Each hybrid exhibited a similar response to planting date and population increase, so only differences between hybrids, populations, and planting dates will be discussed. At the V10 and R2 growth stage, P1352 exhibited 7 to 9% greater net photosynthesis as compared to P1184 (Table 35). However, at R5 the hybrids had similar photosynthetic rates. At V10, stomatal conductance was similar for both

hybrids, but was 27 to 40% lower for P1352 compared to P1184 at R2 and R5 (Table 35). These results suggest the drought-tolerant hybrid was able to maintain or increase its net photosynthesis while reducing its stomatal conductance, which is similar to results observed by Bunce (2010). Roth et al. (2013) observed minor differences in photosynthesis and transpiration rates between drought-tolerant hybrids compared to conventional hybrids, but were more related to maturity than drought-tolerance. The magnitude of net photosynthesis is similar to the results reported by Genty et al. (1989) for barley, but is less than what has previously been reported in maize (Trouverie et al., 2003; Roth et al., 2013). The lower values observed in this study can be attributed to the light intensity selected for the measurements. Previous studies have used the isolation technique, but due to space and time limitations this method was not employed in this study. Increasing population decreased net photosynthesis by 6% at R5, and decreased stomatal conductance at R2 and R5 by 24 and 29%, respectively. Similar results were observed in central Indiana across hybrids (Roth et al., 2013). No differences were observed for photosynthesis or stomatal conductance due to delayed planting.

The $F_v' F_m^{-1}$ ratio was lower at R2 when planting was delayed across all hybrids and planting dates by 1.6% (Table 36). Across planting dates and populations, P1352 exhibited 0.6-2.5% greater $F_v' F_m^{-1}$ than P1184 at V10 and R2, and 1.0% greater when planted in June at R5. Hybrid P1352 also had 1.4% greater Φ_{II} than P1184 at V10, but was 1.1% lower at R2. No difference in Φ_{II} was observed at R5. Increasing population from 59,000 to 104,000 plants ha⁻¹ increased $F_v' F_m^{-1}$

by 0.4% at R2. Conversely, Φ_{II} decreased by 2.2 to 4.6% at R2 and R5, respectively, when population increased. These results suggest that the drought-tolerant hybrid may have experienced less stress at V10 and R2, but the difference between the ratios was small. The larger decrease in Φ_{II} of 2.2 to 4.6% indicates both hybrids became more stressed as population increased from 59,000 to 104,000 plants ha⁻¹. The values reported for Φ_{II} in this study are similar to those reported in maize at similar quantum efficiencies (Genty et al., 1989).

Regardless of stage, P1352 had 5 to 8% lower RCC compared to P1184 (Table 37) within each plant date. Additionally, RCC decreased for both hybrids by 4 to 7% when population increased from 59,000 to 104,000 plants ha⁻¹. Population has been shown to decrease SPAD values in other hybrids, but hybrid differences were less evident (Boomsma et al., 2009; Robles et al., 2012). The increase in net photosynthesis may have been influenced by the RCC of the leaves. Differences in the pigment profile or pigment concentrations of each hybrid as documented in Chapter 3 (A.J. Lindsey, 2015, unpublished data) may have influenced the photosynthetic efficiency of the drought-tolerant hybrid differently than the conventional hybrid.

Light Interception Dynamics and Leaf Characteristics

Light interception was less in the June planting as compared to the May planting at V10 (Table 38), which may be attributed to the decreased height and biomass (Table 39) at V10 for the June planted maize. At R2, height was not

influenced by plant date and light interception was similar across plant dates. Hybrid P1352 was taller than P1184 at V10 and exhibited greater light interception. At R2, the hybrids were similar in height but P1352 had greater interception in both plant dates. This could be attributed both to increased leaf number as well as greater LAI (Table 38).

The H x P interaction at V10 for light interception was caused by P1352 increasing interception by 0.093 compared to P1184 which increased by 0.082 (Table 38). Conversely, the H x P interaction at R2 for interception was caused by P1184 increasing greater than P1352. The difference in LAI may have impacted interception at R2 because the number of leaves decreased similarly with increasing population for both hybrids. At the low plant population, P1352 exhibited a LAI $>3.5 \text{ m}^2 \text{ m}^{-2}$, whereas the LAI of P1184 was $<3.1 \text{ m}^2 \text{ m}^{-2}$. Light interception has been shown to change little when the LAI exceeds $3.5 \text{ m}^2 \text{ m}^{-2}$ (Westgate et al., 1997; Cox and Cherney, 2012). Conversely, Roth et al. (2013) observed no difference or a lower LAI for the drought-tolerant hybrid compared to two conventional hybrids. Additionally, as population changed from 59,000 to 104,000 plants ha^{-1} the drought-tolerant hybrids experienced a greater increase in LAI compared to the conventional hybrid. Increases in LAI with increasing population has also been recorded in Indiana and New York (Cox and Cherney, 2012; Robles et al., 2012). Delaying planting increased the LAI response to population for both hybrids, which differs from previous research (Van Roekel and Coulter, 2011).

The SLA of both hybrids was greater when planting was delayed until June as compared to the May planting (Table 38). However, the drought-tolerant hybrid was greater than the conventional hybrid across populations regardless of planting date. The increase in population increased the SLA for both hybrids, indicating leaf characteristics changed similarly. A lower SLA has been shown to be more common in other drought-tolerant species (Poorter and Garnier, 2007), but this plant characteristic was not evident for the evaluated drought-tolerant hybrid compared to its conventional counterpart.

Plant Biomass and Flowering Synchrony

Plant biomass at V10 experienced a PD x H x P interaction because biomass of P1184 did not decrease with increasing population when planted in June (Table 39). Aside from this, biomass was typically greater for the May planting than the June planting. Delayed planting has been shown to decrease total biomass (Tsimba et al., 2013). Biomass of P1352 exceeded that of P1184 at both stages. Additionally, as population increased the dry biomass decreased to a greater degree for P1352 compared to P1184 at both V10 and R2. Stalk diameter was greater for P1352 (2.37 cm) compared to P1184 (2.28 cm), and both hybrids exhibited a 12% decrease in diameter as population increased from 59,000 to 104,000 plants ha⁻¹ (data not shown). This is similar to results observed in Minnesota (Van Roekel and Coulter, 2011), and suggest that increasing population caused the plant stalks to become thinner. The drought-tolerant hybrid was more affected than the conventional hybrid,

and the reduced stalk diameter paired with greater biomass may have led to the greater percent stalk lodging at harvest for P1352 grown at 104,000 plants ha⁻¹ (Table 39).

Hybrid P1352 exhibited shorter ASI than P1184 by 1.4 to 2.4 days within each population. These results suggest the drought-tolerant hybrid exhibited a shorter ASI than the conventional hybrid, which may improve grain yield. Previous research has demonstrated a strong correlation of ASI with yield, especially under drought conditions (Bolaños and Edmeades, 1996). Increasing population lengthened the ASI for P1352 by 1.8 days, and 0.8 days for P1184. Regardless of planting date, P1352 at 59,000 plants ha⁻¹ exhibited the shortest ASI. When grown at 104,000 plants ha⁻¹, P1352 had a similar ASI to P1184 at 59,000 plants ha⁻¹, and P1184 at 104,000 plants ha⁻¹ had the longest ASI. Previous research has demonstrated a similar effect of increasing population on ASI (Robles et al., 2012).

Grain Yield and Quality

Hybrid P1352 planted at 104,000 plants ha⁻¹ in May exhibited the greatest yield, but was not significantly different than the May planted P1184 at the same population and P1352 planted at 59,000 plants ha⁻¹. Similar trends were present in the June planting, but the yields were not statistically different from one another. Both hybrids planted at 104,000 plants ha⁻¹ and P1352 planted at 59,000 plants ha⁻¹ in the June planting had similar yields to the conventional hybrid planted in May at 59,000 plants ha⁻¹. Other researchers have observed a more consistent response to

population from the drought-tolerant hybrids compared to conventional hybrids across environments, but the populations tested only ranged from 30,000 to 80,000 plants ha⁻¹ (Cooper et al., 2014). These results suggest that increased net photosynthesis, plant biomass, LAI and SLA, and reduced stomatal conductance and RCC in the drought-tolerant hybrid elicited a 3.0% increase in grain yield across populations and plant dates. Gaffney et al. (2015) observed a 1.9 or 6.5% yield advantage from drought-tolerant hybrids compared to conventional hybrids under favorable or drought conditions, respectively. These results differ from those of Roth et al. (2013), who observed minimal differences in grain yield between the drought-tolerant and conventional hybrids. This may have been due to the smaller difference in populations measured (79,000 plants ha⁻¹ as compared to 59,000 plants ha⁻¹ in this current study). Across all planting dates and hybrids, yields were 5.8% greater at 104,000 than 59,000 plants ha⁻¹. The decreases in per plant biomass and RCC from increasing population were offset with the increase in plant number. However, Roth et al. (2013) observed a yield decrease when populations increased from 79,000 to 104-109,000 plants ha⁻¹, which may be attributed to the proximity of the low population to the optimum population for the environment.

At harvest, grain moisture was greater in P1352 compared to P1184 across plant dates and populations (Table 39). Grain moisture at harvest was also greater in the June planted maize compared the May planting, but the difference between hybrids was similar (around 20 g kg⁻¹) regardless of plant date. Delayed planting reduced kernel weight (reported at 155 g kg⁻¹ moisture) as seen in Table 33, possibly

due to the shorter growing season (Eichenberger et al., 2015). The differences in hybrid yield could be attributed to a yield partitioning difference. Hybrid P1352 produced slightly more kernel rows (16.2) compared to P1184 (15.7) (Table 40). Hybrid P1184 produced more kernels per row resulting in more kernels per plant, which was more evident in the May planting. However, the per plant kernel number reduction for P1352 was offset by greater weight per kernel, resulting in yields per plant that mirror the yield results in Table 39. Kernel weight was positively correlated to yield ($r = 0.196$, $P < 0.001$) whereas kernel number was not correlated to yield ($r = 0.107$, $P = 0.069$). Kernel number has been shown to be less stable under late plantings as compared to kernel weight (Tsimba et al., 2013), and that long-term yield gains have been attributed to increasing kernel weight rather than increasing kernel number (Hay and Porter, 2006). However, multiple other researchers have identified the importance of kernel number contributing to yield is greater than kernel weight (Bolaños and Edmeades, 1996; Cox and Cherney, 2012).

Delaying planting until June decreased protein and increased starch in P1352 compared to P1184 (Table 41). However, when planted in May no differences between hybrids was evident. Protein levels in the grain were 2.6 g kg^{-1} less and oil content was 1.0 g kg^{-1} greater for P1352 compared to P1184 across populations and plant dates. Ali et al. (2010) also observed lower protein and greater oil content in a drought-tolerant maize line compared to a drought-sensitive control. While starch content was similar for both hybrids, an increase in population increased starch content by 3.5 g kg^{-1} . The increase in starch caused by increasing population was

accompanied by a 3.2 g kg^{-1} decrease in protein, which has been documented by previous researchers (Cook et al., 2012).

Plant Trait Correlations to Grain Yield

Of all the traits evaluated, plant components that contributed to increased light interception exhibited the strongest correlations to yield (Table 42). Light interception, plant height, green leaf number, and early-season biomass exhibited strong positive correlations to grain yield, whereas specific leaf area exhibited a negative correlation. These results suggest that more light interception resulted in greater grain yield. Net photosynthesis was positively correlated with yield only at R2, and stomatal conductance was negatively correlated to yield at V10. A positive relationship between $F_v' F_m^{-1}$ and yield at R2 and R5 suggests that as efficiency during grain fill increases, grain yield also increases.

CONCLUSIONS

Across all planting environments, the drought-tolerant hybrid exhibited different physiological traits as compared to the conventional hybrid. However, in the nine site-years most of the physiological and morphological responses to population and planting date were similar regardless of hybrid. The differences in physiological traits like net photosynthetic rate, stomatal conductance, and $F_v' F_m^{-1}$, and morphological traits such as biomass, light interception, leaf number, SLA, and ASI may have contributed to the 3% yield advantage averaged across populations

and planting dates. The drought-tolerant hybrid produced greater yield compared to the conventional hybrid by increasing kernel weight rather than kernel number. The hybrids exhibited a similar yield response within each planting date to increasing population, and stalk lodging was greater for the drought-tolerant hybrid when population increased from 59,000 to 104,000 plants ha⁻¹. The differences in protein and oil concentrations in the grain may be related to the drought tolerance of the hybrid, but further research on these characteristics is needed. Based on these field trials, innate physiological and morphological differences of the drought-tolerant hybrid may have provided a yield advantage over conventional hybrid, but did not influence the response to increasing plant population and delayed planting.

TABLES

Site	Year	Previous Crop	OM g kg ⁻¹	CEC cmol(+) kg ⁻¹	pH	P -----mg kg ⁻¹ -----	K
NWARS	2012	soybeans	47	17.3	5.8	52	218
	2013	wheat	30	22.2	6.2	23	185
	2014	soybeans	32	22.4	6.6	20	176
WARS	2012	soybeans	31	16.4	6.0	32	108
	2013	soybeans	31	17.4	6.6	15	133
	2014	soybeans	34	23.7	5.3	37	165
WST	2012	maize	20	8.0	6.1	60	123
	2013	soybeans	13	7.0	5.5	46	145
	2014	soybeans	25	6.5	6.4	54	131

Table 30. Organic matter (OM), cation exchange capacity (CEC), pH, Bray P₁, and K soil levels in 2012-2014 for the Northwest Agricultural Research Station (NWARS), Western Agricultural Research Station (WARS), and the Ohio Agricultural Research and Development Center in Wooster (WST).

Characteristic	Hybrid	
	P1184	P1352
GDU to silk (°C)	770	740
GDU to maturity (°C)	1470	1430
CRM	111	113
Drought Tolerance	7	9
Traits	YXR, XR, XR	AMX-R, YXR, CYXR

Table 31. Hybrid characteristics for 2012-2014, including growing degree units (GDUs) to silking, GDUs to maturity, comparative relative maturity (CRM), drought tolerance rating, and insect and herbicide resistance traits.

Site	Year	PD	Planting Date	Flowering Date	Harvest Date
NWARS	2012	May	3 May	16-23 July	5 Nov.
		June	7 June	1-9 Aug.	7 Nov.
	2013	May	7 May	9-19 July	22 Oct.
		June	7 June	30 July-9 Aug.	5 Nov.
	2014	May	20 May	16-25 July	29 Oct.
		June	14 June	10-17 Aug.	19 Nov.
WARS	2012	May	14 May	10-19 July	5 Oct.
		June	8 June	30 July-7 Aug.	8 Nov.
	2013	May	20 May	15-22 July	29 Oct.
		June	12 June	1-12 Aug.	25 Nov.
	2014	May	30 May	24-31 July	22 Oct.
		June	16 June	9-15 Aug.	10 Nov.
WST	2012	May	7 May	13-20 July	26 Oct.
		June	6 June	27 July-6 Aug.	11 Nov.
	2013	May	16 May	18-28 July	15 Nov.
		June	15 June	12-19 Aug.	15 Nov.
	2014	May	25 May	26 July-2 Aug.	12 Nov.
		June	13 June	17-23 Aug.	5 Dec.

Table 32. Dates of planting, flowering, and harvest at each site and planting date treatment (PD).

Site	Year	PD	Cumulative Precipitation			Cumulative GDU		
			V10- R2	R2-R5	Season Total	V10- R2	R2- R5	Season Total
			-----mm-----			-----°C-----		
NWARS	2012	May	56.6	139.4	479.3	462	310	1753
		June	161.3	94.2	413.3	382	314	1436
	2013	May	225.6	38.1	571.0	377	335	1615
		June	107.7	19.3	550.2	284	343	1368
	2014	May	48.8	101.6	345.7	376	453	1473
		June	39.9	99.6	317.5	358	338	1206
	30-yr average		106.4	95.7	463.7	464	404	1585
WARS	2012	May	122.7	33.5	354.6	509	230	1661
		June	120.1	8.4	390.9	438	308	1450
	2013	May	131.6	37.3	486.9	340	367	1535
		June	67.6	130.3	495.3	332	405	1338
	2014	May	74.7	90.9	377.2	335	403	1363
		June	34.3	108.0	344.7	299	342	1205
	30-yr average		126.0	77.5	453.7	468	420	1561

continued

Table 33. Cumulative precipitation, growing degree unit (GDU) accumulation, and 30-yr averages for each growing season for each planting date (PD) at each site.

Table 33. continued

Site	Year	PD	Cumulative Precipitation			Cumulative GDU		
			V10- R2	R2-R5	Season Total	V10- R2	R2- R5	Season Total
			-----mm-----			-----°C-----		
WST	2012	May	57.4	47.0	369.3	433	269	1580
		June	56.4	119.9	397.3	323	422	1380
	2013	May	165.1	51.1	562.6	347	419	1479
		June	43.4	91.7	476.5	295	315	1222
	2014	May	64.0	111.0	417.1	323	410	1349
		June	136.7	25.9	393.4	351	287	1173
	30-yr average		105.6	97.8	443.6	446	393	1427

PD	H	P	2012						2014		
			10 ³ ha ⁻¹	NWARS			WARS			WST	
			V10	V14	R2	R2	V10	V14	R2	V10	
May	P1184		3.4	2.8	6.4	7.3	7.1	7.8	7.4	--	
		P1352	3.3	2.8	6.6	7.8	7.4	7.9	7.9	--	
			H	0.56	0.70	0.44	<0.01	0.21	0.39	<0.01	--
			59	3.7	3.1	7.0	7.5	7.6	7.8	7.8	--
			104	3.0	2.5	6.1	7.5	6.9	7.9	7.4	--
			P	0.03	0.19	0.03	1.00	0.29	0.32	0.07	--
June	P1184		--	6.6	7.1	7.6	6.3	7.5	6.6	7.8	
		P1352	--	7.2	7.5	7.7	6.9	7.8	6.8	7.6	
			H	--	<0.01	0.06	0.29	0.02	0.06	0.57	0.36
			59	--	7.3	7.4	7.5	6.7	7.8	6.9	8.0
			104	--	6.5	7.3	7.8	6.4	7.6	6.5	7.4
			P	--	0.03	0.62	0.10	0.39	0.49	0.19	0.08

Table 34. Visual drought ratings for each hybrid (H) across population (P), and each P across hybrids. Ratings are on a 9 to 1 scale (9 = no stress and 1 = irreversible stress symptomology).

PD	H	P	Net Photosynthesis			Stomatal Conductance		
			V10	R2	R5	V10	R2	R5
		10^3 ha^{-1}	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$			$\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$		
May	P1184	59	3.75	4.19	4.72	225	143	154
		104	4.29	4.38	4.29	211	138	128
	P1352	59	4.11	4.66	4.65	210	128	111
		104	4.24	4.88	4.26	200	111	90.5
June	P1184	59	3.49	4.06	4.71	242	175	129
		104	3.63	4.41	4.61	226	119	93.2
	P1352	59	3.95	4.65	4.71	234	122	90.8
		104	4.11	4.58	4.59	243	91.6	63.8
		PD	0.51	0.58	0.17	0.30	0.82	0.10
		H	<0.01	<0.01	0.75	0.57	<0.01	<0.01
		LSD _{0.05}	0.22	0.15			16.3	12.9
		P	0.07	0.08	0.01	0.32	<0.01	<0.01
	LSD _{0.05}			0.20		17.7	13.1	
	PD x H	0.16	0.57	0.83	0.24	0.22	0.56	
	PD x P	0.49	0.72	0.12	0.58	0.06	0.54	
	H x P	0.40	0.31	0.94	0.34	0.69	0.56	
	PD x H x P	0.36	0.23	0.88	0.48	0.21	0.90	

Table 35. Photosynthetic measurements for each hybrid (H) at each population (P) for both planting dates (PD) at V10, R2, and R5 collected in 2013 and 2014.

PD	H	P	$F_v' F_m'^{-1}$			Φ_{II}		
			10^3 ha^{-1}	V10	R2	R5	V10	R2
May	P1184	59	0.698	0.721	0.704	0.687	0.677	0.636
		104	0.692	0.726	0.698	0.672	0.659	0.602
	P1352	59	0.712	0.726	0.703	0.692	0.669	0.634
		104	0.715	0.728	0.695	0.685	0.648	0.596
June	P1184	59	0.702	0.710	0.689	0.684	0.670	0.646
		104	0.705	0.713	0.693	0.684	0.666	0.626
	P1352	59	0.721	0.716	0.698	0.698	0.670	0.640
		104	0.721	0.717	0.701	0.690	0.655	0.619
		PD	0.61	0.02	0.66	0.76	0.68	0.46
		LSD _{0.05}		0.008				
		H	<0.01	<0.01	0.09	<0.01	0.01	0.24
		LSD _{0.05}	0.005	0.003		0.005	0.006	
		P	0.95	0.03	0.45	<0.01	<0.01	<0.01
		LSD _{0.05}		0.003		0.005	0.006	0.010
		PD x H	0.86	0.59	<0.01	0.80	0.48	0.77
		LSD _{0.05}			0.006			
		PD x P	0.64	0.54	0.07	0.17	0.12	0.13
		H x P	0.48	0.37	0.65	0.92	0.27	0.79
		PD x H x P	0.27	0.68	0.91	0.16	0.54	0.85

Table 36. Efficiency of open photosystem II reaction centers for excitation capture ($F_v' F_m'^{-1}$) and quantum efficiency of photosystem II (Φ_{II}) at V10, R2, and R5.

PD	H	P	RCC		
			V10	R2	R5
		10 ³ ha ⁻¹			
May	P1184	59	0.942	0.944	0.937
		104	0.901	0.876	0.870
	P1352	59	0.895	0.893	0.871
		104	0.852	0.820	0.801
June	P1184	59	0.931	0.951	0.945
		104	0.904	0.892	0.903
	P1352	59	0.906	0.914	0.894
		104	0.870	0.847	0.825
		PD	0.75	0.49	0.41
		H	<0.01	<0.01	<0.01
		LSD _{0.05}	0.009	0.006	0.008
		P	<0.01	<0.01	<0.01
		LSD _{0.05}	0.010	0.008	0.010
		PD x H	0.04	0.06	0.75
		LSD _{0.05}	0.032		
		PD x P	0.28	0.38	0.20
		H x P	0.54	0.33	0.07
		PD x H x P	0.67	0.77	0.15

Table 37. Relative chlorophyll content (RCC), plant height, stalk diameter, and biomass in each planting date (PD) for each hybrid (H) at each population (P).

PD	H	P	Light		Height		Green	LAI	SLA
			Interception				Leaf		
			10 ³	V10	R2	V10	R2	R2	R2
			ha ⁻¹	cm		m ² m ⁻²		cm ² g ⁻¹	
May	P1184	59	0.646	0.898	135	253	13.0	3.02	116
		104	0.719	0.946	137	253	12.7	4.26	120
	P1352	59	0.720	0.937	145	253	13.5	3.57	124
		104	0.816	0.973	148	253	13.1	5.19	134
June	P1184	59	0.589	0.927	122	250	12.7	3.09	136
		104	0.679	0.963	125	253	12.8	4.79	145
	P1352	59	0.657	0.949	131	252	13.3	3.64	141
		104	0.747	0.976	134	255	13.1	5.63	153
		PD	0.04	0.06	<0.01	0.90	0.64	0.23	<0.01
		LSD _{0.05}	0.047		6.5				15
		H	<0.01	<0.01	<0.01	0.21	<0.01	<0.01	<0.01
		LSD _{0.05}	0.012	0.006	1.3		0.1	0.10	3
		P	<0.01	<0.01	<0.01	0.26	<0.01	<0.01	<0.01
		LSD _{0.05}	0.013	0.007	1.8		0.1	0.14	3

continued

Table 38. Light interception, plant height, green leaf number, leaf area index (LAI), and specific leaf area (SLA) for each hybrid (H) and at each population (P) in both plant dates (PD).

Table 38. continued

	Light Interception		Height		Green	LAI	SLA
					Leaf		
					Number		
	V10	R2	V10	R2	R2	R2	R2
PD x H	0.28	0.02	0.14	0.28	0.81	0.59	0.10
LSD _{0.05}		0.016					
PD x P	0.75	0.16	0.81	0.14	0.06	<0.01	0.10
LSD _{0.05}						0.46	
H x P	0.50	0.11	0.73	0.80	0.17	<0.01	0.13
LSD _{0.05}						0.17	
PD x H x P	0.50	0.83	0.24	0.97	0.65	0.61	0.60

PD	H	P	Dry Biomass		ASI	Lodging	Yield	Moisture
			V10	R2				
		10 ³ ha ⁻¹	g		d	%	Mg ha ⁻¹	g kg ⁻¹
May	P1184	59	33.4	185	0.69	1.4	12.56	220
		104	25.7	134	1.32	3.4	13.31	221
	P1352	59	37.7	210	-0.36	2.9	12.84	241
		104	30.0	147	0.25	11.3	13.82	235
June	P1184	59	23.7	153	0.55	3.9	10.89	269
		104	22.1	117	0.73	4.5	11.84	275
	P1352	59	31.7	174	-0.76	3.9	11.59	292
		104	25.4	128	0.43	8.5	11.84	292
		PD	0.01	<0.01	0.54	0.79	0.01	<0.01
		LSD _{0.05}	4.2	10.6			1.14	28
		H	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		LSD _{0.05}	1.1	4.9	0.24	1.7	0.23	4
		P	<0.01	<0.01	<0.01	<0.01	<0.01	0.98
		LSD _{0.05}	1.4	5.0	0.28	2.2	0.30	

continued

Table 39. Dry biomass, anthesis-silking interval (ASI), percent stalk lodging at harvest, grain moisture at harvest, and grain yield for each hybrid (H) at both populations (P) for each planting date (PD) across sites.

Table 39. continued

	Dry Biomass		ASI	Lodging	Yield	Moisture
	V10	R2				
	g		d	%	Mg ha ⁻¹	g kg ⁻¹
					1	
PD x H	0.25	0.51	0.29	0.12	0.83	0.51
PD x P	0.01	<0.01	0.82	0.24	0.38	0.15
LSD _{0.05}	4.0	10.5				
H x P	0.04	0.04	0.04	<0.01	0.31	0.07
LSD _{0.05}	1.8	6.9	0.37	2.8		
PD x H x P	0.04	0.87	0.03	0.48	0.04	0.95
LSD _{0.05}	4.2		0.85		1.07	

PD	H	P	KW	KRW	KPR	KPP	YPP
		10^3 ha^{-1}	mg kernel^{-1}		Count		g plant^{-1}
May	P1184	59	357	15.9	41.6	660	207
		104	325	15.5	32.6	504	188
	P1352	59	389	16.3	37.8	616	217
		104	348	16.1	30.7	493	195
June	P1184	59	328	16.0	37.8	601	162
		104	307	15.4	30.1	463	169
	P1352	59	359	16.2	36.6	592	199
		104	330	16.1	28.5	456	170
		PD	<0.01	0.65	0.03	0.03	0.01
		LSD _{0.05}	15		2.2	36	19
		H	<0.01	<0.01	<0.01	<0.01	<0.01
		LSD _{0.05}	3	0.2	0.6	9	7
		P	<0.01	<0.01	<0.01	<0.01	<0.01
		LSD _{0.05}	4	0.2	0.8	12	7
		PD x H	0.90	0.71	0.02	0.04	0.14
		LSD _{0.05}			2.0	33	

continued

Table 40. Individual kernel weight (KW), kernel row number (KRW), kernel number per row (KPR), kernels per plant (KPP), and yield per plant (YPP) for each hybrid (H) at both populations (P) for each planting date (PD) across sites.

Table 40. continued

	KW	KRW	KPR	KPP	YPP
	mg kernel ⁻¹		count		g plant ⁻¹
PD x P	<0.01	0.88	0.83	0.88	0.17
LSD _{0.05}	13				
H x P	<0.01	0.18	0.08	0.07	<0.01
LSD _{0.05}	14				10
PD x H x P	0.97	0.05	0.43	0.11	0.02
LSD _{0.05}					2.2

PD	H	P	Protein	Oil	Starch
		10 ³ ha ⁻¹	-----g kg ⁻¹ -----		
May	P1184	59	98.3	36.9	690
		104	95.0	36.7	693
	P1352	59	98.4	38.0	688
		104	93.7	37.2	693
June	P1184	59	95.5	39.5	689
		104	93.5	39.4	692
	P1352	59	91.5	40.7	691
		104	88.9	40.6	693
		PD	0.03	<0.01	0.88
		LSD _{0.05}	3.6	1.8	
		H	<0.01	<0.01	0.44
		LSD _{0.05}	0.6	0.3	
		P	<0.01	0.07	<0.01
		LSD _{0.05}	0.8		1
		PD x H	<0.01	0.24	<0.01
		LSD _{0.05}	0.9		1
		PD x P	0.03	0.30	0.08
		LSD _{0.05}	3.3		

continued

Table 41. Grain quality of each hybrid (H) at each population (P) and planting date (PD).

Table 41. continued

	Protein	Oil	Starch
H x P	0.16	0.46	0.56
PD x H x P	0.50	0.40	0.22

Stage or Parameter	<i>r</i> , <i>P</i> -value	Stage or Parameter	<i>r</i> , <i>P</i> -value
V10		R2	
Plant Height	0.522, <0.001	Green Leaf Number	0.219, <0.001
Dry Biomass	0.170, 0.004	LAI	0.027, 0.680
Light Interception	0.264, <0.001	SLA	-0.340, <0.001
RCC	0.025, 0.691	Net Photosynthesis	0.217, 0.003
Net Photosynthesis	0.024, 0.741	Stomatal	-0.100, 0.170
		Conductance	
Stomatal Conductance	-0.146, 0.048	$F_v' F_m'^{-1}$	0.259, <0.001
$F_v' F_m'^{-1}$	-0.060, 0.417	Φ_{II}	-0.112, 0.124
Φ_{II}	-0.037, 0.615	R5	
VT-R1		RCC	0.054, 0.359
ASI	-0.006, 0.925	Net Photosynthesis	-0.058, 0.423
R2		Stomatal	0.052, 0.479
		Conductance	
Plant Height	0.326, <0.001	$F_v' F_m'^{-1}$	0.196, 0.007
Dry Biomass	0.083, 0.200	Φ_{II}	-0.081, 0.263
Light Interception	0.202, <0.001	R6	
RCC	-0.034, 0.568	Stalk Lodging	-0.000, 0.999

Table 42. Pearson's correlation coefficients (*r*) for each growth parameter and yield.

CHAPTER 6: SUMMARY

Grower adoption of the new generation of drought-tolerant hybrids in the Eastern U.S. Corn Belt may be dependent on whether a yield advantage or yield penalty is incurred. These field studies conducted in Ohio were sufficient to detect physiological, morphological, and yield differences between the evaluated drought-tolerant and conventional hybrids. In environments where the yield potential for conventional hybrids was below 12.2 Mg ha^{-1} , the drought-tolerant hybrids produced greater yields 66% of the time (Figure 9a). When the conventional hybrids produced grain yield greater than 12.2 Mg ha^{-1} , the drought-tolerant hybrids produced less yield 60% of the time (Figure 9b). Additionally, the yield losses incurred were greater in magnitude when the yield potential was greater than 12.2 Mg ha^{-1} (losses were $0.5\text{-}2.9 \text{ Mg ha}^{-1}$ greater). These results suggest that in lower yielding environments in Ohio (below 12.2 Mg ha^{-1} average yield), the drought-tolerant hybrids have a greater potential to limit yield loss than their conventional counterparts of similar maturity at the same population. Additionally, the drought-tolerant hybrids exhibited more stable yield than the conventional hybrids in that the low yield was not as low, and the highest yield was not as high. This supports the statements presented by Cooper et al. (2014) that these drought-tolerant hybrids have been bred for improved yield stability across environments.

The yield advantage observed in lower yielding environments may be related to plant physiological and morphological differences. However, overall conclusions are limited because only the full-season hybrid pair was evaluated intensively for most physiological characteristics. Plant biomass, specific leaf area, and plant height were correlated to yield, which can all influence light interception. Net photosynthetic rates were greater by 7 to 9% and stomatal conductance was lower by 27 to 40% for the drought-tolerant hybrid, but this was solely on a per meter basis. If entire leaf area is considered, the drought-tolerant hybrid had 16% greater green leaf area at R2 (5,650 cm²) compared to the conventional hybrid (4,730 cm²). The decrease in stomatal conductance per unit area was still enough to offset the increased leaf area if stomatal conductance were assumed constant across all leaf area, and may have resulted in greater water use efficiency (equivalent production of biomass while using less water). Increased leaf area may have increased net photosynthesis as well.

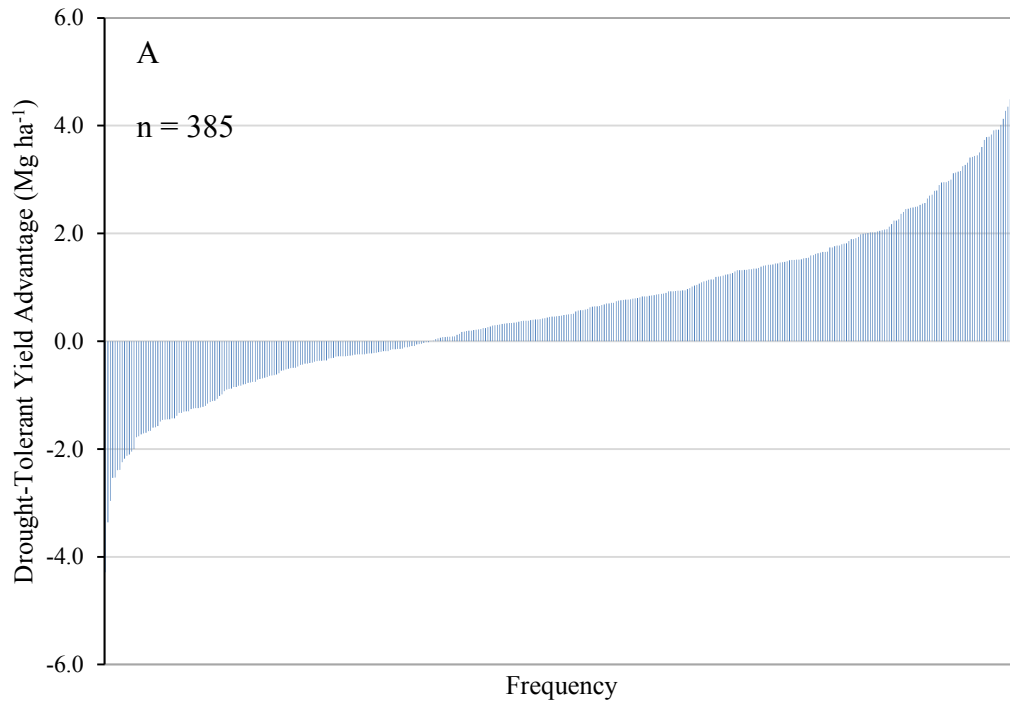
The reduced stomatal conductance may suggest improved water use efficiency, which may have influenced N uptake. When evaluating hybrid response to N application rate, a lower concentration of ear-leaf N in the drought-tolerant hybrids at R2 was observed. No differences in soil moisture were observed in 2013 and 2014, which may not imply reduced water uptake was occurring. However, soil moisture measurements were collected only at each sampling time, and differences may have appeared if continuous logging methods had been employed to track soil moisture changes.

Based on their increased stress tolerance, it was hypothesized that the drought-tolerant hybrids would maintain yield levels at greater populations, and maintain yield levels even with delayed planting. However, the drought-tolerant hybrids did not respond as hypothesized to agronomic management. Delayed planting reduced yield for the drought-tolerant hybrids to a greater degree than the conventional hybrids. The agronomic optimum plant population to maximize yield was less than the conventional hybrids, and lodging was increased for the drought-tolerant hybrids as plant population increased. The optimum N application rate was greater for the drought-tolerant hybrids than for the conventional hybrids. This may be related to the fact that less water is moving through the plant, so the concentration of the nitrogen dissolved in the water needs to increase in order to supply adequate levels to the plant. These results suggest growers would derive marginal benefits from managing these hybrids differently from conventional hybrids with regard to population, planting date, and N application rate. Additionally, the improved water use efficiency seems to have increased the need for N application.

This research has identified potential traits that may be contributing to drought tolerance in this new generation of drought-tolerant hybrids, and provides valuable agronomic information for growers in the Eastern U.S. Corn Belt. Additionally, these studies discerned environmental conditions in which a yield advantage is likely to occur from planting these hybrids as well as conditions where a yield penalty may be incurred from their use.

Future investigations should consider other drought-tolerant hybrids (both transgenic and non-transgenic) to observe if the physiological differences observed in this study are evident across other hybrid pairs. Additional research to discern which traits specifically are causing any yield advantage or penalty should be conducted to better understand the physiological contributions. Physically reducing LAI by removing leaves (both above and/or below the ear) under favorable and drought conditions would allow researchers to examine if the excess leaf material was contributing to the yield stability observed in these studies. Research should be conducted to reduce soil moisture to levels that induce wilting in plants, but field experiments may not be practical for Ohio due to high frequency of spring rainfall and higher humidity during the growing season. These factors may be more successfully manipulated in Ohio using greenhouse environments. Measurements of photosynthetic rate and stomatal conductance as related to vapor pressure deficit and continuous monitoring of soil moisture may provide insights into the interactive role of water levels and hybrid physiology to better understand how varying stomatal conductance will affect yield.

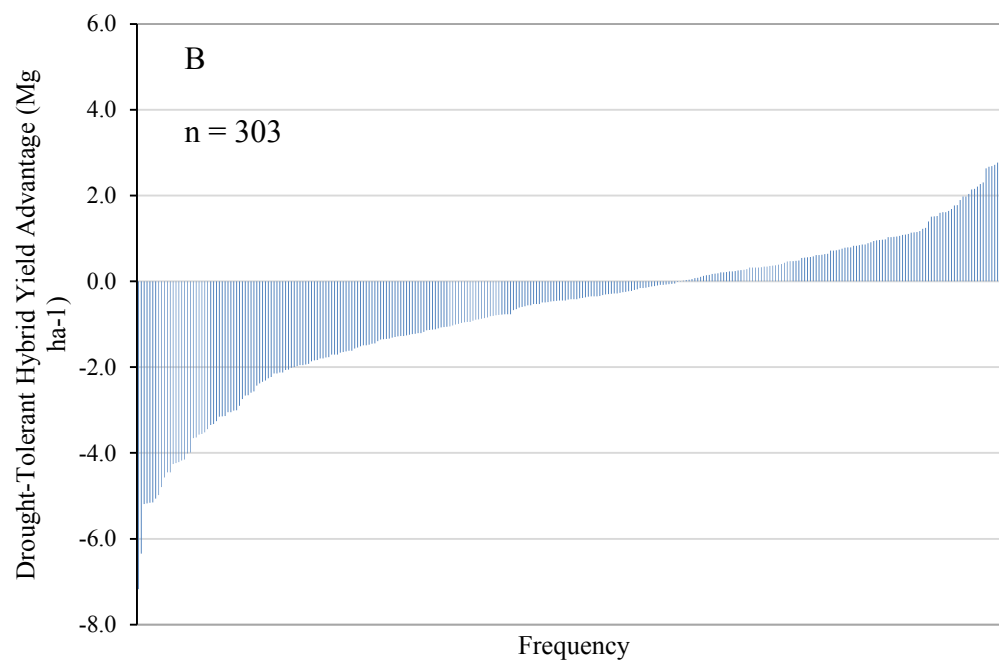
FIGURES



continued

Figure 9. A. Frequency of a yield advantage or disadvantage from planting drought-tolerant hybrids in environments where conventional hybrid yields were less than 12.2 Mg ha⁻¹. B. Frequency of a yield advantage or disadvantage from planting drought-tolerant hybrids in environments where conventional hybrid yields were greater than 12.2 Mg ha⁻¹.

Figure 9. continued



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