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FLORAL TRAIT ARCHITECTURE OF CROP SUNFLOWER (*Helianthus annuus*) UNDER DROUGHT CONDITIONS

A Thesis

Presented to

The Graduate Faculty

Central Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Biology

by

Lauren Evangeline Ormsby Segarra

March 2020

CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

We hereby approve the thesis of

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Candidate for the degree of Master of Science

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Dr. Eric Graham

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Dean of Graduate Studies

ABSTRACT

FLORAL TRAIT ARCHITECTURE IN CROP SUNFLOWER (*Helianthus annuus*) UNDER DROUGHT CONDITIONS

by

Lauren Evangeline Ormsby Segarra

March 2020

Longer and more intense droughts are predicted to become more common in the coming century due to anthropogenic climate change. Drought can reduce crop yield and decrease food security. In order to mitigate the negative effects of drought on crop production, it is important to elucidate the underlying mechanisms that promote drought stress resistance in crop plants. Floral traits impact yield, especially in oilseed crops such as sunflower (*Helianthus annuus*), but their susceptibility to drought stress is understudied. The goal of this study was to describe the floral trait architecture of *H. annuus* crop lines under drought versus well-watered conditions and examine the relationship between these traits and drought resistance. Forty *H. annuus* lines from the Sunflower Association Mapping population were assessed for size traits (height, stem diameter, head diameter and mass) and floral traits (floret lengths, nectar volume and concentration) in a field experiment under well-watered and drought conditions. Drought stress resulted in a decrease for most size traits, as well as shorter corollas and styles, and a decrease in average nectar volume. Floral sucrose concentration was unaffected by drought stress; however, line and line by treatment variation was observed for this trait and for average nectar volume. Line effects were highly significant for each trait, indicating that all traits measured have a strong genetic component. Lines differed significantly in their response to drought for head diameter at time of flowering, anther length, and days to flower. Larger size generally increased drought resistance. Nectar sucrose concentration had a significant positive correlation with final height of the plant and seed total in the watered treatment, indicating that larger plants with higher seed totals had higher nectar sucrose concentrations by volume than those in the drought treatment. The results involving shortened corolla and style lengths during drought should be studied further to determine whether there is an advantage for agricultural pollinators. Anther length was the only floral trait correlated with drought resistance. Anther length should be studied further in order to determine if its conservation across treatments can be useful for improving future *H. annuus* marker assisted selection efforts.

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CHAPTER I

INTRODUCTION

Drought and its Agricultural Implications

Drought, which is defined by the World Meteorological Organization (1992) as a “prolonged absence of marked deficiency of precipitation,” is an ecological, agricultural, and humanitarian issue. Water is the most important component for growing crops, and periods of persistent drought can severely decrease harvests (Barr 1981). More than 60% of global food production is grown with rain-fed systems (Tirado and Cotter 2010), and areas where ambient precipitation is depended upon to irrigate crops may become hotter and drier in the coming years due to anthropogenic climate change (Funk et al. 2008). Arid, semi-arid, and dry-subhumid regions are the most vulnerable to predicted climate changes, because they have the highest risks of desertification and frequent instances of drought (Falkenmark and Rockström 2008). Climate change is thus likely to increase food insecurity in regions that are already experiencing limited agricultural production. In the face of a changing climate and potential periods of more frequent and intense droughts, it is necessary to improve our understanding of how drought affects the yield of crop plants and how these effects could be mitigated.

Drought Resistance

Modern agriculture aims to continually increase yield. Historical plant breeding efforts focused on achieving high yields under optimal (well-watered) growing conditions have resulted in the loss of natural drought resistance of many crop species (Donald 1968, Mayrose et al. 2011). This lack of resistance in highly-selected, fast growing crop plants has been inferred to be due to tradeoffs between traits that are focused on acquiring resources (such as root depth and size) and traits that are linked to drought tolerance (genes, metabolites, early maturation) and the conservation of resources (Cattivelli et al. 2008, Mayrose et al. 2011, Koziol et al. 2012). It is therefore imperative to identify traits in the crop germplasm and from wild relatives of crops that may still be involved in retaining size and yield while also contributing to drought stress resistance.

Marker Assisted Selection

If we are able to identify traits or genetic lines that confer drought resistance, then those could be utilized in breeding programs through marker assisted selection. Marker-assisted selection (MAS) involves identifying specific DNA fragments, which are referred to as “markers,” that are linked to specific genes or quantitative trait loci (QTLs) that influence a trait, such as drought resistance (Tirado and Cotter 2010). In order to identify markers that may infer resistance to drought stress, crop plants are grown under these stress conditions to ascertain which genetic lines exhibit the preferred traits (Cobb et al. 2013). One such crop species that has been under investigation during the past two decades for its ability to resist abiotic stresses is crop sunflower (*Helianthus annuus* L.) (Burke et al. 2002, Rieseberg 2006, Kane and Rieseberg 2007, Koziol et al. 2012, Badouin et al. 2017). Valuable resistance genes identified during MAS can then be selected during crop breeding in order to increase the ability of the species in question to endure challenges imposed by drought stress (Baack and Rieseberg 2007, Cattivelli et al. 2008).

CHAPTER II

LITERATURE REVIEW

Climate Change and Drought in Agricultural Systems

One of the predicted effects of anthropogenic climate change is longer and more intensive droughts (Backhaus et al. 2014, Neilson et al. 1989, Trenberth et al. 2013). Drought is a primary cause of yield reduction in agricultural systems (Cattivelli et al. 2008, Shao et al. 2009). More severe droughts thus increase potential for crop failure, weakening food security and threatening widespread famine for the growing worldwide human population (Bita and Gerats 2013). In order to mitigate such dire consequences, there is increasing demand for the identification and study of cultivars that can maintain stable, high yields under drought stress (Fulda et al. 2011). It is therefore imperative to study a diversity of economically important crop species under realistic, low water field conditions in order to help farmers and agricultural stakeholders cope with the threat of climatic changes.

Sunflower As a Model Organism

Sunflower (*Helianthus annuus L.*) is an important crop species worldwide, ranking 11th in total area harvested (Kane et al. 2011). *H. annuus* was first domesticated approximately 4000 years ago by Native Americans in the present-day central United States and was grown as a source of edible seeds, dye, and for ceremonial uses (Mandel et al. 2011). Today, 80-85% of *H. annuus* grown as a crop in the United States is solely for the production of oilseed (Mandel et al. 2011). Sunflower is now one of the five most important oilseed crops in the world (Castillejo et al. 2008, Sammataro et al. 1985), and its seeds are valuable sources of oil for food production and cosmetics. Numerous studies have shown that drought stress reduces seed yield, oil production, and oil composition in common *H. annuus* cultivars (Ali et al. 2009, Rauf and Sadaqat 2008, Shao et al. 2009, Stagnari et al. 2016), but the natural history of *H. annuus* and related *Helianthus* species, as well as existing variation in crop-type *H. annuus* genotypes, suggest potential for improving drought resistance in oilseed sunflower (Burke et al. 2002, Kane and Rieseburg 2007).

Wild *H. annuus* is a moderately drought tolerant plant that is weedy in nature, meaning that it grows in disturbed places under suboptimal conditions (Kane et al. 2013). *H. annuus* is also a member of the diverse genus, *Helianthus,* in which some hybrid species have been documented in environments as extreme as desert regions in the southwest United States (*H. deserticola*), sand dunes (*H. anomalus*) and Texas salt marshes (*H. paradoxus*) (Rieseburg et al. 2003, Rieseburg 2006). These wild hybrid species are self-incompatible, meaning that they cannot produce seeds without cross pollination, which is a major difference between wild *Helianthus* species and domesticated *H. annuus* (Rieseburg 2006). The wild *H. annuus* is the same species as crop *H. annuus*; however, the growth habit of wild *H. annuus* differs from that of the cultivated in several ways. Wild *H. annuus* plants can have 40-50 flowering heads that shatter to release their seeds, while on the contrary, cultivated lines have one main inflorescence and the seeds remain on the head until harvest (Burke et al. 2002, Kim and Rieseburg 1999). Additionally, the achene weight of cultivated *H. annuus* ranges from 55-65 mg, while the average achene weight of wild *H. annuus* ranges between 9-10 mg (Burke et al. 2002, Kim and Rieseburg 1999). Discoveries of wild relatives of *H. annuus* that were found to be more stress tolerant (such as *H. petiolaris*) led to the production of commercial *H. annuus* hybrids (Leclercq 1969, Seiler et al. 2017). *H. anomalous*, a hybrid species that is native to desert sand dunes that has been recognized as drought tolerant, has large achenes with high oil content and is a good candidate for improving abiotic stress tolerance of cultivated *H. annuus* (Nabhan and Reichhardt 1983, Seiler et al. 2006). *H. annuus* is often grown as a dryland, rainfed crop because it has deep roots and therefore able to extract water from depths that are not reachable by other crops (NDSU 2007, Zheljazkov et al. 2008). The natural drought resistance exhibited by *Helianthus* species and select cultivated *H. annuus* suggest great potential for identifying causal loci that could be used in crop improvement through marker-assisted selection (Cattivelli et al. 2008, Coop et al. 2010).

Sunflower and Drought

Several recent studies have begun to unravel the complex trait relationships and associated genetic architecture underlying drought stress response on crop *H. annuus* (Mayrose et al. 2011, Masalia et al. 2018, Owart et al. 2014, Seiler 2007). In 2007, Seiler collected seed from *H. anomalous,* a desert wild relative of *H. annuus*, and found that it had the largest seeds and the highest oil concentration of wild *Helianthus* species. Because this wild relative has the same chromosome number as cultivated *H. annuus*, it is a good candidate for the introgression of desirable drought tolerance traits from the wild germplasm into the crop *H. annuus* (Seiler 2007). Owart et al. (2014) studied the genetic architecture and how phenotypic selection acted upon vegetative, reproductive, and physiological characteristics of crop-wild hybrids of *H. annuus* during low-water conditions. They found that some crop derived traits were preferential in the low-water treatment and suggest that these alleles could spread into wild sunflower populations during periods of water stress (Owart et al. 2014). Masalia et al. (2018) found that water stress conditions caused a reduction in seedling size and a shift towards deeper rooting, and that these effects varied across the group of genotypes they were studying. These studies mainly focused on seed production, size traits, and root traits for the improvement of crop *H. annuus* grown during periods of drought.

Floral Traits and Drought

The aforementioned drought studies in *H. annuus* have largely ignored floral traits which are considered to be understudied in the Asteraceae (Torres and Galetto 2002). Studies in other wild and cultivated species show that drought influences several floral traits that would likely impact yield in oilseeds (Seiler 2007, Hussain et al. 2018, Descamps et al. 2018). Flowering can be a costly process for plants in terms of water usage, and plants often produce smaller flowers in times of drought (Carroll et al. 2001, Caruso 2006). This could mean that certain floral traits, such as corolla and anther length, may be affected detrimentally by drought stress and could affect the ability for pollinators to access nectar, which is produced by nectaries at the base of the style deep in the corolla tube of each disc floret (Sammataro et al. 1985, Torres and Galetto 2002). Honeybees (*Apis mellifera* L.) are the most common managed pollinator of agricultural crop *H. annuus*, and it is well known that presence of pollinators increases oil yield, seed yield, and seed oil percentage for most *H. annuus* crop hybrids (NDSU 2007). Tongue length of bees can limit which bee species are able to gain nectar rewards as corolla depths increase (Mallinger and Prasifka 2017). Common honeybees (*Apis mellifera* L.) have tongue lengths of 6.6 ± 0.3 mm and some wild bees (*Bombus* spp.) have much longer tongue lengths that range from 7.8 mm to 9mm or longer (Apatov 1929, Balfour 2013, Inouye 1980). Abnormal anther morphology, compounded by decreased nectar volume from periods of water stress, can lead to reduced pollen transfer, which can decrease the amount of plant-pollinator interaction and ultimately lead to decreased crop yield (Descamps et al. 2018).

Floral nectar volume and sugar concentration have also been shown to be negatively affected by abiotic stress. Floral nectar production is positively correlated with soil moisture levels (Waser and Price 2016). Decreased precipitation could thus lead to less nectar, which may result in fewer pollinators and reduced crop yield (Phillips et al. 2018, Stagnari et al. 2016). In a study by Carroll et al. (2001) on *Epilobium angustifolium* (Fireweed), drought conditions led to a threefold decrease in nectar volume and a 33% flower size decrease when compared with fully watered controls, suggesting that drought can indirectly influence floral traits that function as pollinator advertisements.

Previously mentioned studies involved plants with inflorescences consisting of single larger flowers on a terminal raceme or cyme (Caruso 2006, Caroll et al. 2001, Descamps et al. 2018, Waser and Price 2016). The flowers involved in these studies do not produce copious amounts of seeds such as those in the Asteraceae with their specialized inflorescences. Although *H. annuus* is pollinated by many different insect species in the Hymenoptera and the Lepidoptera (Knopper et al. 2016), honeybees (*Apis spp.*) are the main pollinators used for the pollination of crop sunflower when grown in large-scale agricultural settings (Mani and Saravanan 1999,Vear et al. 1990, Zajacz et al. 2006, NDSU 2007). The inflorescence of *H. annuus* is especially attractive to honeybees, whose main diet consists of floral nectar (Knopper et al. 2016), due to the inflorescences' strong aroma and secretion of high quantities of sugar-rich nectar (Sammataro et al. 1985, Vear et al. 1990). If some genetic lines of sunflower are able to maintain their floral nectar resources for pollinators while under drought stress, then future research may be able to elucidate which genes may be associated with drought resistance in order to maintain nectar rewards for optimal yield. In comparison to other plant families, there are only a few studies involving nectar production and *H. annuus* (Vear et al. 1990, Sammataro et al. 1985, Zajacz et al. 2006), and none of these studies address the response of floral traits to drought conditions. The inflorescence of *H. annuus* is a capitulum: a radiate head containing peripheral ligulate ray florets (petals) and numerous disc florets in the center of the inflorescence (the corolla tube containing anthers, style, ovary, and nectaries) (Funk et al. 2009, Mani and Saravanan 1999). The capitulum behaves as a single blossom; the large, brightly colored ray petals attract a diversity of pollinators to visit the disc florets (Mani and Saravanan 1999). Once pollinated, the disc florets mature to seeds (achenes) which contain valuable oil (Funk et al. 2009). Further research is necessary in order to understand how drought reduces seed production in *H. annuus* and if the floral traits involved in the unique capitulum inflorescence structure are in any way relevant to drought resistance.

The majority of studies examining floral traits under drought conditions were conducted in a greenhouse (Carroll et al. 2001, Caruso 2006, Descamps et al. 2018, Waser and Price 2016) or in the field with rain-out shelters (Phillips et al. 2018). It is imperative to understand how floral architecture may be impacted by drought in a field experiment as it is the closest scenario to what many farmers may experience in the coming years due to more frequent droughts brought on by climate change.

In this study, previous drought experiment data (*unpublished data* 2018) was analyzed in order to rank crop *H. annuus* lines from the Sunflower Association Mapping (SAM) population (Mandel et al. 2011) by drought resistance and to select 40 lines for floral assessment. Several floral and size traits were assessed under well-watered and drought conditions in an agricultural field experiment, and the relationship between floral traits and drought resistance was examined. These data describe how drought affects floral trait architecture and identify floral traits that predict drought resistance in cultivated *H. annuus*. Additionally, seed total data was used to rank the SAM lines by drought resistance. Resistance across the SAM lines can be compared to other studies in this population in order to identify lines that consistently resist stress or that may harbor genes that confer resistance to multiple abiotic stresses.

CHAPTER III

LINE SELECTION

When planning this thesis project, we determined that we had space and resources to examine 40 SAM lines. These were selected using data from a previous years experiment in which nine replicates each of 60 SAM lines were grown under drought and well-watered conditions in the field in Ellensburg, WA from June to October 2018. At physiological maturity, each individual was harvested and the apical inflorescence (API) was dried for at least 48 hours and then weighed. These data were used to estimate drought resistance as the residuals from a linear model regressing API mass for each line in the drought treatment versus its API mass in the watered treatment (Figure 1) (R Core Team 2012). In order to select the 40 lines to be used in my 2019 thesis field research, we excluded lines that fared poorly in the 2018 field and exhibited low drought resistance – those with mean API mass values in the drought treatment less than 5 g. This removed 15 lines (Figure 1). The remaining five that we chose to remove from my thesis research were randomly selected from a group of eight lines that had low residual values.

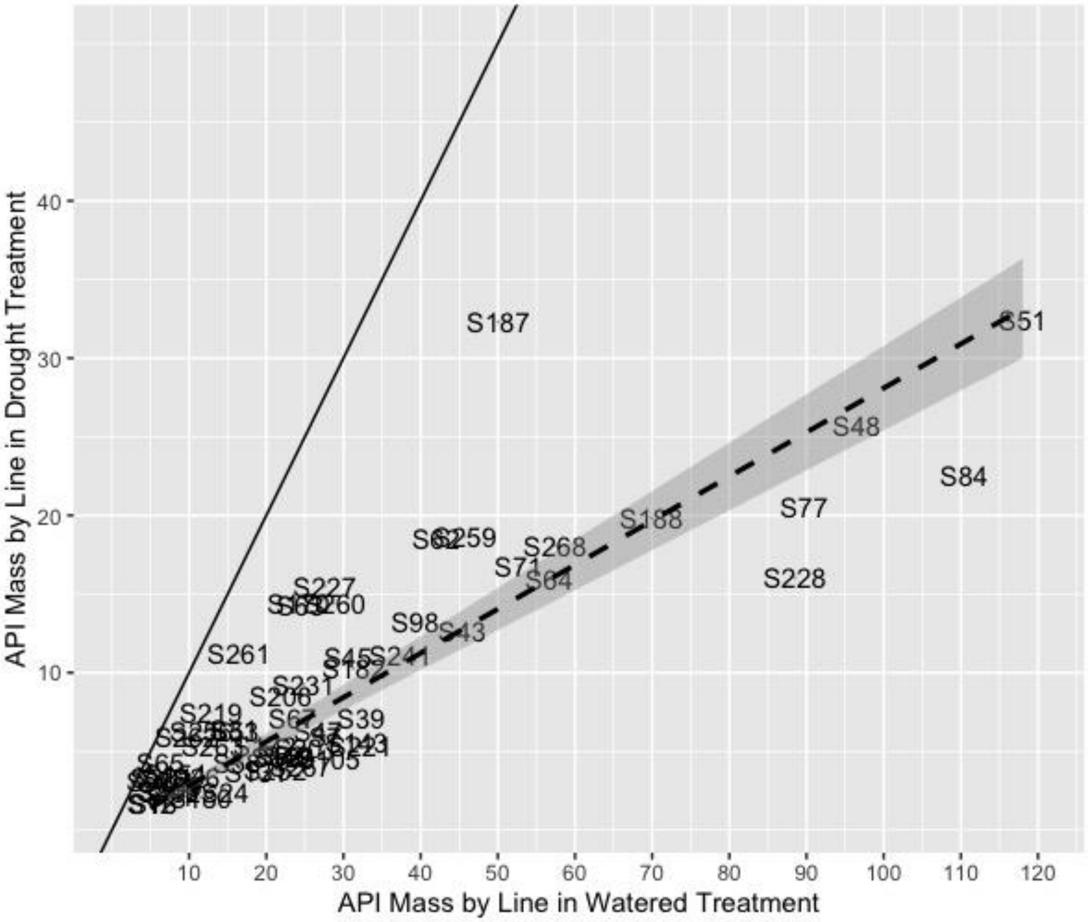


Figure 1: Line selection scatterplot. A scatter plot showing mean API mass for each 2018 SAM line (numbers shown) in the watered treatment (X-axis) by its mean API mass in the drought treatment (Y-axis). The solid black line shows a slope of 1. The dashed black line shows the trend line for the linear regression and the gray surrounding area shows 95% confidence intervals. The group of lines in the bottom left of the figure were removed for my 2019 thesis experiment.

CHAPTER IV

METHODS

Study System

As described in Chapter III, we selected 40 lines from the Sunflower Association Mapping (SAM) population (Mandel et al. 2011) that ranged in drought resistance and produced seed in a 2018 drought experiment at our field site. The full SAM population of 288 lines represents nearly 90% of the allelic diversity within the germplasm of cultivated sunflowers (Mandel et al. 2011). The lines used in this study represent a range of resistances to various abiotic stressors (Masalia et al. 2018, Gao et al. 2019) and include the *H. annuus* line XRQ that has been fully sequenced (Badouin et al. 2017).

Study Location

The 40 SAM lines were grown to maturity in a 12.2 m by 22.6 m fenced field site in Ellensburg, WA (47°00’50”N, 120°31’28”W) during May - October 2019. The average annual high temperature of Ellensburg is 15.5 C, the average low is 2.1 C, and the average annual precipitation is 22.6 cm (US Climate Data 2019). The soil in the field is a combination of Opnish Ashy Loam (a Vertisol) and Mitta Ashy Silt Loam (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture 2019).

Field Design

On May 18, 2019, eight replicates of each of the 40 SAM lines were planted in a randomized block design. Two blocks, (block A and block B) were each divided into a drought and a watered treatment (sub-block). There were 160 plants per sub-block, and they were organized into three double rows, totaling 640 individuals in the experiment (Figure 2). Each individual was planted 30.5 cm apart. A standard cultivated hybrid *H. annuus* line provided by the United States Department of Agriculture was planted along the border of each sub-block in the same manner. The purpose of the border plants was to reduce edge effects and buffer between watered and droughted sub-blocks.

The field was irrigated with two overhead oscillating sprinklers at planting for 12 days until seedlings were established, then the oscillating sprinklers were removed and replaced with drip hoses that were placed in the center of each double row. The drip hoses were set to water each day for 45 minutes. Once seedlings were established (June 21st, 35 days after planting), the drip hoses for both of the drought blocks were turned off so that those plants did not receive any water other than ambient precipitation throughout the rest of the experiment. Soil moisture and conductivity data were monitored once a week during the field season with WaterScouts that were installed between rows in each block on May 24th (Spectrum Technologies 2017). All of the WaterScouts failed and had to be replaced on July 17th, so all water content data was taken between July 17th and August 23rd (time period between the midpoint census and the tail end of flowering). For June, July, and August 2019, the cumulative ambient precipitation recorded at a weather station adjacent to the sunflower field was 5.82cm (Weather Underground).

Seedlings began to emerge six days after planting, and we first censused on May 27, 9 days after planting. Emergence was monitored for 10 days. Seedlings were thinned on June 7 and extras of the same line that were removed from thinning were transplanted to

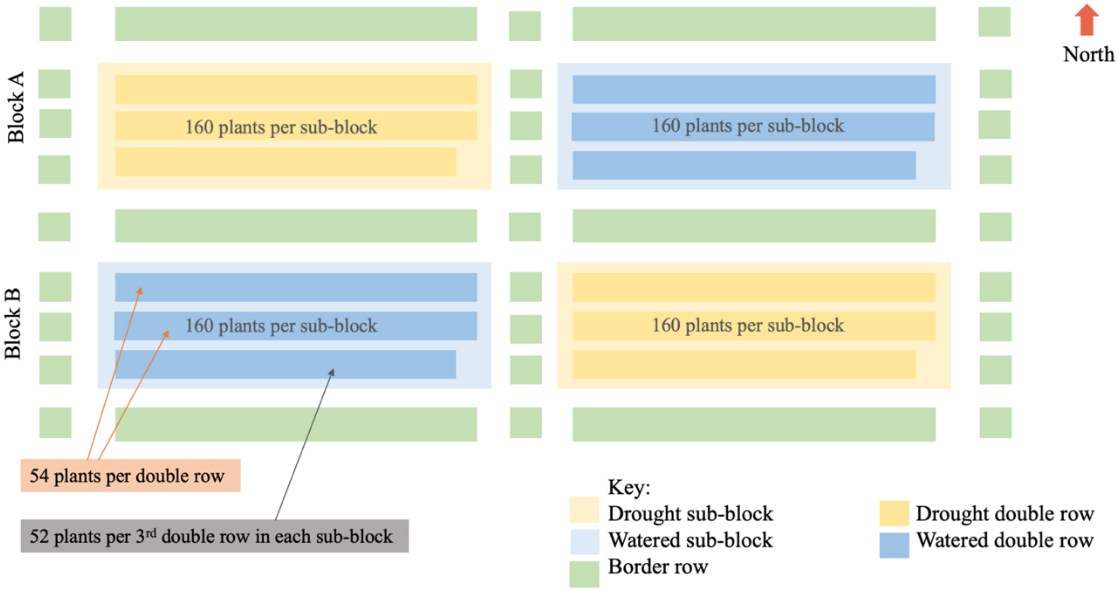


Figure 2: Field design diagram. Two blocks (A and B) with 160 individual plants per sub-block. Drought sub-blocks are shown in yellow and watered sub-blocks are shown in blue. Border rows are shown in green.

other planting sites that had not germinated when possible. The field was fertilized on June 20th when most of the seedlings were at the four to six leaf stage. Osmocote Blend 20-8-4 (Osmocote 2017) was added at a rate of 12.5 pounds per acre using a rolling push spreader.

Twelve soil samples (collected on June 18th) that were taken evenly across the field showed soil nitrate levels that ranged from 6 to 78 ppm (low to high) (MidWest Laboratories 2019). Phosphorous across the field was found to be 7-25 ppm (low to high), and potassium levels ranged from 204-270 (high to very high). The pH of the soil ranged from 6.4-7.1 pH. Prior to planting, we estimated the water table to be greater than 4.5 ft (1.37 meters) at the far north side of the field and at the southern end of the field.

Both soil classes (Opnish Ashy Loam and Mitta Ashy Silt Loam) identified for the field location of the experiment were rated as medium in terms of soil susceptibility to compaction, which indicated that there was a significant potential for soil compaction (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture). Soil compaction measurements were taken after planting at five intervals along transects that were 680cm in length for each double row of seedlings with a penetrometer (Soil Compaction Tester, Agratronix™). It was noted that some sections in Block A Dry, Block B Watered, and Block B Dry had average compaction at depth greater than 500 PSI.

Flowering Date and Floral Trait Measures

The midpoint census of height and stem diameter was completed two months after the planting date on July 18th, the approximate halfway point of the growing season (Table 1). Height was measured from the base of the stem to the base of the API. Stem diameter was taken with digital calipers at 3 cm above the soil line. During this census, 39% of plants were in the R3 stage (the immature bud of the apical inflorescence is greater than 2 cm above the most recently expanded leaf) and 2% had reached the R5.1 stage (the beginning of flowering in which at least one row of disc florets have reached the staminate stage where pollen is being presented) (NDSU 2007).

Plants began flowering on July 15th. The flowering date was recorded for each individual when it reached the R 5.1 stage (NDSU 2007). Days to flower was calculated as the number of days that elapsed between the planting and flowering dates (Table 1). Nectar and disc floret collection occurred when plants reached the R 5.3-R 5.6 stage (the flowering stage in which three to six rows of disc florets are presenting pollen or stigmas), which is approximately 1-2 days after the R 5.1 stage (NDSU 2007). Head diameter was taken during this stage as well (during the R 5.3-5.6 stage). All nectar measurements were taken during 6:00 – 10:00 am on days 2 - 4 of each API's bloom. The nectar measurements were taken during this time frame in order to minimize nectar predation from bees and other insects which are more active during warmer, sunnier parts of the day. Nectar was collected from 10 - 20 haphazardly selected disc florets from each individual plant's API that were in the pistillate (female) stage. One μL microcapillary tubes (Drummond Microcaps®) were gently inserted to the base of the corolla, and we measured the length of the withdrawn nectar with a millimeter ruler (Burquez and Corbet 1991, Descamps et al. 2018, Mallinger and Prasifka 2017, Morrant et al. 2009, Roubik 1995, Vear et al. 1990, Wist and Davis 2006, Zajacz et al. 2006). The volume of withdrawn nectar was calculated from the height of the nectar column and converted from μL to nL. The average nectar volume per floret was calculated by dividing the total nectar volume collected by the number of florets sampled (Table 1). If all sampled florets were empty, nectar volume was recorded as zero. It is important to note that we chose not to bag API's prior to nectar collection in order to maintain realistic field conditions. In a study by Wyatt et al. (1992), they found that bagging inflorescences before nectar collection from *Asclepias* species changed the microenvironment of the inflorescence inside the bags, causing changes in temperature, relative humidity, and nectar dilution. This was also observed during a study involving nectar secretion and relative humidity in *Epilobium angustifolium* (Bertsch 1983). A caveat is that some nectar may have been removed by pollinators before collection.

After collection, nectar was released onto the prismatic surface of a low volume refractometer (0-50% Bellingham and Stanley, Tunbridge Wells, Kent, UK). At the time of nectar collection, the ambient air temperature (in Celsius) of the field was recorded in order to correct the raw Brix° readings to 20°C. The Brix° reading was recorded and then later corrected to 20°C (manufacturer's reference manual) before converting to ng sugar per nL nectar (NCV) using Búrquez and Corbet's quadratic equation (1991). In order to estimate the nectar sugar quantity per disc floret (Table 1), NCV was multiplied by the average nectar volume per floret for that individual (Wist and Davis 2006).

Nectar collection always occurred first, and then disc florets were collected for anther, corolla, and style length measurements. Ten disc florets in the staminate stage (the “male” stage where the disc floret is presenting only anthers) were collected per plant with tweezers and placed into their own labeled plastic bag in a cooler for transportation from the field to the laboratory. Then, ten florets in the pistillate stage (the “female” stage where the style and stigmas have pushed up through the anther tube and are showing) were collected from each plant and stored in their own labeled plastic bag in the cooler. Since the sunflower disc florets develop centripetally from the outer rim of the capitulum (disc), disc florets of both pistillate and staminate stages occur simultaneously, allowing for collection of both disc floret stages on the same date (Dosio et al. 2011). Some inflorescences were too small to have enough florets to collect ten of each stage. In that case, as many florets of each stage (staminate and pistillate) between flowering stages R5.3-5.6 were collected as possible per individual.

Collected florets were stored at 4°C and dissected within 24 hours after being removed from the field. Anthers were separated from the filament column and placed onto a flatbed scanner along a number line with a ruler for calibration. The corresponding corolla tubes from the staminate florets were also placed along the number line. Corolla length can be measured as a proxy for corolla depth (Portlas et al. 2018). The styles were then gently removed from the pistillate florets and placed along the number line on the scanner bed as well. The dissected floral parts was then scanned for image analysis.

Image Analysis

Image J (Schnieder et al. 2012) image analysis software was used to measure the dissected floret parts from the scanned images. Before measuring, the straight and segmented line tools were calibrated to 10 mm. Each anther was measured from base to tip using the straight line measure tool. The corollas were measured from the base to the indentation between two corolla lobes with the straight or segmented line tool following protocols in a study done by Portlas et al. (2018). Finally, styles were measured from the base to the stigma split point. The ten measurements per trait and plant were averaged to generate corolla, anther, and style length per individual (Table 1).

Harvest and Final Trait Measures

All plants had reached stages R8 - R9 (developed seeds to senescence) and were harvested on October 5th and 6th. Final plant height and stem diameter were measured for each individual following the same procedure as at midpoint. Each API was removed at the point of attachment to the stem, dried in a paper bag for at least 48 hours at 26.7 °C, and then weighed (g). Since birds had scavenged seeds from the field prior to our final harvest, some individual plants had seeds missing. We weighed individual API's that had >70% seed remaining. Even though seed was missing, seed total was still possible to estimate because the indentations from missing seeds were still plainly visible on the API. We calculated seed total for each individual by counting seed insertions across the diameter of its API, dividing this in half to generate a seed number radius, and using πr^2 to estimate seed total (Table 1).

Data Analyses

All data analysis was executed with R statistical software (R Core Team 2012). The final sample sizes were ~600 and ~615 for midpoint and final harvest size traits, respectively. The sample size for head diameter at flowering was 580. The sample size for API mass was 448 and 571 for seed total. The sample size for days to flower was 601. For floral traits, the sample size was ~569. General linear mixed effects models were employed to test the effects of block (fixed factor), treatment (fixed factor), line (random factor), and line x treatment (random factor) for each size and floral trait (lmerTest R package, Kunetsova et al. 2015). For nectar traits, the final sample size was 274. Nectar traits had a gamma distribution and were thus tested using the model (glmmPQL) with block and treatment as fixed factors and line as a random factor with "log" as the link function (MASS package, Venables and Ripley 2002).

Drought resistance was defined as the model residuals taken from the regression of the mean seed total for each line in the drought treatment as a function of that line's seed total in the watered treatment. The resistance graph was created with the R package ggplot2 (Wickham 2016). Relationships between each trait measured and resistance (seed total residual value) were tested using Pearson correlations. Pairwise trait correlations were also tested with Pearson correlations. Differences in environmental variables (soil

Size, Floral, and Nectar Trait Descriptions			
Type	Trait Name	Abbreviation	Description
Size	**Midpoint stem height**		The height of the stem in cm from the base of the stem to the API at the midpoint census
	Midpoint stem diameter		The diameter of the stem measured at 3 cm above the soil line at the midpoint census
	Head diameter at time of flowering		The diameter of the disc portion of the API at time of floret collection: during R 5.3 - R 5.6.
	Final height		The height of the stem in cm from the base of the stem to the API at the final census
	Final stem diameter		The diameter of the stem measured at 3 cm above the soil line at the final census
	Apical inflorescence mass	API	Mass in grams of the dried apical inflorescence (API), including seeds
Other	**Days to flower**		The number of days from planting to the recorded flowering date.
	Seed total		The approximate number of seeds per API. One row of seed insertions was counted across the widest point of the disc portion of the API. This diameter was divided in half to get the radius, and then the area of a circle formula (πr^2) was used to calculate the approximate seed total.
Floral	**Corolla length**		The length of the corolla of staminate disc florets: measured from the base of the corolla to the base of where two corolla lobes meet.
	Anther length		The length of the anther of staminate disc florets.
	Style length		The length of the style of pistillate disc florets.
Nectar	**Nectar volume per floret**		The total volume of nectar per floret: an average. The total nectar volume was divided by the number of florets that nectar was collected from.
	g/nL sugar per floret	NCV	Nectar solute concentration by volume per floret. This was calculated with a quadratic equation using the raw data from the refractometer measurement.
	Nectar sugar quantity per disc floret	NSQ	Nectar sugar quantity per disc floret: a product of the multiplication of the measured NCV of the individual and the nectar volume per floret.

Table 1: Size, Floral, and Nectar Trait Descriptions and abbreviations.

compaction and water data) between blocks were tested for significance with a Welch Two Sample *t*-test in R.

CHAPTER V

RESULTS

Effects of Block and Environmental Factors

Block effects were highly significant for all traits. The average water content of blocks A and B was 8.02 and 8.94 percent saturation, respectively, but this difference was not statistically significant (Figure 3). For both soil types in the field, Opnish ashy loam and Mitta ashy silt loam, the soil susceptibility to surface sealing was rated as high (a high susceptibility to form a surface seal) and the soil susceptibility to compaction was rated as medium (the potential for compaction is significant) (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture). Average soil compaction (averaged for all depths measured) was found to be 373.6 PSI for block A and 360.9 PSI for block B, which was not statistically different (*P* = 0.666). However, there were five measurements in the first double row of block A Drought that were measured as 1000 PSI, which indicates extreme compaction. Root growth is usually inhibited at penetrometer readings higher than 300 PSI (Duiker 2002).

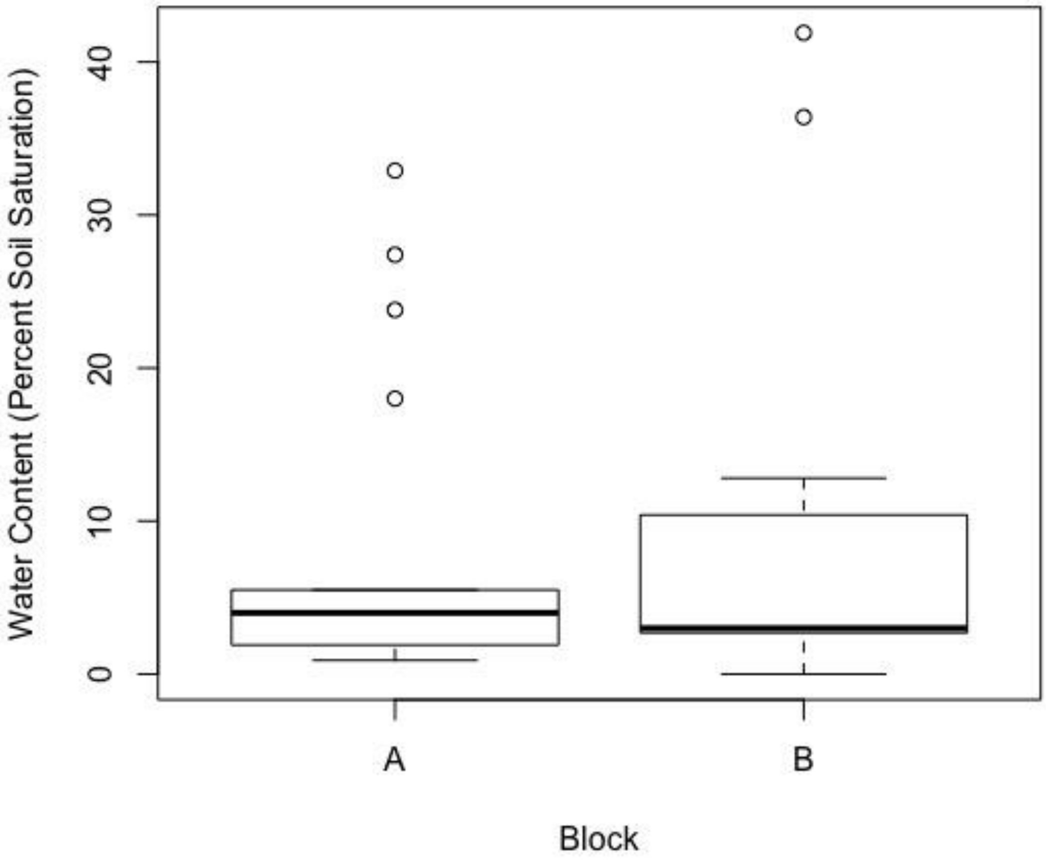


Figure 3: Boxplot of relative water content by block (percent soil saturation). The difference is not statistically significant (*P* = 0.801).

Trait Responses to Drought Treatment

All size traits differed significantly by treatment (Tables 2, 3). Of the floral, nectar, and other traits, days to flower, corolla length, style length, and nectar volume per floret differed significantly by treatment. Plants were larger in the watered treatment, with wider and taller stems throughout the growing season, larger heads, and approximately 70% more seeds. Plants flowered earlier and had longer corollas and styles in the watered treatment. Nectar volume per floret showed a significant decrease in the drought treatment. Plants with average nectar volumes of zero were not included in the data analysis because the cause of a lack of nectar was confounded by the possibilities that either the floret did not produce any nectar or the floret had already been visited by a pollinator. Nectar sucrose concentration was unaffected by drought.

Line effects were highly significant for each trait, indicating that all traits measured have a strong genetic component. Lines differed in their response to drought (significant line by treatment effects) for head diameter at time of flowering, anther length, and days to flower (Table 3). For these traits, SAM lines varied in their response to treatment; i.e., some lines had larger head diameters in the drought treatment, whereas other lines showed the opposite effect (Figure 4). It was not possible to perform an additional ANOVA on the type of general linear mixed effects model that was used to analyze the nectar data, so only block and treatment effects are included in Table 4. Although nonsignificant or untested (nectar), reaction norms show some variation in how lines responded to treatment for floral and nectar traits, suggesting that sunflower floral architecture exhibits plasticity to drought (Figure 5).

Means and Standard Errors by Trait

Trait	Watered Mean (Standard Error)	Drought Mean (Standard Error)
Size Traits		
Midpoint stem height (mm)	992.48 (16.58)*	684.99 (16.17)
Midpoint stem diameter (mm)	18.95 (0.31)*	14.86 (0.29)
Head diameter (mm)	85.12 (1.30)*	72.96 (1.25)
Final stem height (mm)	1345.31 (19.43)*	1043.00 (19.90)
Final stem diameter (mm)	20.09 (0.35)*	16.92 (0.34)
Days to flower (days)	75.44 (0.36)*	78.76 (0.46)
Final API mass (g)	71.61 (3.45)*	51.61 (3.83)
Seed total (# seeds)	909.77 (29.37)*	644.08 (25.45)
Floral Traits		
Corolla length (mm)	7.81 (0.043)*	7.57 (0.048)
Anther length (mm)	4.53 (0.035)	4.60 (0.028)
Style length (mm)	8.48 (0.080)*	8.29 (0.084)
Nectar Traits		
Nectar volume per floret (nL)	23.0 (1.9)*	19.3 (1.8)
NCV	0.0209 (0.0012)	0.0207 (0.0011)
NSQ	0.586 (0.071)	0.704 (0.060)

Table 2: Untransformed trait means and standard errors by treatment (Watered, Drought). Asterisk (*) denotes traits that differed significantly by treatment.

Results of Linear Mixed Effects Models for Size and Floral Traits

	Midpoint stem height	Midpoint stem diameter	Head diameter at time of flowering	Final stem height	Final stem diameter	Days to flower
Block (F, p-value)	33.63, 1.13e^{-8}***	24.57, 9.57e^{-7}***	29.83, 7.42e^{-8}***	43.3, 1.12e^{-10}***	70.9, 3.42e^{-16}***	22.81, 2.32e^{-6}***
Treatment (F, p-value)	175.02, 6.99e^{-6}***	95.98, 4.97e^{-12}***	32.91, 1.28e10^{-6}***	32.26, 1.38e^{-6}***	53.69, 8.68e^{-9}***	41.64, 1.25e^{-7}***
Line (χ^2)	8.67e^{-12}***	6.7e^{-5}***	0.000547***	3.08e^{-8}***	2.9e^{-7}***	1.38e^{-11}***
Line by Treatment (χ^2)	0.055	0.27	0.0025**	0.19	0.63	0.032*
Sample size	628	628	580	615	617	601

	Final API mass	Seed total	Corolla length	Anther length	Style length
Block (F, p-value)	29.56, 9.52e^{-8}***	8.77, 0.0032**	15.17, 0.00011***	57.12, 1.99e^{-13}***	7.85, 0.0053***
Treatment (F, p-value)	32.26, 1.38e^{-6}***	57.87, 3.71e^{-9}***	25.97, 9.36e10^{-6}***	2.36, 0.13	4.56, 0.039*
Line (χ^2)	3.08e^{-8}***	2.75e^{-6}***	8.67e^{-12}***	8.54e^{-5}***	1.59e^{-11}***
Line by Treatment (χ^2)	0.19	0.89	0.47	0.015*	0.77
Sample size	448	571	569	568	570

Table 3: Results of linear mixed effects models for size and floral traits: block and treatment as fixed effects, line and line by treatment as random effects. The table shows F and p-values for fixed effects and chi-square values (χ^2) for random effects. Sample sizes are included. Significance: ^*P* < 0.1; **P* < 0.05; ***P* < 0.01; ****P* < 0.001

Results of General Linear Mixed Effects Models for Nectar Traits

	Nectar volume per floret	NCV	NSQ
Block (t-value, p-value)	-3.19, 0.0015**	0.47, 0.64	-1.70, 0.089
Treatment (t-value, p-value)	-2.41, 0.016*	-0.004, 1.0	-1.54, 0.12
Sample size	368	274	274

Table 4: Results of general linear mixed effects models for nectar traits: block and treatment as fixed effects, line as a random effect. The table shows t- and p-values for fixed effects. Sample sizes are included. Significance: ^*P* < 0.1; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

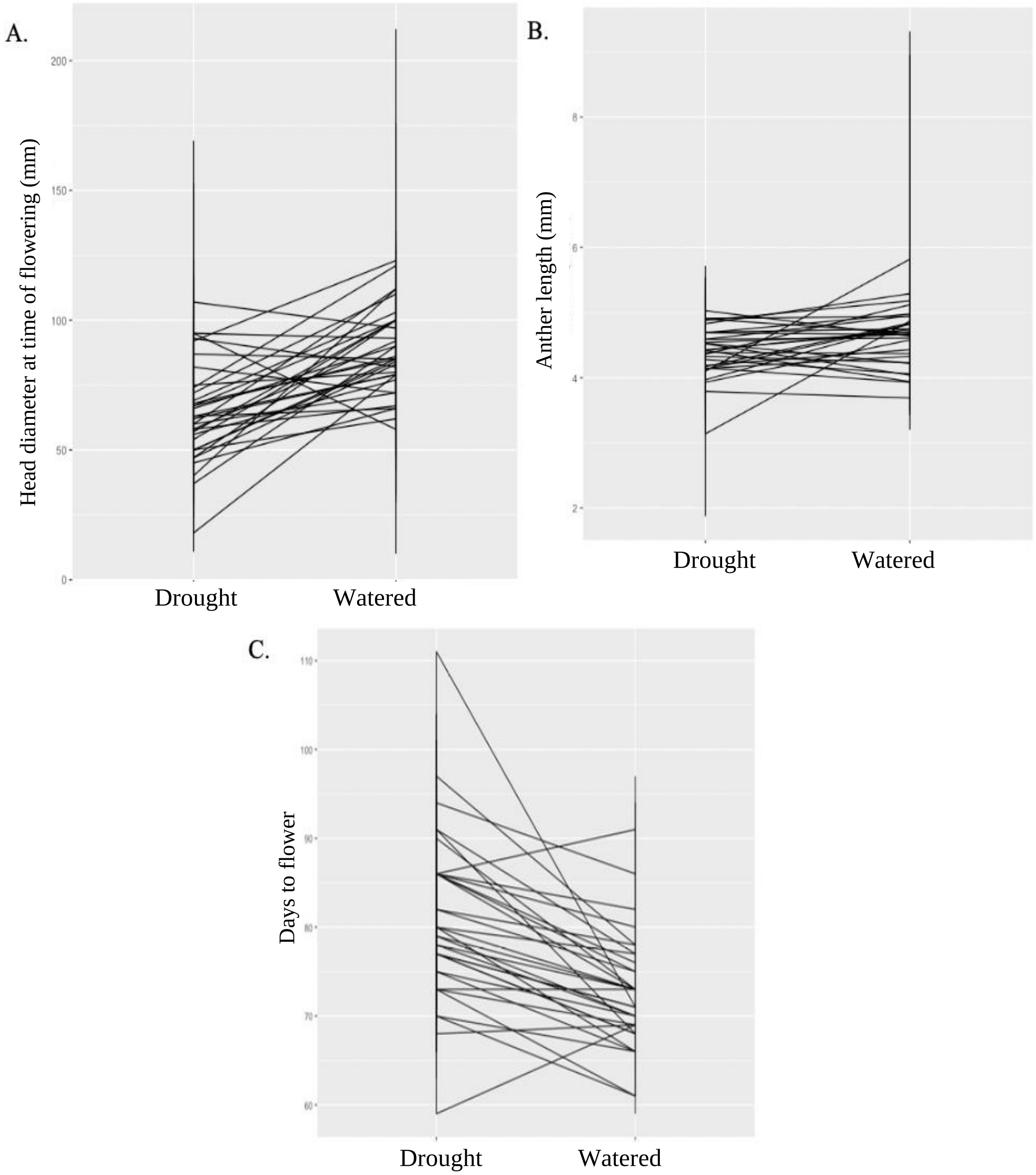


Figure 4: Reaction norm plots for traits with significant line × treatment effects: head diameter (A), anther length (B), and days to flower (C) for each of the 40 SAM lines in the drought (D) and watered (W) treatments. Each black line represents a SAM line.

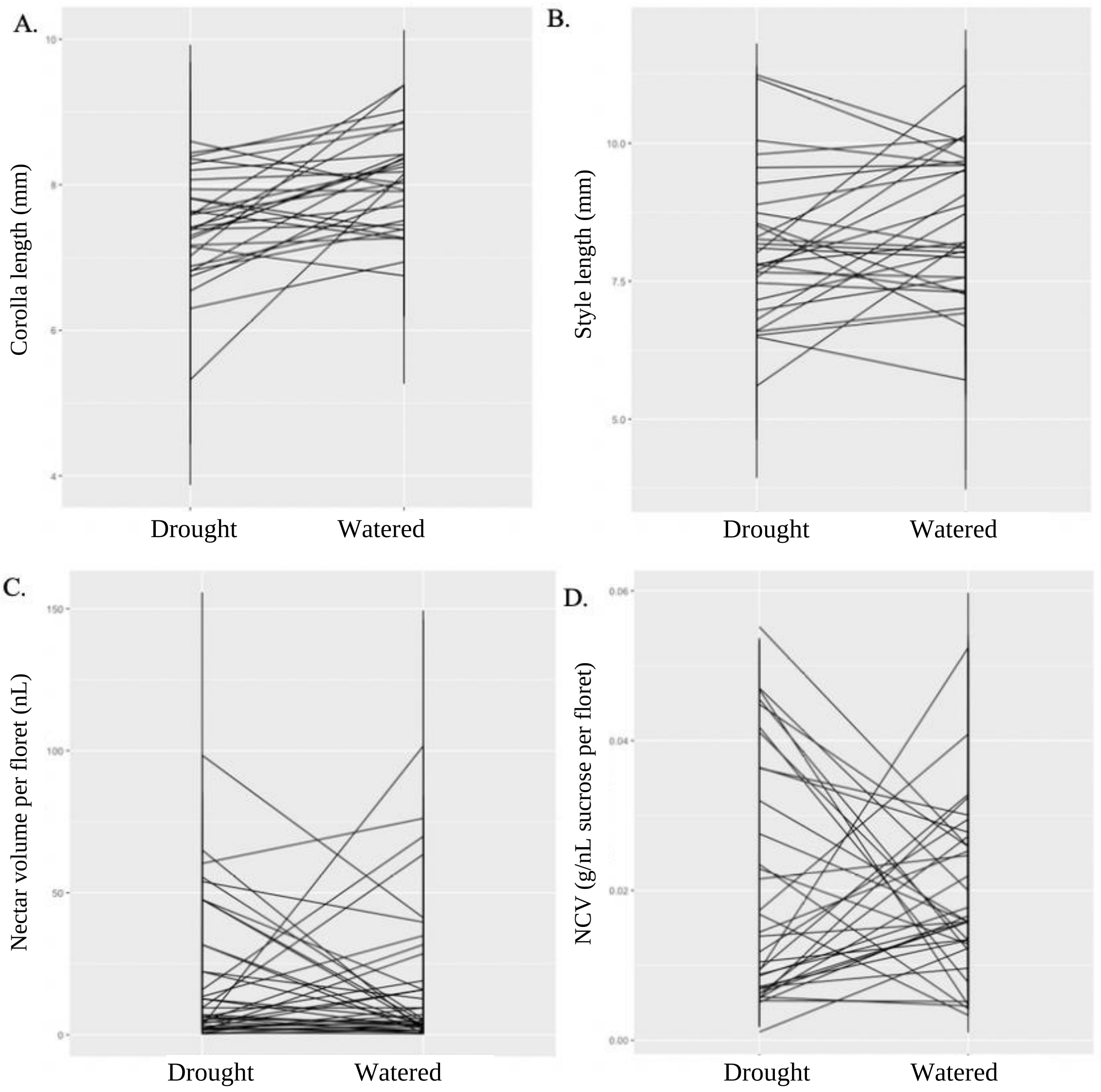


Figure 5: Reaction norm plots showing line response to treatment for floral and nectar traits that had nonsignificant or untested line x treatment effects: corolla length (A), style length (B), nectar volume per floret (C), and NCV (D) for each of the 40 SAM lines in the drought (D) and watered (W) treatments. Each black line represents a SAM line.

Defining Resistance

We defined relative resistance as the residuals from a linear mixed effects model in which each SAM line's mean seed insertion estimation (seed total) in the drought treatment was regressed on its mean seed insertion estimation in the watered treatment (Figure 6). This estimation defines resistance as relative to the genotypes included in this study. The black one-to-one line in Figure 6 denotes the null hypothesis: that each SAM line's seed total is the same regardless of treatment. The dashed regression line fell further below the black one-to-one line as seed total increased. SAM lines that had the highest (more positive) residuals were defined as most resistant to drought stress. The five most resistant SAM lines were S71, S48, S262, S51, and S131 (Table 5). All of these, except S131, had slightly higher seed totals in the drought treatment than in the watered, indicating that they were able to fully maintain seed production under drought. The lines that had the lowest (more negative) residuals were defined as those that were least resistant to drought stress. The five least resistant SAM lines were S231, S26, S63, S259, S188. These lines produced median to high numbers of seeds in the watered treatment, but were unable to maintain seed production under drought.

We also estimated resistance as percent change in seed total under drought. Percent change was calculated for each line by subtracting its mean seed total under drought from its mean seed total when watered, and dividing the difference by the watered mean seed total (Table 5). Using this estimation, the five most resistant SAM lines were S261, S262, S221, S48, and S39. The five most susceptible SAM lines were S63, S231, S26, S259, and S188.

Interestingly, only one SAM line (S262) falls into the top five most resistant to drought stress using both resistance measures (although 7 lines are in the top 10 most resistant on both lists). The two resistance measures differ because lines that produced low numbers of seeds in watered and maintained (even increased) seed production under drought (e.g., S261, S39) has positive percent change but small residuals.

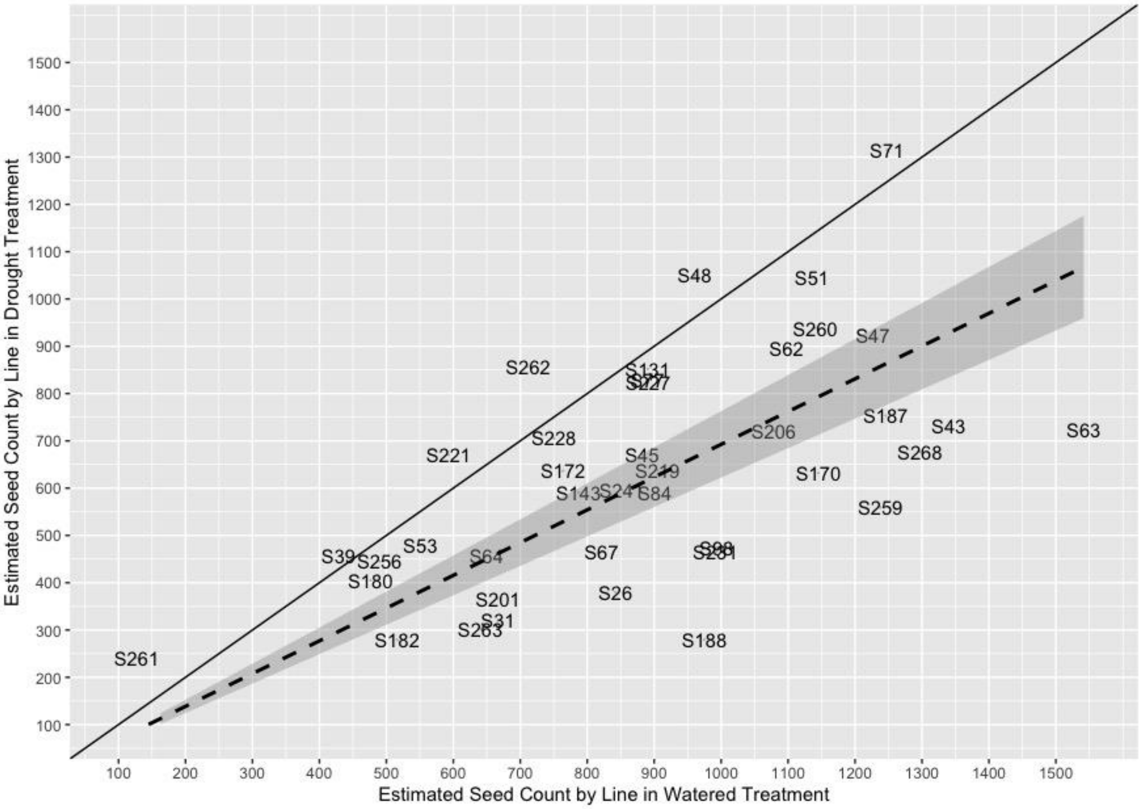


Figure 6: Defining resistance scatterplot. A scatter plot showing mean seed insertion estimations for each line (numbers shown) in the drought treatment by the mean seed insertion estimation for each line in the watered control treatment. The black line has a slope of one. The black dashed line indicates the line of best fit for the regression. The higher above the dashed line each genetic line is, the more resistant to drought stress it is. The further below the dashed line each genetic line is, the more susceptible to drought stress it is. The gray area indicates the 95% confidence intervals for the linear regression.

SAM Lines Ordered by Resistance and Seed Percent Change			
Line ranking	**Linear model residuals**	**Line ranking**	**Percent change with drought**
S71	505.24	S261	89.39
S48	381.16	S262	20.50
S262	311.18	S221	13.06
S51	289.66	S48	9.32
S131	217.66	S39	6.40
S77	194.84	S71	5.42
S227	187.73	S131	-4.31
S221	182.37	S228	-5.95
S260	180.83	S77	-6.85
S62	160.17	S227	-7.70
S228	140.18	S51	-8.01
S47	123.26	S256	-9.00
S172	64.43	S53	-13.02
S39	48.91	S180	-15.00
S45	40.51	S172	-16.68
S53	11.83	S260	-17.74
S256	8.40	S62	-18.33
S143	7.01	S45	-23.95
S219	-3.00	S47	-24.75
S206	-5.33	S143	-24.97
S241	-17.99	S219	-29.45
S261	-18.23	S64	-29.77
S180	-25.77	S241	-29.86
S84	-49.50	S206	-33.10
S187	-55.83	S84	-34.51
S64	-59.64	S187	-39.53
S43	-123.82	S67	-43.33
S170	-127.86	S170	-44.83
S67	-134.89	S201	-45.02
S268	-156.95	S43	-45.39
S201	-157.68	S182	-45.90
S182	-170.94	S268	-47.77
S31	-203.89	S31	-51.95
S263	-209.78	S98	-52.21
S98	-210.25	S263	-52.91
S231	-219.95	S63	-53.06
S26	-230.18	S231	-53.19
S63	-231.17	S26	-54.83
S259	-246.27	S259	-54.84
S188	-396.44	S188	-71.33

Table 5: SAM lines ordered by resistance. The most resistant lines are shown at the top. Columns one and two show the SAM lines ordered by residuals generated from a linear regression of seed total in the drought versus the watered treatment (Figure 6). Columns three and four show the SAM lines ordered by percent change in seed total for that line when grown in the watered versus the drought treatment. Positive values indicate an increase in seed production under the drought treatment for those six lines.

Correlations with Resistance

Of thirteen traits examined, five were significantly correlated with relative drought resistance (the residuals), and these correlations were only significant in the drought treatment (Table 6). Four of the significant correlations were for size traits: midpoint height, midpoint stem diameter, head diameter at time of flowering, and final stem diameter. Final apical inflorescence mass (API) was also marginally significantly correlated with resistance. These correlations indicate that plants that were larger in size in the drought treatment were more resistant to drought stress, in that they were able to maintain seed total under drought. The only floral trait that was significantly correlated with resistance was anther length. Plants with longer anthers in drought were more drought resistant. Days to flower had a marginally significant negative correlation with resistance. Plants that flowered earlier were more resistant to drought stress. None of the three nectar traits was correlated with resistance.

The same size traits, midpoint height, midpoint stem diameter, head diameter at time of flowering, and final height were significantly or marginally significantly negatively correlated with percent seed change (Table 7). These correlations were only observed in the watered treatment, suggesting that size when watered had the strongest relationship with percent seed decrease in the drought treatment. No floral or nectar traits were significantly correlated with seed percent decrease.

Correlations Between Traits and Resistance

	Midpoint height	Midpoint Stem Diameter	Head diameter	Final height	Final Stem Diameter	Days to flower	API mass
Correlation (Drought)	0.40***	0.51***	0.50***	0.26^	0.37*	-0.29^	0.29^
Correlation (Watered)	0.019	0.025	0.081	-0.068	-0.003	-0.12	0.13

	Corolla length	Anther length	Style length	Nectar volume per floret	NCV	NSQ
Correlation (Drought)	0.13	0.41**	0.13	0.11	0.027	0.012
Correlation (Watered)	0.018	0.15	0.0016	0.081	0.075	0.096

Table 6: Correlations between each trait and relative drought resistance (residuals) separated by treatment (Drought and Watered). R values shown. Significance: ^$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Correlations Between Traits and Seed Percent Change

	Midpoint height	Midpoint Stem Diameter	Head diameter	Final height	Final Stem Diameter	Days to flower	API mass
Correlation (Drought)	0.08	0.13	0.14	0.04	0.11	-0.10	0.02
Correlation (Watered)	-0.27^	-0.28^	-0.33*	-0.22	-0.31^	0.06	-0.20

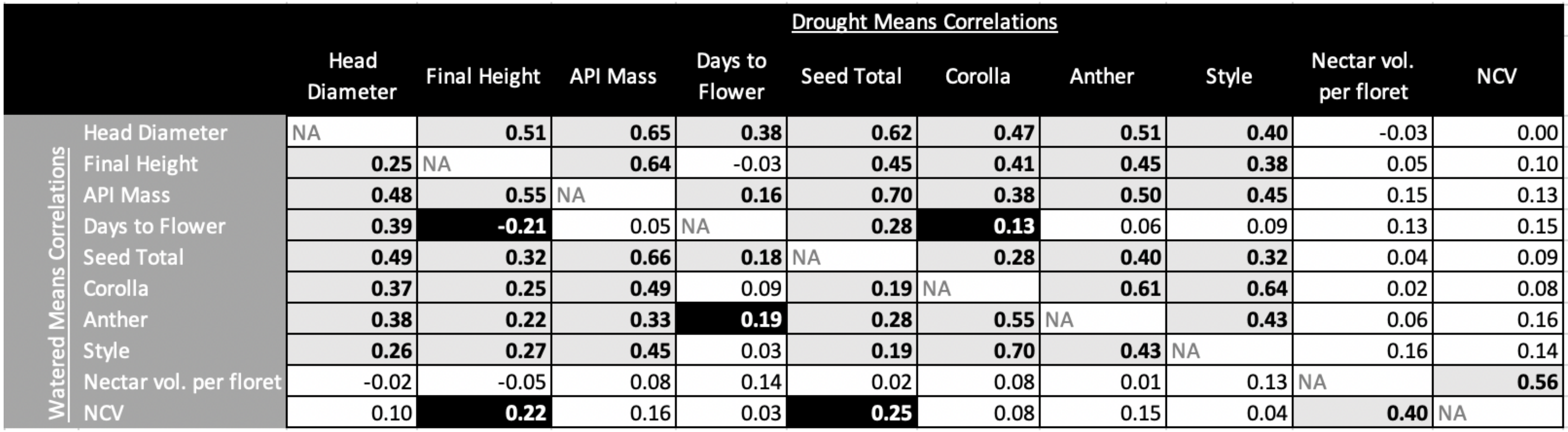
	Corolla length	Anther length	Style length	Nectar volume per floret	NCV	NSQ
Correlation (Drought)	0.01	0.09	0.02	0.23	-0.07	-0.002
Correlation (Watered)	-0.25	-0.14	-0.24	0.11	-0.06	0.07

Table 7: Correlations between each trait and seed percent decrease in drought separated by treatment (Drought and Watered). R values shown. Significance: ^$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Pairwise Correlations of Traits

Most traits within a type (e.g., floral) were positively correlated with other traits of that type within each treatment. For example, anther, style, and corolla length were positively correlated with each other in both the drought and watered treatments (Figure 7). Floral traits were also typically positively correlated with size traits and seed total in both treatments. Nectar traits were mostly uncorrelated with other traits.

A few correlations differed by treatment. Corolla length was positively correlated with days to flower but this was significant only in the drought treatment. In the watered treatment, days to flower was positively correlated with anther length. Plants that flowered earlier were taller at harvest (final height), but this was only significant in the watered treatment. Plants that flowered later had a larger API mass under drought. NCV was significantly positively correlated with final height and seed total only in the watered treatment.



		Drought Means Correlations									
		Head Diameter	Final Height	API Mass	Days to Flower	Seed Total	Corolla	Anther	Style	Nectar vol. per floret	NCV
Watered Means Correlations	Head Diameter	NA	**0.51**	**0.65**	**0.38**	**0.62**	**0.47**	**0.51**	**0.40**	-0.03	0.00
	Final Height	**0.25**	NA	**0.64**	-0.03	**0.45**	**0.41**	**0.45**	**0.38**	0.05	0.10
	API Mass	**0.48**	**0.55**	NA	**0.16**	**0.70**	**0.38**	**0.50**	**0.45**	0.15	0.13
	Days to Flower	**0.39**	**-0.21**	0.05	NA	**0.28**	**0.13**	0.06	0.09	0.13	0.15
	Seed Total	**0.49**	**0.32**	**0.66**	**0.18**	NA	**0.28**	**0.40**	**0.32**	0.04	0.09
	Corolla	**0.37**	**0.25**	**0.49**	0.09	**0.19**	NA	**0.61**	**0.64**	0.02	0.08
	Anther	**0.38**	**0.22**	**0.33**	**0.19**	**0.28**	**0.55**	NA	**0.43**	0.06	0.16
	Style	**0.26**	**0.27**	**0.45**	0.03	**0.19**	**0.70**	**0.43**	NA	0.16	0.14
	Nectar vol. per floret	-0.02	-0.05	0.08	0.14	0.02	0.08	0.01	0.13	NA	**0.56**
	NCV	0.10	**0.22**	0.16	0.03	**0.25**	0.08	0.15	0.04	**0.40**	NA

Figure 7: Correlation matrix of pairwise correlations by treatment. Not all traits are shown for ease of interpretation. The top right shows correlations in the drought treatment and correlations in the watered treatment are shown in the bottom left. Significance is denoted by bold text ($P < 0.05$). Traits that show similarities across treatments are highlighted in light grey. Differences across treatments are highlighted in black with white text.

CHAPTER VI

DISCUSSION

Size Trait Responses to Drought Stress

Plants in the drought treatment were smaller for all size measures and produced fewer seeds than those in the watered treatment. These patterns are expected for plants experiencing water deficit, as water stress can lead to decreased vegetative growth and lowered seed yields from reduced rates of photosynthesis (Anjum et al. 2011, Ferrera et al. 2011, Mahajan and Tuteja 2005, Ramirez-Vallejo and Kelly 1998). Generally, plants under water stress respond by limiting water loss through stomatal closure (halting CO_2 uptake). The rate of photosynthesis thus decreases because there is less CO_2 available to assemble photosynthates (Mahajan and Tuteja 2005, McDowell et al. 2008) and because a decrease in turgor leads to decreased cell expansion.

Drought plants also required more days to reach flowering than watered plants, similar to what Owart et al. found in their 2014 study involving *H. annuus* grown under water-limited conditions. However, this contradicts the findings of Hammad et al. (2002), in which droughted *H. annuus* flowered earlier than watered control groups. Plants avoid drought by either accelerating their life cycle or through the evolution of tolerance mechanisms (Levitt 1972). *H. annuus*, an annual herbaceous plant, would presumably escape drought conditions by flowering earlier if under stress according to its life cycle and the fact that its wild progenitor was adapted to water limited environments (Cattivelli et al. 2008). In a study by Burke et al. (2002), they found that cultivated sunflower generally flowers earlier than wild sunflower. Although, the goal of their study was to compare a single crop line with wild common sunflower in order to identify quantitative trait loci that control phenotypic differences between the two. One reason the crop lines in our drought study may have required more days to flower than those in the well-watered treatment could be attributed to the fact that crop lines were artificially selected for their ability to flower earlier under optimal conditions. Additionally, a study by McAssey et al. (2016) focused on identifying the variation in flowering day across fifteen populations of wild sunflower in the central United States and they found substantial natural variation among the different wild populations. In our study, we were comparing the behavior of 40 different crop lines and their response to drought and significant line effects were observed across all of the 40 lines in our experiment for days to flower. Since these lines are derived from their original wild progenitor, variation in days to flower among these lines would be expected. It is possible that the delay in flowering of drought plants observed in our study may be due to reduced photosynthetic activity and growth while the plants were under stress. In line with this hypothesis, the balance between source and sink organs of the plant (leaves act as source organs, creating photosynthates, whereas the flower acts as a sink organ) can be disrupted by water stress (Lemoine et al. 2013). Restriction of the photosynthetic carbon exported by leaves could lead to a decrease in resources applied to flower production (Descamps et al. 2018, Lemoine et al. 2013).

Floral Trait and Nectar Trait Responses to Drought Stress

Size traits, such as final height and API mass, showed percent decreases in size of 22.5 % and 28 %, respectively, in this experiment. When comparing the percent decrease of size traits with floral traits, such as corolla and style length, changes under drought conditions of these floral traits are much smaller (3.1 % and 2.2 %, respectively). It is important to note that floral traits remain quite stable even when the plant is under artificial selective pressure (Bradshaw 1965, Cresswell 1998) and may not be affected as drastically as size traits under changing environmental conditions. However minute the changes may be, the decreases in length of corollas and styles under drought conditions in this experiment are statistically significant and consistent with previous research showing that drought causes reductions in floral traits due to high water and carbohydrate costs during flowering (Carroll et al. 2001, De la Barerra & Nobel 2004, Descamps et al. 2018, Gallagher and Campbell 2017, Villarreal and Edwards 1990). Two of the aforementioned studies involved herbaceous plants with annual lifecycles (*Ipomopsis longiflora*, Villarreal and Edwards 1990; *Borago officinalis*, Descamps et al. 2018), similar to that of *H. annuus*. Declines in reproductive structure development could be interpreted as an effect of drought from reductions in photosynthate production and the diminished apportioning of carbohydrates to sink organs (floral organs) (Descamps et al. 2018). Additionally, Carroll et al. (2001) claim that drought indirectly influences floral traits that act as pollinator advertisements and rewards. In *H. annuus*, nectaries are located at the base of the style, at the bottommost portion of the corolla tube (Sammataro et al. 1985). Honeybees (*Apis mellifera* L.) have shorter tongues (6.6 ± 0.3 mm) than wild *Bombus* bee species (tongue lengths of 7.8 mm to 9 mm or longer) and nectar collection depends heavily on the depth of the corolla (Apatov 1929, Balfour 2013, Inouye 1980, Mallinger and Prasifka 2017). Pollinators with shorter tongues, such as honeybees, could benefit from the shorter corollas caused by drought because nectar rewards are more accessible. A study by Portlas et al. (2018) concluded that inbred and hybrid sunflowers with smaller florets that are more accessible to honeybees could augment sunflower pollination. This could be beneficial agronomically because honeybees are used to pollinate large scale *H. annuus* crop fields and seed oil yields are higher when pollinators are present (NDSU 2007). Native bees (Bombus spp.) would most likely be unaffected by changes in corolla length since their tongues are long enough to access nectar from deeper *H. annuus* corollas. It is important to note that the average corolla length in both treatments of our study was longer than 7.5 mm, indicating that bees with tongue lengths of 7.5 mm and greater are more suited for accessing nectar rewards in the crop lines involved in this experiment. Studying the pollinator preferences for each SAM line would be interesting to understand more about which species of Hymenoptera prefer which genetic lines and the lengths of their corollas. Additionally, identifying the genetic markers that are associated with shorter corollas should be pursued in future research.

Floral nectar, which is a derivative of phloem solution (De la Barrerra and Nobel 2004), has been shown to have a positive relationship with soil moisture, meaning that it will decrease if soil moisture decreases (Carroll et al. 2001). Plants that are capable of maintaining higher water potentials during well-watered conditions have been shown to have higher secretion rates for their nectar (Zimmerman 1983, Carroll et al. 2001). Additionally, some field studies indicate that a lack of precipitation reduces the secretion rate of nectar (Cruden et al. 1983, Waser 1978, Pleasants 1983). In our study, nectar volume was reduced significantly for plants in the drought treatment, which aligns with previous studies of drought and nectar volume production (Carroll et al. 2001, Descamps et al. 2018, Gallagher and Campbell 2017, Petanidou et al. 1999, Villarreal and Freeman 1990). Other studies found that nectar volume is unaffected by drought (Phillips et al.

2018, Clearwater et al. 2018, Waser and Price 2016). Phillips et al. (2018) reasoned that the soil moisture content may have not been low enough during their drought treatment to induce nectar volume changes. The total ambient precipitation in our field, which amounted to approximately 5.82 cm during the months of June-August (Weather Underground), was small enough to allow for a significant drought treatment effect across size traits, floral traits, and average nectar volume per floret.

Nectar sugar concentration did not change in response to drought in agreement with other studies (Carroll et al. 2001, Gallagher and Campbell 2017, Phillips et al. 2018, Villarreal and Freeman 1990). Villarreal and Freeman (1990) found that nectar sucrose was unaffected by water stress and they attributed this to the tubular morphology of the corolla; the amount of corolla water lost to evaporation is low, hence allowing the conservation of nectar sugar in the solution regardless of temperature or water status. On the contrary, a study by Descamps et al. (2018) shows that nectar sugar concentration decreases when under water stress. They attributed the decrease in nectar sugar to a lack of carbohydrate production, starch transport declines, and drought-induced phloem transport failure (Descamps et al. 2018, Sevanto 2014).

All size and floral traits exhibited significant line effects, indicating they have a genetic basis with alleles segregating across the SAM population. Line effects and line by treatment effects can help provide insight into the genetic basis of traits and can be utilized to identify and select valuable genetic markers (Cobb et al. 2013). Reaction norms showed variation in the line by treatment interaction for all nectar traits. This was especially true for NCV, in which there are several lines that increase NCV under drought, and nearly as many that increase NCV in the well-watered treatment. Further study of the lines that displayed enhanced nectar sugar concentration under drought conditions are warranted because increased honeybee visits are linked with higher nectar sugar concentrations, which in turn leads to higher seed yields (Prasifka et al. 2018, Rabinowitch et al. 1993, Silva and Dean 2000).

Pairwise Correlations of Traits

Floral traits were significantly positively correlated with several size traits and seed total in both treatments. This pattern indicates that larger plants also have larger floral organs regardless of drought. In terms of nectar traits, NCV and nectar volume were positively correlated with each other but nothing else in both treatments, meaning that plants with higher nectar volumes also had higher nectar sugar concentrations. Nectar sugar and volume have been found to be positively associated with pollinator visits (Prasifka et al. 2018). The relationship between nectar sucrose and nectar volume could be important for bee foraging and research has shown that pollination of crop sunflower by bees provides a substantial benefit for seed yield in both confectionary sunflower (Prasifka et al. 2018) and oilseed (Degrandi-Hoffman and Chambers 2006).

Interestingly, some trait correlations differed between treatments. Corolla length was significantly positively correlated with days to flower in the drought treatment, but this relationship was non-significant in the watered treatment. This may indicate that plants in the drought treatment with longer corollas took more days to flower than those with shorter corollas. This aligns with the source:sink hypothesis: floral organs may have taken longer to develop due to restricted photosynthate supply during water stress

(Descamps et al. 2018, Lemoine et al. 2013). It is worth noting that neither of these correlations were very strong. Another interesting relationship was observed among two size traits, final height and seed total, and a nectar trait, NCV. Final height and seed total had a significant positive correlation with NCV in the watered treatment, but not in the drought treatment. This suggest that taller plants with higher seed totals also had higher nectar sucrose concentrations by volume when watered, and this relationship was not retained under drought. This relationship, again, could have to do with larger plants having more photosynthetic resources to expend on enhanced nectar sugar production under well-watered conditions (Descamps et al. 2018, Lemoine et al. 2013). The only significant negative correlation of note was the relationship between final height and days to flower in the watered treatment. Interestingly, plants that were taller in the watered treatment took fewer days to flower than those in the drought treatment. This could reinforce the hypothesis that larger plants are more resistant to drought: they are able to acquire more resources more quickly and also flower earlier.

Resistance to Drought Stress

The linear regression model we used to approximate resistance was based on each line’s ability to maintain relative seed total (the estimated seed total from counting seed insertions) under drought conditions. From this model, we identified the most and least resistant of the 40 SAM lines so that they may be focused upon as candidates for future phenotypic and genetic analyses. Forty-five percent of the 40 SAM lines in the experiment had positive residual values, meaning that they were more able to maintain seed totals in the drought treatment than those with negative residual values (less able to maintain seed totals in the drought treatment). The mean seed total of the top five most resistant lines in the watered treatment was 989.2 seeds versus 1023.8 seeds under drought. These lines were able to maintain seed total regardless of treatment; they were able to accumulate biomass in reproductive organs (carbohydrate sinks) better than other lines under drought stress and therefore should be considered for future selection as drought resistant lines (Lemoine et al. 2013). In another study, the ability for plants to tolerate drought was also defined as being able to maintain seed numbers while under water stress (Fussell et al. 1991). For the five most susceptible lines, the mean seed total in the watered treatment was 1,117.6 seeds versus 481.3 seeds in drought. Seed yield decreased significantly when these lines were subjected to drought. It is important to note that the economically important traits, seed size, oil percentage, and oil content, were not assessed in this study. The lines identified as most resistant in this study thus warrant further investigation of their seed traits, as well as the consistency of their resistance to other abiotic stressors.

The ranking of lines by resistance changed when resistance was defined as percent change in seed production under drought. SAM lines that showed a percent increase in seed production in the drought treatment ranked as the most resistant. It was interesting that only two lines ranked in the top five in both resistance analyses: S48 and S262. These lines produced more seed under drought than when watered and had high seed totals in both treatments. Line S261, on the other hand, ranked as the most resistant when examining percent change but was considered susceptible using the model residuals. This line was physically small and had seed totals less than 200 in the watered treatment and less than 300 in the drought treatment. In comparison, one of the most resistant lines that was identified using the regression method (S71) had seed totals of approximately 1250 in the watered treatment and 1300 in the drought treatment, making this line a more desirable cultivar, because it has high seed output regardless of water regime. However, line S261 should not be ignored when considering lines to utilize for marker assisted selection of drought resistance. A line with a smaller size may contain more genetic material from wild *H. annuus* progenitors, which are used as sources of genes that confer resistance and stress tolerance (Seiler et al. 2017). Then again, this line may have simply avoided/escaped drought because of its small height (Fischer and Wood 1979, Cattivelli et al. 2008). This line's usefulness as a source of genetic material for marker-assisted selection depends on the possibility of breaking the linkage between loci conferring drought resistance and those conferring smaller size.

Size Traits and Resistance

All size traits were at least marginally significantly, positively correlated with resistance (measured using residuals) in the drought treatment. A study by Owart et al. (2014), found that higher fecundity in sunflower recombinant inbred lines was associated with size traits such as height and head diameter. Consistent with these results, lines that were larger under watered conditions experienced less percent decrease of seed total in the drought treatment with the exception of line S261and S39. These data indicate that plants that were large and able to maintain size in both treatments were ultimately the most drought resistant, again reinforcing the hypothesis that drought resistance lies in the plant's ability to accumulate biomass in sink organs (floral organs, seeds) (Lemoine et al. 2013). A more nuanced examination of the residual resistance correlations reveals that midpoint height and stem diameter were more strongly correlated with resistance than these traits measured at harvest. Gaining size before flowering may be more advantageous to plants experiencing drought than investing photosynthetic resources in increased size during and after flowering. In other studies, it was found that plants with lower rates of growth survive for longer periods of time during drought stress (Givnish 1979, Donovan and Ehleringer 1992, Heschel et al. 2002). In Heschel et al.'s (2002) study involving genetic lines of *Impatiens capensis* (a weedy annual plant), they found that lines derived from drier populations were more adapted to increase their water use efficiency under dry conditions. In our study, the lines that were able to invest in size gain prior to flowering, rather than the tallest lines at their final height, should be the focus of future marker assisted selection studies. Stem diameter also had higher correlations with resistance than height at both the midpoint and harvest time points. Investing in stem diameter instead of height could be advantageous for plants experiencing drought because it implies that there is more stem surface area for water transport (Ramirez-Vallejo and Kelly 1998). A study involving *Phaseolus vulgaris* (common bean) by Ramirez-Vallejo and Kelly (1998) claimed that stem diameter is a heritable trait that could be useful when selecting for drought resistance because it had a strong association with biomass traits. However, this could be attributed to the hypothesis that crop lines that are large fare better than smaller lines under many stressful and not stressful conditions.

Floral Traits and Resistance

The only floral trait that was significantly correlated with resistance was anther length in the drought treatment. More resistance was associated with longer anthers. Anther development and pollen viability are measures of male fitness (Bateman 1948), and the preservation of anther length regardless of treatment may result from the investment of more photosynthetic resources to maintain anther length because this trait enhances pollen removal (Delph and Ashman 2006, Runquist et al. 2017). Our results differ from a study by Su et al. (2013), in which anther development was hindered under water stress. Unlike corolla and style length, anther length did not differ significantly between treatments and reaction norms showed little variation across lines for this trait. These data suggest that many lines were able to maintain anther length under drought and that this ability was associated with drought resistance. Measures of pollen were not included in this study, but future research involving pollen production, viability, and removal by pollinators under drought conditions is merited and could complement provide further insight into the effect of drought on male fitness in sunflower.

CHAPTER VII

CONCLUSION

In this study, *H. annuus* crop lines responded to drought stress with a decrease in overall size, corolla length, style length, and average nectar volume per floret. Nectar sucrose concentration was unaffected by drought stress, but genetic lines differed in their responses to drought conditions and line by treatment effects were observed for this trait. Lines that were larger and better able to maintain their size under drought were generally found to be more resistant to drought stress. Lines that were larger also may have been able to acquire more resources from the soil and produce more photosynthates, allowing them to be able to invest in size gain prior to flowering. Resistance was also related to increased anther length under drought.

Anther length warrants further study as it may influence future crop sunflower selection for breeding drought resistant lines. Additionally, pollen production in crop sunflower should be included in future studies to see how drought affects male fitness. Pollinator preference should also be a topic of further research, in terms of corolla length and genetic line. Identifying the genetic markers that are associated with shorter corollas should be pursued in future research to allow for optimized nectar reward retrieval and pollinator attraction by the agriculturally important pollinator species, *Apis mellifera*. Future field studies involving crop sunflower nectar production under drought conditions should implement measures to improve nectar collection in order to have a more statistically powerful nectar sample size. However, the measures implemented should be screened to reduce effects from increased microhabitat temperature from methods such as bagging heads prior to nectar collection. Also, resistance should be defined with a more informative seed yield trait, such as oil content or seed mass instead of a seed total estimation. Traits such as these will provide more information about the resilience of these crop lines under stress and whether or not they are able to produce adequate amounts of oil during periods of drought. In the future, a field site should be chosen with reliable water availability and less severe soil compaction as these factors may have led to the high variability between blocks in our field desi

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