TIMING OF STRESS AND YIELD DETERMINATION IN MAIZE (ZEA MAYS L.)

by

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ABSTRACT

TIMING OF STRESS AND YIELD DETERMINATION IN MAIZE (ZEA MAYS L.)

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Yield loss in maize (Zea mays L.) is well known to be caused by abiotic and biotic stresses. Previous studies have focused, primarily, on the determination of yield loss as a result of stress occurring during the critical reproductive stages, that is, around silking and the grain fill period. No studies have assessed yield loss caused by differing stresses that occur early during the vegetative phase. Studies were conducted during 2012-13 under controlled and field conditions at two locations in Ontario, Canada. Early season stresses (i.e., up to V9 stage of growth) included drought, light quality (red to far red) and early high-plant density while high plant density was considered a season long stress. In this thesis, the hypothesis was tested that if yield is reduced in response to early season stress, then, resource capture and resource utilization will be reduced proportionally. The relationship between plant dry matter and floret number follows the classic relationship with a minimum dry matter level required and a plateau. Not all stresses impacted these relationships in the same manner. These results confirm that floret number in maize is established well in advance of flowering and also suggests that floret number is related to plant dry weight sampled between V7 to V9-10 stage of growth. Yield loss in the drought and early high-plant density stress treatments, was caused by reductions in dry matter accumulation and kernel number. Growth rates around silking were reduced and flowering delayed in response to early season stress which explained reductions in kernel set. While ASI was only lengthened by early high density, HI remained unchanged in response to early season stress. Season-long high plant...
density stress resulted in reduced plant dry matter, and kernel number. Plants presenting low dry matter accumulation in each stage of growth exhibited lower floret and kernel number. Barren plants at maturity showed very low or no dry matter accumulation during the grain filling period. Overall, early season stress reduced resource capture only, while season long stress defined as high plant density reduced both resource capture and utilization.

**KEYWORDS:** stress, resource capture, resource utilization, timing, yield, yield potential, grain filling period
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List of Abbreviations

ANOVA.......................................................... Analysis of variance
ASI............................................................ Anthesis-to-silking interval
CD........................................................... Conventional density
CG.......................................................... CG108/102
DT........................................................ Drought tolerant
EHD .......................................................... Early high-density
HD........................................................ High density
HI........................................................... Harvest index
LAI........................................................ Leaf area index
NDT........................................................ Non-drought tolerant
R:FR...................................................... Red-to-far red ratio
Introduction

The content of this thesis is organized in i) a literature review, ii) three research chapters and, iii) a summary chapter including the limitations in the research and future lines of work.

In the literature review can be found a status of the knowledge of maize crop physiology and its relationship with abiotic stress at both crop and plant level. The chapter 1 (literature review) is organized temporally, that is, from the emergence of the crop to its physiological maturity. The identified research gaps are a lack knowledge for: i) the relationship between ear development and plant cumulative growth, ii) the effects of contrasting stresses during the vegetative phase on yield determination and iii) the temporal dynamics of individual plants that exhibit barrenness at maturity.

The common theme of chapters 2, 3, and 4 is the impact of abiotic stress in growth and development. Sources of stress were changes in light quality (weeds presence), reductions in water quantity (drought), and reductions in light quantity (high plant density). These three stresses were selected because of how they influence resource acquisition and availability in the maize crop and the differences in their severity. While in chapter 2 and 3 stresses (light quality, water quantity, and light quantity) are applied up to the 12th leaf tip in chapter 4 light quantity stress is imposed throughout the growing season. Contrast between stresses occurring in different times are discussed in chapter 5 along with the limitations of research and future research opportunities. Chapter 2 (The Relationship Between Floret Number and Plant Dry Matter Accumulation Varies with Early Season Stress in Maize (Zea mays L.) has been peer reviewed and published on Field Crops Research Journal 238, pages 129 to 138. Chapters 3 and 4 are to be submitted to the Canadian Journal of Plant Science and Crop Science Journal respectively.
Victor Hugo Gonzalez conceived, planned and executed the research. Victor Hugo Gonzalez, Dr Clarence Swanton, and Dr Elizabeth Lee wrote the manuscripts with support from Dr Lewis Lukens. Dr Clarence Swanton supervised the overall research project.
Chapter 1: Yield Components and their Response to Stress in Maize (Zea mays L.)

Yield in maize (Zea mays L) is the integration of numerous processes throughout the growing season and their interaction with environmental factors and management practices. For this review, four distinct stages describe the crop cycle: the emergence to flowering, flowering, grain filling and the maturity stage. In the first section, each stage and its key yield components will be detailed in the manner that is required to understand the following section. In the second section, stress, its definition and its impact in each stage and on each yield component will be reviewed. Throughout the review and specifically the third section, research questions and objectives will be proposed. Throughout this literature review the discussion will be centered mostly at the crop and plant level.

1.1 The seedling emergence to flowering period

1.1.1 Crop establishment

Seedling emergence to the flowering phase is of central importance for crop establishment and the capture of resources. It is estimated that this phase accounts for approximately half of the seasonal dry matter accumulated by the crop (Tollenaar, 1989; Tollenaar and Lee, 2011; Chen et al., 2015; Gonzalez et al., 2019). Seedlings emerge around a week to ten days after planting when temperatures are above 8°C. The first five to six leaves are dependent on seed reserves since they have pre-differentiated in the seed (Bonnett, 1954; Hunter et al., 1977). After this time-point plants acquire resources without relying on seed reserves transitioning to the autotrophic photosynthetic phase (Cooper and MacDonald, 1970; Pommel, 1990). Subsequent leaf appearance will result in progressive increases in leaf area index, light interception and crop dry matter accumulation.
Rate of leaf appearance and leaf expansion are dependent on temperature and the supply of water and nutrients (Boyer, 1970; Hardacre and Turnbull, 1986; Zur et al., 1989; Uhart and Andrade, 1995; Salah and Tardieu, 1997; Colomb et al., 2000). Temperatures of approximately 30°C will result in maximum rates of emergence (Warrington and Kanemasu, 1983a) and leaf appearance (Warrington and Kanemasu, 1983b). Therefore, the initiation and appearance of vegetative and reproductive structures are related to thermal time (Siemer et al., 1969; Stevens et al., 1986; Otegui and Melón, 1997; Borrás et al., 2003; Smith and Lee, 2016). Leaf area index which is the ratio between leaf area and ground surface area depends on the total number and size of leaves which are driven primarily by the genotype (Kiniry et al., 1983). In corn, values of leaf area index of approximately 4 or more result in interception of photosynthetic active radiation of 80% or more (Allison, 1969; Maddonni and Otegui, 1996; Earl and Tollenaar, 1997; Suyker et al., 2005) (Fig.1.1). Thereby, the leaf area at which light interception is 90% or more is termed the critical leaf area index. Since maize is a determinate species, the leaf area index established before flowering will influence directly and positively the rate of growth during reproductive stages (Williams et al., 1965; Tollenaar and Bruulsema, 1988). Therefore, the seedling emergence to flowering phase is critical for the establishment of a strong supply of resources for subsequent reproductive stages.
Figure 1.1. Absorptance of intercepted photosynthetically active radiation as predicted by leaf area index. Values obtained from Earl and Tollenaar (1997).
1.1.2 Leaves and floral development

The initiation of new leaves and reproductive structures occurs well before the external appearance of flowers. Therefore, leaf initiation (Padilla and Otegui, 2005), leaf appearance, floral initiation (Lejeune and Bernier, 1996) and cumulative plant growth (Bair, 1942) occur in a simultaneous manner. The appearance of leaves is accompanied by leaf primordia and axillary shoots initiation in the stem. The length of the leaf initiation stage is a primary function of the genotype, which influences the total number of leaves and the leaf initiation rate (Padilla and Otegui, 2005). In temperate corn, plants produce new leaves until the plants attain eight or nine visible leaves or approximately five collars (i.e. V5 stage, Hanway, 1966) with considerable interplant (Bonnett, 1940) and genotypic variation (Stevens et al., 1986; Ellis et al., 1992; Padilla and Otegui, 2005). In general, it is established that leaf differentiation ceases when half of the maximum number of leaves for a given genotype are visible in the plant (Padilla and Otegui, 2005). Once leaf differentiation has terminated, the apical meristem transitions to the reproductive phase (Bonnett, 1940; Tollenaar and Hunter, 1983; Russell and Stuber, 1984; Irish and Nelson, 1991). Tassel initiation is accompanied by a rapid elongation of the plant internodes, which in consequence increases plant height. Approximately a week or one leaf collar stage after tassel initiation, the axillary meristems initiate the ear differentiation stage (Siemer et al., 1969; Damptey and Aspinall, 1976). The future harvestable ear is positioned six nodes below the tassel initial, however, ear initiation starts first in the lower axillary meristems (i.e. acropetal). The number of potential ears is equal to the number of axillary shoots which in temperate maize are around six (Bonnett, 1940). As development advances, however, the degree of differentiation in the uppermost axillary meristems dominates over the lower axillary meristems (Bonnett, 1940). This developmental pattern explains the discrepancy between actual vs potential number of ears. For
example, the plant has the potential to differentiate up to six ears, however, commercial grown
maize only presents at most two ears per plant at maturity (Bonnett, 1940; Siemer et al., 1969;
Durieux et al., 1993; Smith and Lee, 2016). The tassel initial begins formation of the basal
branches and the spikelets in the central axis. Spikelet differentiation occurs acropetally in both the
lateral and the main axis of the tassel. The ear follows the same acropetal pattern of spikelet
differentiation presenting rows of paired spikelets, each one, with two florets covered by modified
leaves called glumes. The tassel and the ear, however, differ in the type of flower they develop.
Each flower in the tassel produce three anthers and the pistils abort. In contrast, in the ear, only
one flower per spikelet exhibits a functional pistillated flower with an aborted stamen (Bonnett,
1954). The developmental difference between the tassel and the ear and the lower vs higher
spikelets positioned in the ear, is maintained through the crop cycle. The former relates with the
first appearance of the tassel relative to the ear and the latter with the late appearance of silks,
resulting in small or absent kernels in the ear tip (Tollenaar and Daynard, 1978; Carcova et al.,
2003). Interestingly, although developmental and growth process occur simultaneously, little effort
has been made to relate plant cumulative growth with developmental processes (Pagano et al.,
2007).

1.2 The flowering period

1.2.1 Fertilization of florets and kernel number establishment

The flowering period is central for the determination of kernel number, which is a determinant
for yield in maize. Therefore, environmental conditions during the sensitive 30-day flowering
period are critical for kernel setting (Early et al., 1967; Tollenaar and Daynard, 1978; Tollenaar et
al., 1992; Andrade et al., 2002; Kiniry and Ritchie, 2010). The flowering period, particularly, female flowering (i.e. silking) lasts from ten to fifteen days depending on conditions and genotypes (Bassetti and Westgate, 1993a; Turc et al., 2016; Yan et al., 2018). The key mechanism driving kernel set is the fertilization of the ear florets by the pollen grains of the tassel and the proportion of these potential kernels that attain active grain fill (Herrero and Johnson, 1980, 1981; Westgate and Boyer, 1986a; Kiniry and Ritchie, 2010; DeBruin et al., 2018). The tassel appears first because of its apical dominance and temporal advancement in the initiation process, followed by the silks in the ear husk. While the appearance of the male flower first is the most frequent observed pattern (protandry), the anticipation of the silks appearance relative to the tassel (protogyny) has a substantial probability of occurrence in modern germplasm (Uribelarrea et al., 2002). The temporal separation of tassel appearance and silks exposure is known as the anthesis-to-silking interval (Bolaños and Edmeades, 1996). This interval provides a measure (in days or cumulative temperature) of synchronicity between the male and female flower appearance. This synchronicity relates narrowly with the number of pollinated flowers, that is, the more synchronous the flowers’ appearance the larger the fraction of fertilized florets (Hall et al., 1982; Cárcova et al., 2000). This dependency of kernel establishment on silks earliness is explained, in part, by a progressive reduction in silks growth rate and receptivity to pollen grains (Bassetti and Westgate, 1993b; Anderson et al., 2004; DeBruin et al., 2018). Therefore, pollen availability does not appear to be a primary factor for kernel set. For example, it is estimated that pollen availability of approximately of more than 50% of total pollen shedding will not contribute to further silks fertilization and kernel set (Lizaso et al., 2003). In addition, experiments conducted by Westgate and Boyer, (1986b) and Otegui et al., (1995) have shown that late appearing silks did not respond to artificial fertilization when fresh pollen was added. Thereby, under normal conditions, delay in silks
exposure, explains largely the discrepancy between kernels at maturity and the number of potential fertilized florets (Cárcova et al., 2000, 2007; Gustin et al., 2018). Reduced kernel number is then explained by failures in the fertilization during silking (Turc and Tardieu, 2018). Therefore, conditions that favour growth during this critical period are expected to necessarily improve kernel establishment.

1.2.2 Kernel establishment and growth

Supply of assimilates around the critical silking period associates positively with kernel number at maturity. For example, studies conducted by Edmeades and Daynard, (1979) showed a positive association between assimilate availability per plant around silking and kernel establishment. Similarly, Schussler and Westgate, (1991a) showed a positive association between kernel number at maturity and plant photosynthetic rates around silking. These associations showed an increased number of kernels per plant which increased hyperbolically when photosynthetic rates were maximized. Similarly, subsequent studies related kernel number with growth rates around silking (Tollenaar et al., 1992). The estimation of growth rates around silking most commonly refers to a 15 to 30 days period around flowering with variations among studies (Vega et al., 2001a; D’Andrea et al., 2008). Similar to Edmeades and Daynard, (1979), this relationship exhibits a minimum rate of growth per plant in order to present kernels greater than zero. This value is commonly referred to as threshold growth rate for kernel set and its value ranges from 1 to 2 g plant\(^{-1}\) day\(^{-1}\). Above this value range kernel number increases, however, at growth rates of approximately of 4 g plant\(^{-1}\) day\(^{-1}\), kernel number plateaus at values varying between the 600 to 900 kernels per plant depending on the genotype (Andrade et al., 1999)
(Fig. 1.2). Successive increases in kernels per plant, then, will depend in the number of ears per plant (typically less than two) which is influenced by the genotype (Durieux et al., 1993). Similarly, crop growth rates around silking explain variability in kernel number per unit area. Modal values for crop growth rates around silking and kernel number per unit area are 35 g m$^{-2}$ day$^{-1}$ and 3500 kernels m$^{-2}$ respectively (Andrade et al., 1999). In summary, the key features of the kernel number vs growth rate around silking framework are its plasticity to explore different sources of variation for the dependent variable (kernel number) and the independent variable (growth rate around silking). High crop or plant growth rates around silking, then, will result in increased number of fertilized florets attaining the onset of the grain filling period.
Figure 1.2. Kernel number as a function of plant growth rate at silking for the hybrid Pioneer 38N87 grown at Elora, Ontario, Canada. Modified from Gonzalez et al., (2019).

\[ y = \frac{slope(X - threshold)}{1 + curvature(X - threshold)} \]

Slope = 550  
Curvature = 0.1  
Threshold = 1 g day\(^{-1}\)  
X = growth rate
1.3 The grain filling period

The period between the silking stage and physiological maturity is commonly referred to as the grain filling period. Its duration in temperate corn lasts from 7 to 8 weeks (Ying et al., 2000). This phase is subsequent to the determination of kernel establishment and critical for the attainment of potential yield. The grain filling period can be subdivided into three major stages i) the lag phase, ii) the linear grain filling phase and iii) the drying phase (Fig. 1.3) (Johnson and Tanner, 1972). The lag phase is characterized by an increase in the number of cells in the kernel endosperm and lasts around 10 to 15 days depending in the genotype and temperature (Reddy and Daynard, 1983; Maddonni et al., 1998). This phase is critical to the maximization of grain volume for subsequent filling. After the lag phase is completed, individual kernels enter the linear phase of dry matter accumulation which usually last from 40 to 50 days depending on the genotype and conditions. Assuming maximum kernel establishment around silking, the rate and the duration of the grain filling period will be related positively with grain yield (Daynard et al., 1971; Poneleit and Egli, 1979).

The metrics used to quantify growth during the linear phase of the grain filling are more variable than those used for the bracketing silking period (i.e. g day\(^{-1}\) or g thermal unit\(^{-1}\)). For example, ear growth rates of 160 to 200 kg ha\(^{-1}\) day\(^{-1}\) have been reported by Daynard et al., (1971) which reflect only part of average kernel weight. The most classical measurement of the rate of kernel growth, is in mg day\(^{-1}\) as shown by Reddy and Daynard, (1983) with values of 2 to 10 mg day\(^{-1}\) kernel\(^{-1}\). Similarly, gains in plant dry weight per kernel range from 100 to 400 mg plant\(^{-1}\) kernel\(^{-1}\) (Cirilo and Andrade, 1996). Alternatively, values of dry matter accumulation during the grain filling period range from 50 to 300 mg kernel\(^{-1}\) (Hisse et al., 2019) or from 5000 to 10000 kg
ha$^{-1}$ (Chen et al., 2015; Gonzalez et al., 2019). These latter ranges represent very well the variation that can be expected in kernel weight and yield per unit area respectively. Kernel weight has been related satisfactorily to assimilate availability per kernel at the onset of the grain filling period. Its expression equates to growth rate around the critical silking per kernel with values of 6 to 15 mg day$^{-1}$ kernel$^{-1}$ (Gambín et al., 2006). This metric attempts to describe the availability of assimilates per individual kernels to attract assimilates. One advantage of this approach, relative to the others is that it is one of the very few that account for variation among individual plants, however, still does not reflect events during the grain filling period but rather before its occurrence (i.e. before linear phase of grain fill).

Dry matter accumulation per kernel during grain fill appears to be attributable in most part to current supply of assimilates. Although reserve carbohydrates (i.e. pre-grain fill carbohydrates) have been recently reported as a possible contributor of assimilate supply during the grain filling period (D’andrea et al., 2016), it is accepted that the main contributor to grain fill under non-stress conditions is current photoassimilates from photosynthesis (Kiniry et al., 1992; Westgate, 1994). For example, maximum rates of leaf net photosynthesis of 45-50 mmol m$^{-2}$ s$^{-1}$ around silking are very stable during approximately half of the whole grain fill period (Ying et al., 2000). After this time point, declines in rates of photosynthesis are accompanied by leaf senescence (Echarte et al., 2008). Progressive dry down in short season maize hybrids is driven by genotypic and environmental factors and is a central trait for Ontario maize (Ma and Dwyer, 2001). Values of grain moisture of less than 40% (Sala et al., 2007) are associated with the attainment of physiological maturity.
Figure 1.3. Kernel weight as a function of days after fertilization. Modified from Borrás et al., (2009).
1.4 Maturity

The two primary components of yield are seasonal aboveground dry matter accumulation and the proportion of dry matter that is allocated to yield weight, that is, the harvest index (Tollenaar and Lee, 2006) \( (Eq.[1.1]) \). Both traits, seasonal dry matter accumulation and harvest index are dependent on environmental conditions, management practices, genetics and their interactions (Woli et al., 2017). In absence of abiotic or biotic stress, the environmental determinants of dry matter accumulation are the integration of i) the intercepted radiation by the crop canopy ii) the efficiency of conversion of this radiation into biomass (Kiniry et al., 1989; Sinclair and Muchow, 1999), and iii) the duration of light interception by the crop canopy (Muchow et al., 1990) \( (Eq. [1.2]) \). Harvest index is very stable under a wide range of conditions ranging from 0.45 to 0.55 for maize (Hütsch and Schubert, 2017) and other summer annual crops such as soybeans \( (Glycine max \) (L.) Merr.) (Tamagno et al., 2017; Adams et al., 2018) and sunflower \( (Helianthus annuus \) L.) (Andrade, 1995). Therefore, the interpretation of harvest index by its value alone may not translate strictly into high yield, rather, to an equal proportion between yield dry matter and overall seasonal dry matter. In contrast, any substantial reduction in harvest index from approximately of 0.50 g g\(^{-1}\) can be attributed to a net yield reduction (Rattalino Edreira and Otegui, 2012). The harvest index can be further dissected into simpler components, that is, i) number of kernels and the ii) dry weight per kernel (Tollenaar and Lee, 2006) \( (Eq. [1.3]) \). Kernel dry weight contributes to yield (Wang et al., 1999; Echarte et al., 2000; Gambín et al., 2006), however, kernel number is the most impactful yield component (Andrade et al., 1999; Borrás et al., 2004). Yield and dry matter accumulation, then, are the result of the integration of several processes during the growing season.
\[ Eq.[1.1] = Yield = DM \times HI \]

\[ Eq.[1.2] = DM = \int_{t=planting}^{t=harvest} SR \times ABS \times E \]

\[ Eq.[1.3] = HI = \frac{KN \times KW}{DM} \]

Where DM is dry matter, HI is harvest index, SR is solar radiation, ABS is fraction of the solar radiation absorbed by the canopy, E the efficiency of conversion of the solar radiation absorbed into carbon, KN is kernel number and KW is kernel weight.

1.5 Stress and yield determination

The negative effects of stresses in maize yield are related to their type, timing and severity. The definition of stress is variable in the literature, however, its quantification at the crop level is often related with yield reductions or variations in biological rates. For example, stress can be defined as a single or several factors that alter functions in plants (Wang et al., 2003) or reduce yield in crops (Tollenaar and Lee, 2002). Alternatively, stress can also be defined as any factor that reduce capture and utilization of resources by the crop (Tollenaar and Wu, 1999). This later definition corresponds to resource dependent response to stress. While timing of the stress is
related with the stage of the crop when it occurs, the severity of a stress can be quantified in terms of the variation in yield, dry matter and leaf area (Lorens et al., 1987).

### 1.5.1 Resource dependent response to stress

Resource dependent response to stress is associated with reductions in resource capture and resource utilization. For example, reductions in seasonal dry matter accumulation and harvest index caused by drought stress would be a classic example of a negative effect on resource capture and utilization respectively (Earl and Davis, 2003). Among the most studied resource dependent stress are: drought, nutrient deficiency, extreme temperatures, interspecific and intraspecific competition. The commonalties among these stresses is their direct effects on the mechanisms of resource acquisition (e.g., light interception) or utilization (e.g., efficiency of conversion of light into dry matter) and their negative effect on yield. Reductions in yield, however, are not restricted strictly to reduced capture and utilization of resources.

### 1.5.2 Resource independent response to stress

A resource independent response to stress is defined as a factor or a series of factors that alter the capacity of the plants to capture the pool of available resources without affecting the pool of available resources (i.e., light, water, and nutrients). Most resource independent responses to stress are reported to occur during early stages of growth. In plant competition, a classic example of this is the shade avoidance syndrome (Ballaré et al., 1990; Wies et al., 2019). At early stages of growth, well before direct competition for light occurs, neighboring plants change the spectral
distribution of light by absorbing, reflecting or transmitting different wavelengths of the photosynthetically active radiation (Casal and Ballaré, 2000). Preferential absorption by plants of light in the red portion (630-690 nm) relative to the far-red portion (710-760 nm) causes a change in the red to far red ratio (Holmes and Smith, 1975). This results in an enriching of the environment with far red light. Departures to values below approximately 1.2 (Smith, 1982) in the red to far red ratio changes qualitatively the light environment. This enriched far red light environment is sensed by phytochrome pigments in the plants (Smith and Whitelam, 1997), primarily by the phytochrome B (Morgan and Smith, 1978; Casal and Smith, 1989; Franklin et al., 2005), which in turn initiates a set of plant signaling events at numerous levels of organization. At the molecular level the complexity of signaling spans from upregulation of genes to increases in reactive oxygen species (Afifi and Swanton, 2012). At the external plant level, morphological changes such as increased height (Ballaré et al., 1990), shoot to root ratio (Liu et al., 2009), specific leaf area (Rajcan et al., 2004), and earlier flowering (Devlin et al., 1999) are among the multiple symptoms of the shade avoidance syndrome. In maize, the most consistent reported changes in morphology are increased height, shoot to root ratio and delayed rate of leaf appearance (Kasperbauer and Karlen, 1994; Liu et al., 2009). Response to environmental cues, however, are not restricted to changes in the light environment. Physical contact, organic volatile compounds, and allelopathic chemicals have also been proposed as signals for mechanisms of adaptation upon impending competition (reviewed by Pierik and De Wit, 2014). Regardless of the response on direct or indirect acquisition of resources, the consequence of any given stress is associated with the timing, duration, and the severity of the stress during the growing season.
1.6 Timing of stress and crop development

1.6.1 Stress and the seedling emergence to flowering period

Stresses that occur early during seedling emergence to flowering phase cause kernel number to be reduced. This response is common across several types of early season stress. For example, yield reductions in response to pre-silking drought are most commonly attributable to low kernel number (Wang et al., 2017) and reduced dry matter accumulation (Denmead and Shaw, 1960; Lorens et al., 1987; NeSmith and Ritchie, 1992a; Pandey et al., 2000; Wang et al., 2017). Reductions in dry matter accumulation can be associated with reductions in leaf expansion, resulting in lower leaf area and less light interception (Boyer, 1970). Similarly, intraspecific competition or supra-optimal plant density reduce yield. For example, differences in individual plant dry matter caused by plant density stress occur as soon as the V6 and become permanent approximately at V9 stage of growth when comparing contrasting densities (Page et al., 2010a). Studies conducted by Pagano and Maddonni, (2007) showed that densities of 12 plants m\(^{-2}\) thinned at V9 stage to 6 plants m\(^{-2}\) reduced irreversibly growth rates during both the vegetative phase and the around silking period. These reductions in growth rates throughout the growing season were attributed to the establishment of permanent differences among more competitive and less competitive individuals within the crop (Maddonni and Otegui, 2006). In addition, increases in competitive asymmetry of individuals is often reflected in reduced interplant uniformity which indicates unequal sharing of resources within the crop (Donald, 1968; Edmeades and Daynard, 1979b; Hara, 1988).

Interspecific or weed competition which occurs early during the season has been shown to reduce yield consistently regardless of management optimization in maize (Subedi and Ma, 2009). The high variability of yield loss in response to weeds presence is associated with numerous
factors (Kropff, 1988). Examples of these are weeds density (Bosnic and Swanton, 1997), time of growth relative to the crop growth (Hall et al., 1992) and the pre-existing stress level of the crop (Tollenaar et al., 1994). As well as with other early season stress, reduced kernel number is the common response to weed competition (Evans et al., 2003; Cox et al., 2006). Along with poor kernel set (Cerrudo et al., 2012; Reid et al., 2014), yield loss caused by weeds has also been associated with reduced leaf area and overall dry matter accumulation of the crop (Hall et al., 1992). Reductions in yield caused by weed competition, however, are not limited only to direct competition for resources.

The shade avoidance syndrome has been shown to have effects beyond the time of exposure to enriched far red light. The earliness of the response led to the hypothesis that the sensing of plant competitors could influence the onset of the critical period for weed control (i.e. the time window the crop should remain weed free to ensure maximum yield) (Rajcan and Swanton, 2001). For example, in an experiment conducted by Rajcan et al., (2004), plants that were exposed to enriched far red at the 4th leaf tip stage exhibited increases in plant height, root to shoot ratio, and plant leaf area that were consistent by the 9th leaf tip stage of growth. In a later study, Page et al. (2009) showed that these changes in morphology were accentuated when exposure to far red light occurred during earlier rather than later stages of crop growth. This temporal decline in the response, was attributed to the naturally occurring far red environment at later stages of growth when shading intensifies in the canopy (Liu et al., 2009). The negative effects of increased far red light extended to traits such as biomass at silking, kernel number, harvest index and plant uniformity at maturity (Page et al., 2010b, 2011). Studies conducted by Maddonni et al., (2001) showed that changes in canopy architecture by means of row spacing and plant density changed the orientation of leaves relative to the rows indicating different strategies to maximize light
capture. More recently, Wies et al., (2019) showed that maize genotypes lacking the capacity to sense competitors exhibited lower rates of growth when compared with their wild type counterparts. Similar responses to enriched far red have been reported for other crops such as soybean (*Glycine max* L. Merr) (Green-Tracewicz et al., 2011) and wheat (*Triticum aestivum* L.) (Ugarte et al., 2010).

Nutrient deficiency during the pre-silking phase, particularly nitrogen deficit, has been shown to negatively affect yield. Nitrogen is the nutrient that most limits growth since it has a central role in protein synthesis and growth (Ding et al., 2005a). The net effects of nitrogen deficiency have been shown to extend throughout the growing season (Mueller and Vyn, 2018). For example, nitrogen deficiency resulted in reduced growth rates, low leaf area and less light interception during the pre-silking phase (Vos et al., 2005). Nitrogen deficiency reduce plant growth rates around silking (Andrade et al., 2002) and the proportion of growth that is allocated to ear growth (Jacobs and Pearson, 1992). For example, reductions in kernel number under nitrogen deficiency are associated with low plant growth rates and reduced partitioning to ear growth around silking relative to fertilized maize at 400 kg of nitrogen ha$^{-1}$ accumulated throughout the growing season in Pergamino, Argentina (D’Andrea et al., 2008). Similarly, fertilization with nitrogen favors processes during the grain filling period. For example, fertilized maize hybrids above 300 kg of nitrogen ha$^{-1}$ at planting and at V9 stage resulted in mean kernel weights of 274 mg kernel$^{-1}$ while unfertilized treatments showed 230 mg kernel$^{-1}$ (Hisse et al., 2019). This increased grain weight in response to nitrogen fertilization was attributable to increased biomass accumulation during the grain filling period as shown by Chen et al., (2015). In addition, lengthened duration of grain filling is related positively with nitrogen status (Moll et al., 1994; Melchiori and Caviglia, 2008; Boomsma et al., 2009). Other macro-nutrients such as phosphorus have also been shown to impact
leaf area, cumulative dry matter (Colomb et al., 2000), root extension (Mollier and Pellerin, 1999), and kernel number (Barry and Miller, 1989). The net effects of nutrient deficiency on yield appear to be related to reduced seasonal dry matter accumulation.

The response of floral development to early season stress has been studied using contrasting approaches and has yielded varying results. Moss and Downey, (1971) found an increased percentage of abnormal florets when hybrids were exposed to pre-anthesis drought. In a similar study, Damptey and Aspinall (1976) imposed water deficit as soon as 21 days after germination and observed reduced rates of growth in apical and axillary shoots. These inflorescences, however, showed recovery. In contrast, Edmeades et al. (1993) comparing different selection populations showed no effect of drought stress on spikelet number or the length of the initiation period. In contrast, Rossini et al., (2016) showed that floret number was reduced from 754 to 718 florets plant⁻¹ in response to less than 50% of plant available water (100%=well-watered) around silking in one of two years. In the same study, floret number was reduced to 654 florets plant⁻¹ in non-fertilized conditions as compared to 726 florets plant⁻¹ in maize fertilized with 200 kg of nitrogen ha⁻¹ during two consecutive years. Similarly, nitrogen limitation resulted in reduced number of florets relative to the fertilized controls in a study conducted by Jacobs and Pearson (1991). Cirilo and Andrade (1994) and Otegui (1997) showed that floret number per plant was not affected by delays in planting date and increases in plant density (5 and 9 plants m⁻²). Pagano et al., (2007) showed increased relative rates of floral development when plant growth rates increased from 0 to 1.5 g day⁻¹ during the pre-silking stage. Results of this study, however, were expressed in relative terms and the observations corresponded to categories of dominant and dominated plants exhibiting high and low growth rates respectively. Few attempts have been made to relate floret number development with cumulative growth which is simultaneous and related positively with
both thermal time (Otegui and Bonhomme, 1998; Carcova et al., 2003) and phenology stages (Smith and Lee, 2016). The integration of cumulative growth with ear and floret development could account for variations among studies. To date there are no reports showing the relationship between floral development and cumulative growth during early stages of development and how (if existent) this relationship may respond to stress.

1.6.2 Stress and the flowering period

Stresses occurring during the flowering period disrupt normal fertilization and survival of florets causing yield to be reduced. Drought stress during this critical period has been shown to reduce yield (Denmead and Shaw, 1960; Lobell et al., 2014). At the crop level, mechanisms of yield loss in response to drought have been related primarily with reduced photosynthesis (Nissanka et al., 1997), growth rates (Sinclair et al., 1990; Earl and Davis, 2003), and asynchronous flowering (Herrero and Johnson, 1981; Hall et al., 1982; Bolaños and Edmeades, 1993). Around silking, mechanisms such as growth, fertilization of florets, and survival of florets are inter-related (Borrás and Vitantonio-Mazzini, 2018) and affected simultaneously across organization levels. For example, Schussler and Westgate, (1991) reported reduced growth, reduced allocation to reproductive structures (i.e. fertilized florets) and reduced kernel number. In this study, reductions in rates of photosynthesis were 50% when leaf water potentials were reduced to less than -1 MPa. This reduced rate of photosynthesis was accompanied by reductions in dry matter accumulation and kernel number of 40 and 48% respectively. In later studies it was reported that supply of assimilates exhibited a minimum that if not met resulted in abortion of fertilized florets (Boyle et al., 1991; Zinselmeier et al., 1995, 1999). Comparable results have been shown in
different studies imposing drought around the silking period (reviewed by Boyer and Westgate, 2004).

Under field conditions, drought has been shown to reduce growth rates around silking as well as kernel establishment (Otegui et al., 1995; Andrade et al., 2002). Furthermore, delayed silking and increased anthesis-to-silking interval under water deficit (Herrero and Johnson, 1981; Hall et al., 1982) have been related to reduced partitioning to ear growth especially in tropical germplasm (Edmeades et al., 1993, 1999; Bruce et al., 2002). Recent studies addressing the exclusive effect of drought on reproductive partitioning around silking, however, are not extensive (Cooper et al., 2014). Associated with drought, above optimum temperatures (i.e. heat stress) have also been shown to reduce kernel set around flowering. Physiological mechanisms influencing the sensitive kernel establishment period are reduced partitioning to ear, reduced growth rates, increased anthesis interval (Cicchino et al., 2010). In addition it has been shown that heat stress causes also reductions in assimilate supply (Cantarero et al., 1999) mainly by negatively impacting the efficiency of use of intercepted radiation (Rattalino Edreira and Otegui, 2012).

Plant density or intraspecific competition reduce kernel set per plant. Although plant density can be considered a season-long stress its effects are evident around flowering. Crowding stress has been shown to affect negatively individual plant growth rates around silking (Tollenaar et al., 1992), ear growth rates (Pagano and Maddonni, 2007), and rates of silk appearance (Borrás et al., 2007). The main driver for these reduced growth rates around silking in response to density is the intensification of interplant competition for resources, especially light per plant (Maddonni et al., 2001). Comparable reductions in light availability per plant have been reported for shading stress, however, reductions in kernel number caused by plant density are more severe (Andrade et al.,
At the crop level, growth rates per unit area around silking increase in response to density as well as kernel number (Andrade et al., 1999). However, the combined reduction in kernel number per plant and the inability to fill these fewer kernels cancels the yield benefit of increases in the number of plants. If reductions in plant growth rates are severe, that is below 1 to 2 g day\(^{-1}\), plants fail to set kernels and barrenness occurs. Reduced yield, then will be proportional to the frequency of plants growing at very low and below minimum growth rates for ear fertility (Rossini et al., 2011). In a similar manner, the frequency of plants with low partitioning to ear growth and increased anthesis-to-silking interval contributes to increased interplant variability which relates negatively with yield (Borrás et al., 2009a).

Clearly, kernel set defined as the relationship between kernel number and growth rates around silking per plant and silking dynamics (anthesis to silking interval) are useful frameworks to measure the impact of different stresses. Interestingly, the quantification of these parameters occurring around flowering have been almost exclusively studied in response to around silking stresses. In contrast, less attention has been paid to kernel set and flowering dynamics in response to pre-flowering stress, especially, for drought and changes in light quality.
1.6.3 Stress and the grain filling period

Stresses occurring during the grain filling period and their consequences on yield are explained by their effect on rate and duration of grain filling. In general, stresses occurring during the lag phase are more detrimental to yield because of the overlap with the kernel establishment period (Jurgens et al., 1978). In contrast, as grain filling progresses the relative impact of a given stress on yield diminishes. For example, during the lag phase, temperatures above 30°C increased the proportion of aborted kernels (Cheikh and Jones, 1994) and final kernel weight by reducing the rate of starch deposition and number of endospermatic cells in cultured kernels (Jones et al., 1984). Drought stress imposed from the lag phase to physiological maturity, resulted in reduced potential kernel volume, rates of kernel growth, and shortening of grain filling (Ouattar et al., 1987).

Similarly, Nesmith and Ritchie, (1992) showed that reduced yield when water deficit was imposed early during grain fill was attributable to reductions in rates of kernel growth while at later stages the duration of linear grain fill was shortened. Similarly, reductions in light interception per plant by means of shading cause rates of kernel growth to be reduced, however, the duration of the grain fill is not affected (Reed et al., 1988; Andrade and Ferreiro, 1996; Tanaka and Maddonni, 2009). In contrast, reductions in resources per plant caused by supra-optimal density, seem to reduce more the duration of the grain filling period than the rate of kernel growth (Poneleit and Egli, 1979; Borrás and Otegui, 2001; Monneveux et al., 2005). Similarly, Singletary and Below, (1990) and Seebauer et al., (2010) found a reductions in kernel weight and changes in metabolic activity (enzymes and starch concentration) upon nitrogen deprivation during the onset of kernel fill. In contrast with the critical 30-day period for kernel set, growth during grain fill has been given little attention in terms of individual plant growth. This is surprising since the interplant variation improves considerably the analysis of resource capture and utilization within the crop community
(Vega et al., 2000, 2001b; Vega and Sadras, 2003). In addition, improved dry matter accumulation during the grain filling period has been a large contributor to improved yields in modern hybrids (Tollenaar and Lee, 2011) (Fig.1.4). The association between reproductive and vegetative growth in response to stress during the grain fill stage provides a suitable framework to study source to sink relations.
Figure 1.4. Relationship between grain yield and dry matter accumulation during grain fill for old and newer hybrids grown at 80 000 plants ha\(^{-1}\). Experiments conducted at Elora, Ontario Canada during 2007, 2008, and 2009. Each point represents a plot (n=60, \(R^2=0.79\)). Modified from Gonzalez et al., (2019).
1.7 Source to sink relations

The relation between the supply and the demand of assimilates determines the source to sink relationship. This concept applies to organization levels that include cellular, organ, plant and crop levels (Borrás et al., 2004). In crop production, the source is commonly associated with photosynthate supply from the leaves while the sink is related to the reproductive sinks or ear grains and their demand capacity (Tollenaar, 1977; Borrás et al., 2004). Alternatively, other organs can be considered sinks such as for example, the roots (Brouwer, 1962). Most frequently, both the source and the sink are studied during the grain filling period since both components exist simultaneously. In general, the source to sink relation is considered as balanced when the relative strength of each component is equal. The strength of the source is associated with its capacity to supply in full the assimilates demanded by the sink while sink strength is driven by the capacity of the sinks to demand and accommodate these photoassimilates (Westgate et al., 2004; Tollenaar and Lee, 2011). In general, three scenarios of the source-sink relationship can be distinguished (Fig.1.5). The first one is a source limited scenario, where the supply of resources does not satisfy the demand of the sink or a plant within a community grown under optimum plant densities (Sarlangue et al., 2007). The second scenario is a balanced supply and demand between source and sink which represents the co-limitation scenario which would be exemplified by an individual plant grown within a crowded stand (Gonzalez et al., 2019). Finally, the third scenario is where the supply of assimilates is larger than the sink demand, that is, the sink limited scenario. An example of this would be a plant within a crop of maize grown at very low plant densities were reproductive growth will be favored relative to vegetative growth (Tollenaar, 1992; Andrade and Abbate, 2005). The source to sink ratio is strictly numerical, however, it has been reported using variable metrics.
Figure 1.5. Schematic relationship between sink demand and source supply for a maize crop using growth rates during the grain filling period. Three scenarios are defined: source limited scenario, source-sink co-limited scenario, and sink limited scenario. Modified from figure 6 in Bonelli et al., (2016).
The source to sink balance can be disrupted by affecting the strength of any of its components. For example, increasing planting densities beyond an optimum favor source strength by increasing crop dry matter accumulation. Beyond an optimum yield per plant is reduced severely resulting in extreme cases in plant barrenness and crop yield (Tollenaar, 1989; Gonzalez et al., 2019).

Similarly, delayed planting dates have been shown to lower source strength by reducing growth rates during the grain filling period relative to earlier planting dates (Bonelli et al., 2016). This source to sink balance can also be disrupted in a more artificial manner. Ear removal during the silking stage results in no demand from the sink causing plants to concentrate sugars and reduce growth rates during the grain filling period (Allison and Weinmann, 1970; Tollenaar and Daynard, 1982; Paul and Foyer, 2001). In contrast, plant defoliation which favors sink strength results in less stem dry matter and a consequent increase in assimilate remobilization to the ear or plant self-destruction (Ceppi et al., 1987; Rajcan and Tollenaar, 1999a). In addition, the balance between source and sink strengths is temporally dynamic which poses a challenge when studying its response in response to environmental stress. Thereby, absence of sinks or barrenness relates negatively to dry matter accumulation from silking to maturity. Although plant barrenness is intensified under density stress, little research attention has been paid to the relation between plant barrenness, and plant dry matter accumulation during the grain filling period in maize.
1.8 Individual plant variability

Individual plant variability for a given trait occurs naturally in every crop community (Donald, 1968). In maize, yield and its components exhibit this same variability which is intensified in response to stress (Tollenaar and Wu, 1999). This variability can be quantified by the coefficient of variation (Eq. [1.4]), which is the ratio the standard deviation (numerator) of, say, plant yield and the average plant yield of the crop (denominator) (Edmeades and Daynard, 1979b).

\[
Eq.[1.4] = \frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{\frac{n}{\bar{X}}} \times 100
\]

Thereby, any factor that reduces average yield per plant will often increase this interplant variability (Gonzalez et al., 2019). Therefore, it follows that stresses will increase interplant variability. Perhaps, the most attractive feature of using an individual plant approach is the usefulness to obtain narrower relations among yield traits by exploiting the naturally occurring variability when compared to plot measurements (see example in Fig. 1.6). This phenomena has been shown most frequently in response to plant density (Glenn and Daynard, 1974; Vega et al., 2001b; Tollenaar et al., 2006; Liu and Tollenaar, 2009). Other stresses such as changes in light quality (Page et al., 2010b), nitrogen deficiency (Rossini et al., 2011), weed competition (Cerrudo et al., 2012), and drought (Echarte and Tollenaar, 2006) have been shown to reduce yield and plant uniformity. The most common plant traits found in the literature, however, are growth rates around silking (Borrás and Vitantonio-Mazzini, 2018), days to flowering (Borrás et al., 2007), and yield components at maturity (Sarlangue et al., 2007). Yet, little information less information or none, is found for traits showing interplant variability for the early pre-silking stage and the grain filling
stage. This information, especially in the low end of resource capture of plant dry matter, for stresses occurring from emergence to flowering, and stresses other than plant density, is scarce in the literature.

Figure 1.6. Relationship between grain yield vs dry matter accumulation for plot data (A) and plant data (B). Hybrid P38N87 grown at 160000 plants ha\(^{-1}\). Elora Research Station from 2007-09. Modified from Gonzalez et al. (2019).
1.9 Research objectives

This research will study the effects of three distinct stresses on yield and its determinants when applied early in the season (i.e. before flowering). These stresses differ in the type of limitation they impose to growth. Stresses consist on reductions in light quality (weeds presence), light quantity (increased plant density), and water quantity (drought). The first objective was to elucidate the association between ear development and cumulative growth under early season stress. The second objective was to assess physiological mechanisms underlying yield loss when exposed to three contrasting early season stress. The third objective of this thesis was to understand the dynamics of plant dry matter accumulation in low yielding and barren plants when grown under season-long density stress. Together these three objectives will bring new knowledge in the relationship between ear development and growth, the physiological mechanisms underlying yield loss, and the association between plant barrenness and per plant cumulative growth.
Chapter 2: The Relationship Between Floret Number and Plant Dry Matter Accumulation Varies with Early Season Stress in Maize (Zea mays L.).

2.1 Abstract

The number of fertilized florets around silking determines the number of kernels in maize (Zea mays L.). In this study, we examined the relationship between plant dry matter accumulation, ear initial length and floret number. What does this relationship look like between the V7 to V9 growth stages and how does it change when the plants are subjected to stress events? We tested the hypothesis that if floret number per ear row, is related positively to plant dry matter accumulation during early stages of growth then the occurrence of stress during this period will result in a loss of floret number. Three modern hybrids were subjected to three different stresses, drought, plant population density and light quality. Light quality and drought stresses were applied for two seasons in our field hydroponics system, while the plant population density stress was conducted for two field seasons. The relationship between plant dry matter and floret number follows the classic relationship with a minimum dry matter level required and a plateau. Not all stresses impacted these relationships in the same manner. Drought stress was most severe resulting in a reduction in dry matter, ear length and floret number for the sampled period. Plant density stress was similar to drought, resulting in a reduction in plant dry matter and ear length, however, floret number per ear row was unchanged. Both drought and plant density stress resulted in reductions in the predicted minimum dry weight for floret number and the rate of increase of floret number per unit dry matter. Light quality defined as a reduced red-to-far red ratio caused reductions in dry matter up to and including the V7 stage of growth, however, the relationship between floret number and plant dry matter accumulation did not vary from the control. These results confirm
that floret number in maize is established well in advance of flowering and also suggests that floret number is related to plant dry weight sampled between V7 to V9-10 stage of growth. This research presents new knowledge on the relationship between floret number, plant cumulative dry matter and early season stress.

*Field Crops Research, 238, 129-138*
2.2. Introduction

The relationship between floret number and cumulative growth is not well understood in maize (*Zea mays* L). The number of fertilized florets around silking determines the number of kernels in maize. Kernel number, along with potential kernel weight further determines yield (Tollenaar and Lee, 2006). Ear growth, kernel number, and grain yield also relate closely with growth rates around silking (Tollenaar et al., 1992; Andrade et al., 1999; Borrás and Vitantonio-Mazzini, 2018). The kernel number vs plant growth rate framework, explores the existing plant variability in the crop (Edmeades and Daynard, 1979b; Hara, 1986) which allows for characterization of genotypes under varying resource scenarios (Echarte et al., 2000, 2004; Nagore et al., 2017). These changes in resource availability are reflected in variations of either plant growth rates or dry matter accumulation.

The relationship between kernel number and plant growth rate around silking is known to be influenced by several different types of stress. Plant density has been the most common stress used to describe the association between kernel number and individual plant growth rate (Vega et al., 2000, 2001b; Vega and Sadras, 2003). Other stresses such as reduced light interception/shading (Andrade et al., 2000), water (Andrade et al., 2002; Echarte and Tollenaar, 2006), and nitrogen availability (Rossini et al., 2011; Mayer et al., 2012) have also been used as sources of experimental variation for plant growth rate around silking. Common to all these stress responses are: i) the establishment of a threshold growth for kernel set, ii) initial increases in kernels per unit growth and iii) a stabilization of kernel number when resources per plant are plentiful. Kernel set has also been shown to be affected by stresses occurring before silking. An example of this is the detrimental effects of a reduction in plant dry matter accumulation and a reduction in stand
uniformity under high plant densities which eventually reduces ear growth during silking (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007). Most research to date has been concerned with the response of source-sink components during the silking and grain filling period phase (Rajcan and Tollenaar, 1999a; Ding et al., 2005b; Echarte et al., 2008; Tollenaar and Lee, 2011); less attention has been paid to the development of floret number. While net number of kernels is determined during the period bracketing silking, floret number is determined during the pre-silking stage of maize development.

The development of floret number is associated positively with the rate of leaf appearance and thermal time (Siemer et al., 1969; Stevens et al., 1986; Otegui and Melón, 1997; Borrás et al., 2003; Smith and Lee, 2016). The apical meristem of the shoot produces leaf initials and axillary meristems at each leaf axil (Vollbrecht and Schmidt, 2009). The growth stage of seven fully expanded leaves (i.e. V6-V7 stage of development, Hanway, 1966) is a general reference point in temperate maize for the transition of the shoot apical meristem to tassel initial (i.e., floral transition), which indicates the termination of further production of leaf initials (Irish and Nelson, 1988, 1991). The ear initials start development approximately ten days after the tassel (Siemer et al., 1969; Jacobs and Pearson, 1991; Edmeades et al., 1993); the two uppermost axillary shoots being likely to form into ears (Durieux et al., 1993). In each ear, differentiation of florets (i.e. potential kernels) and pistils (i.e. silks) progresses from the base to the top (Carcova et al., 2003), with the floret initiation period lasting between five to ten days (Edmeades et al., 1993) and the pistil initiation-appearance period lasting for ten to twenty days (Carcova et al., 2003). Although second and third ears have the potential to differentiate a large part of florets at ultralow densities (Tetio-Kagho and Gardner, 1988a), they frequently seize to develop under conventional densities contributing marginally to plant yield (Gonzalez et al., 2018). Collectively, these developmental
processes occur throughout what is loosely termed the vegetative phase (Lejeune and Bernier, 1996) and is completed upon appearance of the silks. Kernel number is then related to floret number, however, the association of floret number with cumulative growth has not been reported.

In this paper we examine how stresses during early stages of development (herein called the vegetative phase) impact floret number and it is relationship with cumulative growth on a per plant basis. In order to address this, we examined the length and floret number of the primary ear as it relates to individual plant dry matter accumulation using three different hybrids. Sources of variation in plant dry matter accumulation were induced by selected stress variables which included drought, plant density and reduced red-to-far red ratio. Stress created by drought and plant density were intended to reduce resource availability (i.e., water and light respectively) while the reduced red to far red ratio had the purpose of triggering the shade avoidance response (Kasperbauer and Karlen, 1994; Liu et al., 2009; Page et al., 2010b). Data regarding final yields is subject of a second paper. Specifically, we tested the hypothesis that if floret number per ear row in the primary ear, is related positively to plant dry matter accumulation during the vegetative phase then the occurrence of stress during this period will result in a loss of floret number.
2.3. Materials and Methods

2.3.1. Experiment #1–Drought and Light Quality Stress

The experiment was grown in the field hydroponic system at Arkell Research Station (-43°54´N, 80°18´W and 325 m above the sea level) near Guelph, Ontario, Canada during the 2012 and 2013 growing seasons. The field hydroponic system consisted of 22-L plastic pails that were 28 cm diameter and were filled with a baked clay medium, MVP Turface. The concentrated nutrient solution was stored in two 340-L sealed plastic pails of water. The nutrient solution consisted of two components: the first component was 13.6 kg of 28–14–14 (N, P, K), and 7 kg of NH₄NO₃; the second consisted of 1.2 kg micronutrient mix and 13.6 kg MgSO₄N₇H₂O. The micronutrient mix consisted of chelated Fe (7%), chelated Mn (2%), chelated Zn (0.4%), chelated Cu (0.1%), Boron (1.3%), Mo (0.06%), EDTA (Ethylene diamine tetraacetate, 42%), DTPA (Diethylene triamine pentaacetate, 14%). The concentrated nutrient solution was then diluted with water at a dilution ratio of 1:100 to deliver approximately 0.12 g N, 0.06 g P, and 0.09 g K per 500 ml of nutrient solution. On a daily basis, the pH of the nutrient solution was adjusted through the addition of HCL to ensure values of 6.5 to 6.8. Each pail was supplied with four fertigation tubes that each delivered a minimum of 100 ml of the pre-mixed nutrient solution over a 10-min time period. Irrigation was programmed to occur three times per day (8 am, 1 pm, 3 pm, and 6 pm). Pail drainage to the soil occurred through holes (4 per pail) in the base of the pail of 1 cm diameter. This field hydroponic system has been used for over three decades to enable maize growth from seedling to maturity in the field under controlled water and nutrient conditions (Tollenaar and Migus, 1984; Earl et al., 2012; McKenzie-Gopsill et al., 2016).
2.3.2. Treatments

Prior to the initiation of this experiment, 22 L pails were filled with Turface® and then covered with a clear plastic sheet that remained in place throughout the growing season. This plastic sheet had the purpose to separate the root systems of the corn and the surrogate weed. A 15-cm diameter hole was cut in the center of the plastic sheet. Each pail was hand sown with four seeds at a depth of 4 cm and thinned to two plants per pail at the 4-leaf-tip stage (ca. V1-2) resulting in a final plant density of 8 plants m\(^{-2}\) in all treatments. Planting dates were 10\(^{th}\) May 2012 and 17\(^{th}\) May 2013.

Three genotypes were used in this study, CG108/CG102, X58945WP and SK5069WP. CG108/CG102 is a hybrid developed by the University of Guelph’s Corn Breeding Program and has been used in previous studies (Lee et al., 2000, 2001). X58945WP and SK5069WP are isoline hybrids from Syngenta that were classified as either a non-drought tolerant hybrid (NDT; X58945WP) or as a drought tolerant hybrid (DT, SK5069WP) (Agrisure® Artesian® Syngenta Crop Protection Inc.). Hybrids are adapted to Ontario conditions. CG108/102 exhibits 2700 crop heat units requirement (Brown and Bootsma, 1993). Isolines exhibit 3000 crop heat units. For further details on the isoline hybrids, see Reid et al., (2014). Treatments were established by filling the area surrounding the maize seedlings above the plastic sheet with either Turface® (control and drought treatments) or with turfgrass (Lolium perenne L.) which acted as a surrogate weed after thinning to final density. Turfgrass was added after 50% emergence of seedlings. The presence of the turfgrass resulted in a reduced red-to far red ratio treatment of 0.37 compared to 0.86 in the weed free control.

For the light quality treatment, maize seedlings and turfgrass were fed by separate fertigation lines, and the design (plastic sheet separation) prevented any root or shoot contact of corn with the
The turfgrass was maintained at a height of 10 cm or less by manual clipping to prevent direct shading of maize seedlings. The light quality environment provided by the two treatments was characterized when the controls reached the 5-leaf-tip stage. The red-to-far red ratio of light reflected 10 cm above the ring of turfgrass or Turface® bordering the maize seedlings centered within the pail was measured using a dual channel red-to-far red ratio sensor (SKR 110, 660 nm /730 nm, Skye Instruments, Llandrindod Wells, Powys, UK). These measurements were recorded on sunny, cloudless days between 0900 and 1300 hrs. In order to ensure the effectiveness of the drought treatment, the base of the stem of the developing seedlings within each pail was wrapped with additional plastic in order to cover the 15 cm diameter hole in the plastic, thereby preventing naturally occurring rainfall from seeping into the base of the pail.

In order to quantify the reduction in water availability for the drought treatment mean water holding capacity was determined in four pails watered up to saturation. Difference between saturation and drained weight yielded available water per pail. Two random pails per replication (six pails total) were selected to track the weight variation upon reductions in irrigation throughout the drought stress period. Pails were kept at constant weight once the drought stress symptoms (rolled leaves) were evident. Clear effects of the drought were reflected in reductions of at least 50% in dry matter accumulation. During 2012 and 2013 available water per pail as a percent of maximum water weight (100%=10 kg water pail\(^{-1}\)) was reduced at 65% (2012) and 85% (2013) from planting up to 12\(^{th}\) leaf-tip stage of the control respectively. During both years those levels were sufficient to reduce plant dry matter accumulation although the decrease in available water over time as substantially higher during 2012. Each pail was irrigated individually with a graded glass of 100:1 water: solution to target available water (65% and 85%) every two days maximum.
Once the 12th leaf tip stage of the CG108/CG102 control was attained full irrigation was restored to allow plants recovery.

2.3.3. Experimental design

The experimental design was a split-plot with three replications. Main plots were assigned to control (i.e., well-watered throughout the entire experiment), drought (i.e., drought until the 12th leaf tip stage, then well-watered throughout the entire experiment) and reduced red to far red ratio (i.e., presence of turfgrass from emergence to the 12th leaf-tip stage, turfgrass then removed). Subplot treatments were genotypes, CG108/CG102, X58945WP and SK5069WP. Both the drought and reduced red-to-far red ratio were imposed until the 12-leaf-tip stage or V9 (i.e., nine visible collars) to ensure that the end of the floret differentiation period had been reached. Note that the occurrence of the 12th leaf-tip stage corresponded to the stage of development recorded in the control for CG108/CG102.

Main plot (control, drought, reduced red-to-far red ratio) size was 26 22-L pails long by four pails wide for a total of 104 pails and a total area of 9.1 m by 2.8 m (0.35 between two pails and 0.70 m between rows). Subplots (genotypes) were eight pails long by four pails wide (2.8 m by 2.8 m, 32 pails in total). The experimental unit was the average of three plants in each sampling stage (i.e. two pails). From the two central rows of pails, six pails from each treatment were used for destructive measurements while four pails were left for measurements and final harvest (see Gonzalez, 2019). The remainder six pails and the outer two rows of pails were used as borders when re-arrangement of pails was necessary after destructive sampling.
2.3.4. Experiment #2– Plant Density Stress

This experiment was conducted at the Elora Research Station, Elora, ON (43º38´ N, 80º25´ W, 380 m above sea level). The soil type was a London loam soil (Aquic Hapludalf) with tile drainage and soil organic matter content of 3.8 to 4.0%. Nitrogen, P, and K were applied before planting at rates of 150, 85, and 50 kg ha\(^{-1}\), respectively. Soybean was the previous crop during both years. Weed control was obtained using a pre-plant tank mix of S-metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl]-,(S)], atrazine [6-chloro-N-ethyl-N´-(1-methylethyl)-1,3,5-triazine- 2,4-diamine] and mesotrione [2-[4-(methylsulfonyl)-2- nitrobenzoyl]-1,3-cyclohexanedione] at rates of 1.6, 1.28, and 0.14 kg a.i. ha\(^{-1}\), respectively. Planting occurred on 10\(^{th}\) May 2012 and on 14\(^{th}\) May 2013. The seeds were planted with a precision planter Almaco SeedPro 350 Precision Planter (Almaco, Nevada, Iowa) resulting in 6 m long by 3.04 m wide (4 rows, 0.76 m between rows) plots. Two densities were used in this experiment: 7.5 plants m\(^{-2}\) and 15 plants m\(^{-2}\) to create a wide range of individual plant dry weights (Vega et al., 2000). At the 12-13\(^{th}\) leaf tip stage (ca. V9) stage the 15 plants m\(^{-2}\) treatments were thinned to 7.5 plants m\(^{-2}\) to simulate early season intraspecific competition. The experimental design consisted of a split-plot with four replications. Plant densities were assigned to the main plots and the genotypes (CG108/CG102, X58945WP and SK5069WP) to the sub-plots.

2.3.5. Destructive sampling-both experiments

In experiment #1 three consecutive destructivesamplings (two pails per subplot per sampling date) were made when the first genotype (CG108/CG102) attained seven collars (V7) and was completed when the same genotype attained nine collars (V9). Ear length, florets per ear row and plant dry matter accumulation were recorded for three individual plants/plot/sampling date.
Determinations were made in the primary ears since stresses intended to limit resources per plant which affects the subsequent establishment a second fertile ear (Andrade et al., 1999). In addition, under optimal conditions of both experiments (7.5 to 8 plants m$^{-2}$) almost no frequency of second fertile ears was present. In Experiment #2 three destructive samplings were carried out at V7, V8 and V10 stages in each density-genotype combination. Each sampling stage corresponded to the phenological stage of the 7.5 plant m$^{-2}$ treatment in the CG108/102 genotype. In contrast with Experiment #1, the third sampling was carried out at V10. Phenology was recorded at least weekly in 3 plants per treatment combination in order to monitor development. Delays in leaf appearance were of 2.4 and 0.9 leaf tips at the end of the sampling period in the drought and the weedy treatments respectively while 0.8 leaf tips in the 15 plants m$^{-2}$. Data is presented only for individual dry weights.

The methodology of counting ear and floret initials, measurement of ear length and plant dry matter determination was the same as in Experiment #1. In Experiments #1 and #2 dissections of plants, separation of the uppermost axillary meristem (ear initial) and counting of initial differentiated florets were done in each plant. Florets were counted when they were clearly visible at the base of the ear initial. Row numbers were counted in all primary ears, however, no variation in this trait was observed so data is presented only for ear and floret number per row. During early differentiation, ear length and floret, and row number counts were obtained by photographing each individual ear sample using a camera mounted on a zoom trinocular stereo microscope with a 90X magnification capacity (Cyber Scientific Inc, Kitchener, Ontario; model V434B) and a three-megapixel camera (Cyber Scientific Inc, Kitchener, Ontario, model A1530). Ear length measurements were done on each individual picture using Scope Image Advanced® software. For
dry matter measurements, individual sampled plants were dried at 85°C for at least a week until constant weight was achieved.

2.3.6. Statistical analysis – both experiments

Main treatment effects were analyzed using mixed linear models. The SAS PROC MIXED procedure (SAS® Institute Inc., 2013, Cary, NC, USA, Version 9.4) was used to generate the ANOVA random and fixed effects coefficients and their p-values. Year, replications, and plots were assigned as random factors. Main factors were assigned to controls (Stress free control at Arkell and 7.5 plants m⁻² at Elora), and treatments (drought, reduced red-to-far red ratio and 15 plants m⁻²) and genotypes as sub-factors using the Eq. [2.1]:

\[ Eq. [2.1] = Y_{gsyrp} = \mu + \alpha_g + \beta_s + \delta_y + \rho_r (\delta_y) + \sigma_p (\rho_r) + \alpha_g \beta_s + \varepsilon_{gsyrp} \]

Where \( Y_{gsyrp} \) represents the phenotype of \( g \) at the treatment \( s \) (drought, reduced red to far-red ratio, density) in year \( y \) in plot \( p \) and the replicate \( r \). The \( \mu \) term represents the overall mean of the phenotype, \( \alpha_g \) the genotypic effect; \( \beta_s \) the treatment effect; \( \delta_y \) is the year effect; \( \rho_r (\delta_y) \) is the replicate effect nested within year; \( \sigma_p (\rho_r) \) is the effect of the plot within replicates; \( \alpha_g \beta_s \) is the interaction between the genotype \( g \) and the treatment \( s \); and \( \varepsilon_{gsyrp} \) is the residual error of the genotype \( g \) at the treatment \( s \) in year \( y \) replicate \( r \) and plot \( p \). Means were calculated as best linear unbiased estimators and pre-planned contrasts were used to determine significance at 0.05 level. Non-linear regressions were adjusted using PROC NLIN procedure (SAS® Institute Inc., 2013, Cary, NC, USA, Version 9.4) using the Eq. [2.2]:

46
Eq. [2.2] = Y = \left[ \frac{\alpha(X - t)}{1 + \beta(X - t)} \right] [X > t]

Where Y is the response variable (i.e., ear length or floret number per ear row), \( \alpha \) is the initial slope coefficient, \( t \) is the threshold coefficient (g plant\(^{-1} \) or cm of the ear) for \( Y \geq 0 \), \( \beta \) is the curvilinear degree coefficient, and \( X \) is the dependent variable (i.e., plant dry matter in g plant\(^{-1} \) or ear length in cm. Equations were fitted in order to establish the relationship among ear length, floret number per ear row, and plant dry matter. The non-linear model represented in Eq. [2.2] exhibits three coefficients which are biologically meaningful, that is, the \( t \), \( \alpha \), and \( \beta \) parameter (Vega et al., 2000; Sarlangue et al., 2007; Gonzalez et al., 2018). The \( t \) value for plant dry matter or ear length represents the threshold value for the response variable. The \( \alpha \) slope represents the initial response of the dependent value (i.e. ear length and floret number per ear row) at low value of the independent variable (i.e. plant dry matter and ear length). The \( \beta \) value is the curvilinear response of the relationship (Sarlangue et al., 2007). Coefficients of the non-linear regressions were fitted for all fixed effects and compared using Z test. Each parameter was assessed at a probability level of 0.05 to test the hypothesis of significance within the equation. Goodness of fit was assessed by using coefficient of determination (Archontoulis and Miguez, 2015) calculated as in Eq. [2.3]:

\[
Eq. [2.3] = R^2 = 1 - \frac{SS_{residual}}{SS_{total}}
\]

Where SS means sum of squares.
2.4. Results

2.4.1. Experiment #1

Drought stress which occurred from seedling emergence until the V9 stage reduced consistently plant dry matter, ear length, and floret number; the effect of reduced red-to-far red ratio was found to be more variable. Data on row number is not presented since no variations were detected among stress (Otegui, 1997) and genotypes (Smith and Lee, 2016) with mean values of 14.2 ±0.10 and 14.3±0.08 for Experiment #1 (V9) and #2 respectively (V10). At the V7 stage of development, plant dry matter was reduced by 19 and 50% of the control plant dry matter (30 g plant\(^{-1}\)) in the reduced red-to far red ratio and drought (Table 2.1). Further reduction in plant dry matter at the V8 and V9 stage of development were observed only in the drought. These reductions were of 43 and 53 % relative to the control (41 and 82 g plant\(^{-1}\)). Ear length appeared to increase initially compared to the control during V7 and V8 in the drought (Table 2.1). Ear length was 90 and 110 % higher during the V7 and the V8 stages respectively in the drought when compared to the control (0.21 and 0.62 cm respectively). For floret number, stress by genotype interaction was observed at the V8 stage of development. This interaction was driven by a 90% increase in the number of florets in the DT genotype when compared with the CG and NDT genotypes (not shown). Drought stress appeared to initially increase floret number with respect to the control (stress free), however, by V9 this increase was no longer evident (Table 2.1). For example, at the V9 stage of development drought had reduced floret number per ear row by 14 % relative to the control (data not shown). By the V9 stage of development drought had reduced plant dry matter, floret number per ear row but not ear length. The effect of a stress caused by a reduction in red-to- far red ratio appeared to be temporary, reducing plant dry matter only at V7 stage of development, after which no differences were detected when compared to the control (Table 2.1).
Table 2.1 ANOVA and contrasts for treatment effects on plant dry matter, ear length, and floret number per ear row for control, drought, reduced red-to far red ratio (Reduced R:FR) and three growth stages. Contrasts represent the average of three genotypes. Experiment #1 conducted at Arkell Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Plant dry matter</th>
<th>Ear length</th>
<th>Floret number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V7</td>
<td>V8</td>
<td>V9</td>
</tr>
<tr>
<td>Growth stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Random effects</td>
<td>Pr&gt;Z</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>0.25</td>
<td>0.26</td>
<td>0.25</td>
</tr>
<tr>
<td>Residual</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fixed effects</td>
<td>Pr&gt;F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress(S)</td>
<td>2</td>
<td>16</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Genotype(G)</td>
<td>2</td>
<td>16</td>
<td>0.66</td>
</tr>
<tr>
<td>SxG</td>
<td>4</td>
<td>16</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrasts</td>
<td>Pr&gt;F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drought vs Control</td>
<td>-17***</td>
<td>-19***</td>
<td>-44***</td>
</tr>
<tr>
<td>Reduced R:FR vs Control</td>
<td>-6**</td>
<td>-5†ns</td>
<td>-10ns</td>
</tr>
<tr>
<td>Drought vs Reduced R:FR</td>
<td>-10***</td>
<td>-13.8***</td>
<td>-34***</td>
</tr>
<tr>
<td>Standard error (±)</td>
<td>1.38</td>
<td>1.66</td>
<td>2.26</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant.
2.4.2. Experiment #2

Plant dry matter and ear length were reduced at a density of 15 compared to 7.5 plants m$^{-2}$, while floret number per ear row varied only with genotype. For example, the reductions in plant dry weight at V8 and V10 equated to 22 and 35 % respectively of the dry weight recorded for the same growth stages at density of 7.5 plants m$^{-2}$ (Table 2.2). At V10 ear length was 26 % less of that recorded for the 7.5 plants m$^{-2}$(mean ear length = 2.8 cm). Floret number per ear row during the V7, V8 and V10 stages were of 15, 24 and 35 among plant densities and genotypes. In addition, genotype differences were observed in plant dry matter, ear length, floret number per ear row at each developmental stage sampled (Table 2.2, p value=0.0002). Although plant dry matter, ear length, and floret number per ear row were consistently greater in the CG genotype compared to the two isolines which did not differ between them (not shown), plant dry matter was related positively with the development of ear length and floret number in all genotypes.
Table 2.2. ANOVA and contrasts for treatment effects on plant dry matter, ear length, and floret number per ear row for 7.5 and 15 plants m\(^{-2}\) treatments and three growth stages. Contrasts represent the average of three genotypes. Experiment #2 conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Plant dry matter</th>
<th>Ear length</th>
<th>Floret number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth stage</td>
<td>V7 V8 V10 V7 V8 V10 V7 V8 V10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Pr&gt;Z</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>0.24 0.29 0.39 0.4 0.28 0.44 0.27 0.31 0.47</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>0.005 0.04 &lt;.0001 &lt;.0001 0.02 &lt;.0001 0.001 &lt;.0001 &lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Num df</th>
<th>Den df</th>
<th>Pr &gt; Z</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (Dty)</td>
<td>1 6</td>
<td></td>
<td>0.08</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;.0001</td>
<td>0.891</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>0.46</td>
<td>0.003</td>
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<td></td>
<td>0.535</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>2 6</td>
<td></td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0003</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0002</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Dty x G</td>
<td>2 6</td>
<td></td>
<td>0.62</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.76</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.95</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>g plant(^{-1})</th>
<th>cm</th>
<th>no row(^{-1}) ear(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 vs 7.5 plants m(^{-2})</td>
<td>-3.4**</td>
<td>-7.1***</td>
<td>-24***</td>
</tr>
<tr>
<td>Standard error (±)</td>
<td>2.27</td>
<td>1.45</td>
<td>2.97</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant.
2.4.3. **Ear length and floret number per ear row was associated positively with plant dry matter accumulation**

Ear length and floret number per ear row were positively associated with plant dry matter accumulation during the vegetative phase. A non-linear regression model described adequately ear length ($R^2=0.73$) and florets per ear row ($R^2=0.68$) as a function of plant dry matter (Table 2.3). The dry weight where ears were observed is defined herein as the threshold value of plant dry matter to obtain an ear length greater than zero cm was of 15 g plant$^{-1}$ across experiments. Slope $\alpha$ for ear length vs plant dry matter was of 0.04 cm g$^{-1}$ while the curvilinear response $\beta$ term was of -0.0044 reflecting the exponential-like increase in ear length at higher plant dry weights (Fig. 2.1a). At approximately 80 g plant$^{-1}$ the ear exhibited 50% of the maximum ear length was attained while at 100 g plant$^{-1}$ the ear accumulated 75% of ear length for the sampled plant dry matter. Threshold plant dry matter to obtain florets per row greater than 0 was of 18 g plant$^{-1}$ across experiments (Fig. 2.1b). Initial rates of florets per row appearance ($\alpha$) were of 6 florets g$^{-1}$ with a $\beta$ coefficient of 0.14 which was reflected in the stabilization of florets per row at plant dry matter greater than 50 g plant$^{-1}$. The non-linear regression predicted that at 30 g plant$^{-1}$, 50% of all florets were present in the ear. Additionally, at plant dry matter values of 50 g plant$^{-1}$, the predicted floret number per row attained 75% of all florets.

Florets per ear row were also closely associated with ear length across all experiments and treatments (Table 2.3). Coefficient of determination was 0.90 across all experiments. Threshold ear length to obtain a floret number greater than 0 was of 0.03 cm with initial $\alpha$ of 66 and $\beta$ of 1.64. An ear length of 0.56 cm predicted floret number to be approximately 50% of the maximum and at
1.45 cm the ear was predicted to have obtained 75% of the total florets per row (Fig. 2.2). Both ear length and floret number per row were determined by cumulative plant dry matter with thresholds of 15-18 g plant\(^{-1}\) respectively. The experimental results suggested, that plant dry matter accumulation during the growth stages V7 to V9-10 was critical in the determination of ear length and floret number. Dry matter accumulation, however, during this stage can be influenced by abiotic stresses.
Table 2.3. Models parameters for the relationships of ear length vs plant dry matter, floret number per ear row vs plant dry matter, floret number per ear row vs ear length. Experiments conducted at Arkell and Elora Research Stations during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Experiment #</th>
<th>Treatment</th>
<th>n</th>
<th>Parameter ± standard error</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear length vs plant dry matter</td>
<td>1,2</td>
<td>All</td>
<td>876</td>
<td>0.04 ± 0.0018, 15 ± 3.3†, -0.0044 ± 0.00043</td>
<td>0.73</td>
</tr>
<tr>
<td>Floret number per ear row vs plant dry matter</td>
<td>1,2</td>
<td>All</td>
<td>866</td>
<td>6 ± 0.56, 18 ± 2.1†, 0.14 ± 0.02</td>
<td>0.61</td>
</tr>
<tr>
<td>Floret number per ear row vs ear length</td>
<td>1,2</td>
<td>All</td>
<td>988</td>
<td>65.95 ± 1.73, 0.03 ± 0.01‡, 1.64 ± 0.045</td>
<td>0.90</td>
</tr>
</tbody>
</table>

†Plant dry matter threshold in g plant⁻¹, ‡ Ear length threshold in cm.
Figure 2.1. Ear length (A) and floret number per ear row (B) as a function of plant dry matter accumulation during the V7 to V9-10 stage of growth for different treatments. Treatments are control, drought, reduced red to far red ratio (R:FR), 7.5 plants m⁻² and 15 plants m⁻². Each point represents an individual plant sampled at a specific growth stage (V7,V8, V9-10 stage of growth according to Hanway, 1966). Models and coefficients values are shown in Table 2.3. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations during the 2012-13 growing seasons. Ontario, Canada.
Figure 2.2. Floret number per ear row as a function of ear length during the V7 to V9-10 stage of growth for different treatments. Treatments are control, drought, reduced red to far red ratio (R:FR), 7.5 plants m$^{-2}$ and 15 plants m$^{-2}$. Each point represents an individual plant sampled at a specific growth stage (V7, V8, V9-10 stage of growth according to Hanway, 1966). Models and coefficients values are shown in Table 2.3. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations during the 2012-13 growing seasons. Ontario, Canada.
2.4.4. Ear vs plant dry matter relationship as influenced by stress

Abiotic stresses caused by drought, high density (15 plants m\(^{-2}\)) and changes in the red to far red ratio influenced dry matter accumulation. Ear length and floret number per ear row vs plant dry matter varied with type of stress (Table 2.4). For example, model parameters were significant for each treatment (p<0.05) with an explained variation (R\(^2\)) that ranged from 0.45 to 0.88 for ear length and from 0.35 to 0.88 for floret number per ear row. The threshold values of dry matter accumulation to obtain an ear length greater than zero were 15 and 10 g plant\(^{-1}\) in the drought and 15 plants m\(^{-2}\) treatments respectively, while for the control, reduced red to far red ratio, and 7.5 plants m\(^{-2}\) were of 23, 21, and 20 g plant\(^{-1}\) (Fig. 2.3). Initial slopes \(\alpha\) for ear length vs plant dry matter were of 0.08 cm g\(^{-1}\) for drought vs. approximately 0.03, to 0.05 cm g\(^{-1}\) for the control, reduced red-to-far red ratio, 7.5 and 15 plants m\(^{-2}\) respectively. The higher rate of \(\alpha\) (0.08 cm g\(^{-1}\)) combined with a lower value for \(\beta\) (-0.02) in the drought stress treatment resulted in a significantly greater rate of ear elongation per unit plant dry matter when compared with the rest of the stresses. The 15 plants m\(^{-2}\) treatment presented a \(\beta\) value of -0.0036 which resulted in a linear like response of ear length to plant dry matter when compared to drought, control, and reduced red-to-far red ratio. The control, reduced red-to-far red ratio, and 7.5 plant m\(^{-2}\) had \(\beta\) values of -0.01; suggesting a less linear response compared to the drought and the 15 plants m\(^{-2}\) treatment.

The dry matter thresholds to obtain floret number per ear row greater than zero were 10 and 15 g plant\(^{-1}\) for the drought and 15 plants m\(^{-2}\), respectively. These threshold values differed significantly (see Fig.2.4, b,e). In contrast, the threshold values for the control, reduced red-to-far
red ratio and 7.5 plants m\(^{-2}\) did not differ. For all three treatments, the minimum dry weight threshold value for floret number per ear row was 25 g plant\(^{-1}\). Similar to ear length, the \(\alpha\) values of 9 and 8 florets g\(^{-1}\) for the florets were higher for drought and the 15 plants m\(^{-2}\), compared to 5 florets g\(^{-1}\) in the control, reduced red-to-far red ratio and 7.5 plants m\(^{-2}\) respectively. Additionally, the \(\beta\) coefficients were higher in both drought and 15 plants m\(^{-2}\), thereby increasing the positive curvilinear response for the relationships with values of 0.25 and 0.23 respectively vs 0.13 in the rest of the treatments. When floret number per ear row was regressed against ear length for each treatment the explained variation ranged from to 0.88 to 0.93 (Table 2.4, Fig. 2.5). Values of \(\alpha\) slopes were higher for drought when compared to the control (86 vs 64) but did not differ from 7.5 and 15 plants m\(^{-2}\). Threshold ear length for floret number set was 0.03 and 0.07 cm in the control and drought treatment with no significant threshold terms for the remaining treatments. The reduced red-to-far red ratio exhibited a lower \(\alpha\) slope when compared with the rest of the treatments suggesting a delay in floret set (Fig 2.5a). The \(\beta\) coefficient was lower (1.16) for the reduced red-to-far red ratio when compared to all treatments decreasing the curvilinear response of the relationship and increasing predicted floret number at ear lengths > 3 cm (Fig. 2.5b). Both the drought and 15 plants m\(^{-2}\) showed a departure in the value of the coefficients for both ear length and floret number per ear row. This resulted in a lower threshold dry matter and an increase in ear length and floret number per unit plant dry matter. The association between floret number per ear row and ear length changed for the reduced red-to-far red ratio, showing a lower initial rate but higher floret number at greater ear length.
Table 2.4. Models parameters for the ear length and floret number per ear row vs plant dry matter relationship and floret number per ear row vs ear length for two experiments and five treatments. Treatments are control, drought, reduced red to far red ratio (R:FR), 7.5 plants m\(^{-2}\) and 15 plants m\(^{-2}\). Experiments conducted at Arkell (Experiment # 1) and Elora (Experiment # 2) Research Stations during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Model</th>
<th>Experiment #</th>
<th>Treatment</th>
<th>n</th>
<th>Parameter ± standard error</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(\alpha)</td>
<td>(\beta)</td>
</tr>
<tr>
<td></td>
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<td>(\beta)</td>
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<td>(\beta)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(t)</td>
<td>(\beta)</td>
</tr>
<tr>
<td>Ear length vs plant dry matter</td>
<td>1</td>
<td>Control</td>
<td>181</td>
<td>0.03 ± 0.01 b‡</td>
<td>23.26 ± 3.58 a</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Drought</td>
<td>117</td>
<td>0.08 ± 0.02 a</td>
<td>15 ± 2.15 b</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Reduced R:FR</td>
<td>187</td>
<td>0.04 ± 0.01 b</td>
<td>20.93 ± 2.62 a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.5 plants m(^{-2})</td>
<td>199</td>
<td>0.03 ± 0.01 b</td>
<td>20.01 ± 2.1 a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15 plants m(^{-2})</td>
<td>196</td>
<td>0.05 ± 0.01 b</td>
<td>9.7 ± 2.58 b</td>
</tr>
<tr>
<td>Floret number per ear row vs plant dry matter</td>
<td>1</td>
<td>Control</td>
<td>188</td>
<td>5 ± 0.92 b</td>
<td>25 ± 0.64 a</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Drought</td>
<td>199</td>
<td>9 ± 3.1 a</td>
<td>10 ± 0.62 c</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Reduced R:FR</td>
<td>190</td>
<td>5 ± 0.92 b</td>
<td>25 ± 0.64 a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7.5 plants m(^{-2})</td>
<td>170</td>
<td>5 ± 0.92 b</td>
<td>25 ± 0.64 a</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15 plants m(^{-2})</td>
<td>177</td>
<td>8.06 ± 2.03 a</td>
<td>15 ± 0.76 c</td>
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<tr>
<td>Floret number per ear row vs ear length</td>
<td>1</td>
<td>Control</td>
<td>198</td>
<td>63.56 ± 5.71 b</td>
<td>0.04 ± 0.02 a</td>
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<td></td>
<td>1</td>
<td>Drought</td>
<td>177</td>
<td>85.67 ± 7.76 a</td>
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<tr>
<td></td>
<td>1</td>
<td>Reduced R:FR</td>
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<td>55.45 ± 4.73 c</td>
<td>0.03 ± 0.02 ns‡</td>
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<tr>
<td></td>
<td>2</td>
<td>7.5 plants m(^{-2})</td>
<td>207</td>
<td>76.15 ± 7.94 ab</td>
<td>-0.02 ± 0.03 ns</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15 plants m(^{-2})</td>
<td>200</td>
<td>75.90 ± 8.64 ab</td>
<td>-0.01± 0.03 ns</td>
</tr>
</tbody>
</table>

‡ns: not significant. Different letters among treatments for each model parameter indicate significant differences using Z test (p<0.05).
Figure 2.3. Predicted ear length as a function of plant dry matter accumulation during the V7 to V9-10 stage of growth for different treatments. Treatments are control, drought, reduced red to far red ratio (R:FR), 7.5 plants m$^{-2}$ and 15 plants m$^{-2}$. Each point represents an individual plant sampled at a specific growth stage (V7, V8, V9-10 stage of growth according to Hanway, 1966). Models and coefficients values are shown in Table 2.4. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations during the 2012-13 growing seasons. Ontario, Canada.
Figure 2.4. Floret number per ear row as a function of plant dry matter accumulation during the V7 to V9-10 stage of growth for different treatments. Treatments are control (A), drought (B), reduced red to far red ratio (R:FR) (C), 7.5 plants m$^{-2}$ (D), 15 plants m$^{-2}$ (E), and predicted floret number values for Experiment #1 and #2 (F). Each point represents an individual plant sampled at a specific growth stage (V7,V8, V9-10 stage of growth according to Hanway, 1966). Models and coefficients values are shown in Table 2.4. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations during the 2012-13 growing seasons. Ontario, Canada.
Figure 2.5. Predicted floret number per ear row as a function ear length (A and B) during the vegetative phase for different treatments. Treatments are control, drought, reduced red to far red ratio (R:FR), 7.5 plants m$^{-2}$ and 15 plants m$^{-2}$. Each point represents an individual plant sampled at a specific growth stage (V7,V8, V9-10 stage of growth according to Hanway, 1966). Models and coefficients values are shown in Table 2.4. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations during the 2012-13 growing seasons. Ontario, Canada.
2.5. Discussion

In this study we tested the hypothesis that if floret number per ear row, is related positively to plant dry matter accumulation during the vegetative phase then the occurrence of stress during this period will result in a loss of floret number. Through the testing of this hypothesis, we have explored the association of ear development and cumulative plant dry matter. We observed that individual plant dry matter is a reasonable predictor of ear development. Overall, non-linear regression models described the association between i) ear length vs plant dry matter, ii) florets number per ear row vs plant dry matter, and iii) floret number per ear row vs ear length ($R^2$ of 0.73, 0.61, and 0.93 respectively). The estimated reference dry weight herein defined as threshold dry weight for the initiation of ear elongation and floret appearance varied from an average of 15 to 18 g plant$^{-1}$, recognizing that there was a considerable degree of plant variability around these average dry weights. In contrast, plants that attained more than 50-60 g plant$^{-1}$ had floret numbers closer to the plateau section of the relationship for the period studied (i.e., V7 to V9-10) with a major proportion of florets established (Smith and Lee, 2016). Number of florets per ear plateaued at values greater than 60 g plant$^{-1}$. Interestingly, this range in dry weights was within the reported minimum dry weight at maturity for kernel set (Vega et al., 2000; Vega and Sadras, 2003; Tollenaar and Lee, 2011). These results suggested that plants below this critical value of 50 to 60 g plant$^{-1}$ at maturity may not only fail to set kernels around silking but also may exhibit a developmental delay, early during the growing season. The close association between floret number and ear length suggested a near simultaneous development of both ear
initiation and floret appearance (estimated ear length threshold = 0.03 cm). Variability in
tfloret number per ear row was reduced at ear lengths of 3 cm or greater. For approximately
the same number of florets Otegui, (1997) reported a similar ear length in genotypes of a
longer maturity ratings. Our results, provide a framework to integrate both floret number and
ear length estimation using a single parameter, cumulative plant dry matter. In addition, these
results confirm that potential floret number is established well in advance of flowering time;
that is, when corn plants under stress free conditions, have attained a minimum dry weight of
50-60 g plant\(^{-1}\) and a minimum ear length of 3 cm.

Of the stresses tested in this study, only the severe drought reduced floret number
resulting in a reduction in both floret number and dry matter accumulation for the sampled
period. This partially confirms our hypothesis. Further floret differentiation, however, in the
drought stress may have occurred during subsequent stages. Drought and the 15 plants m\(^{-2}\)
treatment both reduced plant dry matter significantly over time but differed in terms of
magnitude. Severe reductions in plant dry matter caused by drought have been reported in
previous studies (Denmead and Shaw, 1960; Stewart et al., 1975; Çakir, 2004). This plant dry
matter reduction caused by drought may have been initiated by a reduction in leaf expansion
thereby resulting in less dry matter accumulation (Boyer, 1970; Salah and Tardieu, 1997;
Avramova et al., 2016). Reductions in plant dry matter caused by the 15 plants m\(^{-2}\) were
expected as a result of unequal sharing of resources caused by interplant competition during
early vegetative growth (Maddonni and Otegui, 2004; Pagano and Maddonni, 2007; Page et
al., 2010a). The reduced red-to-far red ratio initially reduced plant dry matter accumulation, however, the plants were able to recover and no reduction in floret number was observed. Previous research by Page et al., (2010a) reported a reduction in plant dry matter occurring as a result of reduced red-to-far red ratio prior to 10 leaf tips (approximately V7 stage of growth) which showed subsequent recovery at silking. In addition, although crowding stress includes changes in light quality (Wies et al., 2019), these treatments responded differently. Interestingly, the floret number per ear row vs ear length relationship varied for the reduced red-to-far red ratio by reducing initial $a$ and increasing floret number per ear row. Comparable results have been shown for wheat ($Triticum aestivum$ L.), where reduced red-to-far red ratio accelerated rates of spikelet development but resulted in less fertile spikelets (Ugarte et al., 2010).

The reduction in dry matter accumulation caused by drought or 15 plants m$^{-2}$ resulted in i) a lower threshold dry matter for ear and floret set, and ii) overall higher rates ($\alpha$) of ear elongation and floret appearance per unit dry matter. The increased $\alpha$ value suggested a prioritization of reproductive growth over vegetative growth or possibly, a lower threshold for ear development relative to plant dry matter. No previous research has identified this response in maize to early season stress. This response may be attributed to a larger sensitivity of overall plant dry weight reductions since ear dry weight at this stage is very low as a proportion of overall dry weight (Tetio-Kagho and Gardner, 1988a). This is in contrast to the expectable penalties in either assimilate variation (Edmeades and Daynard, 1979a) or
reproductive partitioning in response to stress around silking (Pagano and Maddonni, 2007; D’Andrea et al., 2008). In addition, normalizing by for temporal variation (i.e. growth per unit time) using a non-destructive approach may account for these differences. For example, Pagano et al., (2007) showed relative floret number development as a function of plant growth rate, however, individual plants were classified by their competitive capacity (dominant vs dominated plants) rather than by the stress applied. In addition, reduced number of florets in response to drought around silking has been reported recently in Rossini et al., (2016), however, the drought applied in our study occurred during the pre-silking stage. This reduction in floret number and plant dry matter caused by severe drought would invariably change the rates of pistil development and subsequent silk emergence (Carcova et al., 2003; Boyer and Westgate, 2004; Fuad-Hassan et al., 2008).

2.6. Conclusions

Three distinct stresses were applied early in plant development (e.g., V7-V10) to generate variation in plant growth. From these responses we demonstrated that floret number was related to cumulative growth per plant early in development. This relationship followed the classic curvilinear response, with a threshold level of plant dry matter accumulation and a plateau. As expected stress during the early developmental stages affected the association between floret number and plant dry matter, however, not all stresses had the same impact on this relationship. Light quality defined as reduced red to far red ratio did not affect dry matter
accumulation, while both drought and plant density reduced dry matter accumulation. Of the stresses applied in this experiment, drought was the most severe in reducing dry matter accumulation. As a result, it was the only stress to reduce floret number.

Only the drought effect resulted in a reduction in floret number, but it was a considerably more severe stress than the others imposed in this study. Neither light quality nor plant density reduced floret number in an absolute or per unit dry matter. These results confirm that floret number in maize is established well in advance of flowering and also suggests that floret number is related to plant dry weight sampled between V7 to V9-10 stage of growth. This research presents new knowledge on the relationship between floret number, plant cumulative dry matter and early season stress.
Chapter 3. Dissection of Physiological Mechanisms in Response to Early Season Stress in Maize (*Zea mays* L.)

3.1. Abstract

The underlying mechanisms of yield loss caused by stresses which occur prior to flowering in maize (*Zea mays* L.) are poorly understood. In this study we tested the hypothesis that if yield is reduced in response to early season stress, then resource capture and resource utilization will be reduced proportionally. Specifically, we expected reductions in dry matter accumulation, growth rates around silking (resource capture), kernel number and, harvest index (HI) (resource utilization) as well as a lengthening in the anthesis to silking interval (ASI). Experiments were conducted during 2012-13 under field conditions at two locations in Ontario, Canada. Early season stresses (i.e., up to V9 stage of growth) consisted of drought, light quality and early high-plant density. Yield loss in the drought and early high-plant density stress treatments, was caused by reductions in dry matter accumulation and kernel number. Growth rates around silking were reduced and flowering delayed in response to early season stress which explained reductions in kernel set. Preflowering dry matter accumulation at V9-10 stage was reduced and explained reductions in growth rates around silking as well as delays in flowering. While ASI was only lengthened by early high density, HI remained unchanged in response to early season stress. Overall, early season stress negatively affected resource capture but not resource utilization.
3.2. Introduction

Physiological mechanisms underlying yield loss are explained by reductions in resource capture and resource utilization in maize (Zea mays L.). For example, at the crop level, resource capture is related with seasonal dry matter accumulation, while resource utilization is related to the proportion of this dry matter that is allocated to yield, i.e., harvest index (HI) (Tollenaar and Wu, 1999; Tollenaar and Lee, 2006). HI can be further dissected into kernel number and kernel weight. Kernel number, however, is the yield component that best explains variation in yield (Borrás et al., 2004) and is positively correlated with rates of dry matter accumulation during the 30-day period around silking (Tollenaar et al., 1992; Andrade et al., 1999). Therefore, reductions in the rate of dry matter accumulation caused by stress around silking will irreversibly reduce kernel number (Kiniry and Ritchie, 2010). This response to stress has been reported for supra-optimal densities (Vega et al., 2001a), lack of water and nutrients (Otegui et al., 1995; Andrade et al., 2002) and shading (Reed et al., 1988). Clearly, such stresses which impact seasonal dry matter accumulation, the period around silking, kernel set, or HI will be detrimental for grain yield.

Variations in kernel number are associated with flowering synchrony. For example, the interval between anthesis (pollen shed) and silks appearance (female flower) provides a measure of synchronicity of floret fertilization (Bolaños and Edmeades, 1996). The shorter this interval, the more synchronous fertilization of florets is expected to be, resulting in more yield at maturity. Among many examples of stresses that lengthen the anthesis-to-silking
interval (ASI) are drought (Fuad-Hassan et al., 2008), supra-optimal plant density (Borrás et al., 2009a), and nutrient stress (Boomsma et al., 2009). Lengthening of the anthesis-to-silking interval or delayed flowering is a typical indicator of stress occurring around the flowering period.

Stresses occurring before the critical silking period (i.e., emergence to anthesis) can also cause yield reductions. For example, yield has been shown to be reduced by pre-silking drought (Lorens et al., 1987; NeSmith and Ritchie, 1992a), intraspecific competition (Pagano and Maddonni, 2007), weed competition (Hall et al., 1992; Cox et al., 2006), and nutrient deficiency (Rossini et al., 2011). From these studies, however, only Pagano and Maddonni, (2007) and Rossini et al., (2011) reported kernel number and its association with rates of dry matter accumulation around silking. In addition, Lorens et al., (1987) and NeSmith and Ritchie, (1992), reported HI and reduced kernel number at maturity in response to pre-anthesis drought. Kernel number is also known to be reduced in response to early season stress caused by weed competition (Evans et al., 2003; Cerrudo et al., 2012). In these studies, however, quantification of growth rates around silking was not reported. Integrated information regarding flowering dynamics, kernel set, dry matter accumulation, and harvest index in response to early season stress (pre-anthesis) is seldom found in the literature.

The goal of this study was to assess the effect of three distinct sources of stress applied before silking (emergence to V9-V10 of growth) on seasonal dry matter accumulation, HI, kernel number and ASI. These three stresses were drought, reduced red-to-far red ratio, and
plant density of 15 thinned to 7.5 plants m$^{-2}$ at V9 (early high-density). The drought was designed to impair resource acquisition directly, early high-density had the purpose of imposing intraspecific competition and the reduced red-to-far red ratio reflected from neighboring weeds was intended to be an example of resource independent plant competition (Harper, 1977; Kasperbauer and Karlen, 1994; Page et al., 2009). We tested the hypothesis that if yield is reduced in response to early season stress, then, resource capture and/or resource utilization will be reduced proportionally. Specifically, we expected reductions in dry matter accumulation at maturity, growth rates around silking (resource capture), kernel number and, HI (resource utilization) as well as a lengthening in the ASI.
3.3. Materials and methods

3.2.1. Experiment #1 – Drought and Light Quality Stress

The experiment was grown in the field hydroponic system at Arkell Research Station (-43°54´ N, 80°18´ W and 325 m above the sea level) near Guelph, Ontario, Canada during the 2012 and 2013 growing seasons. The field hydroponic system consisted of 22-L plastic pails that were 28 cm diameter and were filled with a baked clay medium, MVP Turface®. Irrigation occurred at least four times per day using a nutrient solution described in Tollenaar, (1989). The concentrated nutrient solution was stored in two 340-L sealed plastic barrels of water. The nutrient solution consisted of two components: the first component was 13.6 kg of 28–14–14 (N, P₂O₅, and K₂O), and 7 kg of NH₄NO₃; the second consisted of 1.2 kg micronutrient mix and 13.6 kg MgSO₄N₇H₂O. The concentrated nutrient solution was then diluted with water at a dilution ratio of 1: 100 to deliver approximately 0.12 g N, 0.06 g P, and 0.09 g K per 500 ml of nutrient solution. On a daily basis, the pH of the nutrient solution was adjusted through the addition of HCl to ensure values of 6.5 to 6.8 in order to avoid precipitation of components caused by Ca concentration in the water (>100 g kg⁻¹). Each pail was supplied with four fertigation tubes that each delivered a minimum of 100 ml of nutrient solution over a 10-min time period. This field hydroponic system has been used for over three decades to enable maize growth from seedling to maturity in the field under controlled water and nutrient conditions (Tollenaar and Migus, 1984; Earl et al., 2012; McKenzie-Gopsill et al., 2016).
3.2.2. Experiment #1 Treatments

Prior to the initiation of this experiment, 22 L pails were filled with Turface® and then covered with a clear plastic sheet that remained in place throughout the growing season. A 15-cm diameter hole was cut in the center of the plastic sheet. Each pail was hand sown with four seeds at a depth of 4 cm and thinned to two plants per pail at the 4th leaf-tip stage (ca. V1-2, Hanway, 1966) resulting in a final plant density of 8 plants m⁻² in all treatments. Planting dates were 10th May 2012 and 17th May 2013.

Three genotypes were used in this study, CG108/CG102, X58945WP and SK5069WP. CG108/CG102 is a hybrid developed by the University of Guelph’s Corn Breeding Program and has been used in previous studies (e.g., Lee et al., 2000, 2001). X58945WP and SK5069WP are isoline hybrids from Syngenta that were classified as either a non-drought tolerant hybrid (NDT; X58945WP) or as a drought tolerant hybrid (DT, SK5069WP) (Agrisure® Artesian® Syngenta Crop Protection Inc.). For further details on the isoline hybrids, see Reid et al. (2014). Treatments were established by filling the area surrounding the maize seedlings above the plastic sheet with either Turface® (control and drought treatments) or with turfgrass sod (Lolium perenne L.) which acted as a surrogate weed.
### 3.2.3. Light quality treatment

For the light quality treatment, maize seedlings and turfgrass were fed by separate fertigation lines, and the design (plastic sheet separation) prevented any root or shoot contact of corn with the turfgrass. The turfgrass was maintained at a height of 10 cm by manual clipping to prevent direct shading of maize seedlings. The light quality environment provided by the two treatments was characterized when the controls reached the 5-leaf-tip stage. The red-to-far red ratio of light reflected 10 cm above the ring of turfgrass or Turface® bordering the maize seedlings centered within the pail was measured using a dual channel red-to-far red ratio sensor (SKR 110, 660 nm/730 nm, Skye Instruments, Llandrindod Wells, Powys, UK). These measurements were recorded on sunny, cloudless days between 0900 and 1300 hrs. The presence of the turfgrass resulted in a reduced red-to-far red ratio treatment of 0.37 compared to 0.86 in the weed free control.

### 3.2.4. Drought treatment

In order to ensure the effectiveness of the drought treatment, the developing seedlings within each pail were wrapped with additional plastic in order to cover the 15 cm diameter hole in the plastic, thereby preventing naturally occurring rainfall from seeping into the base of the pail. The reduction in water availability for the drought treatment mean water holding capacity was determined in four pails watered up to saturation. Difference between saturation and drained weight yielded available water (Table B8.1). The weight which represented the
difference between saturation and drained water was used as the reference 100%. Two random pails per replication (six pails total) were selected to track the weight variation upon reductions in irrigation throughout the drought stress period. Pails were kept at constant weight once the drought stress symptoms (rolled leaves) were evident. During 2012 and 2013 available water as a percent of maximum water weight (100%=10 kg water per pail) was reduced by 65% and 85% from planting up to the 12th leaf-tip stage of the control respectively (Table B8.1). During both years those levels were sufficient to reduce plant dry matter accumulation although the decrease in available water was substantially higher during 2012. Each pail was irrigated individually with a graded glass of 100:1 water: solution to target available water (65% and 85%) every two day maximum (Table B8.1). This aimed to maintain the % of water in the set of pails corresponding to the drought stress. Once the 12th leaf tip stage of the CG108/CG102 control was attained full irrigation was restored to allow plants recovery.

3.2.5. Experiment #1 Experimental design

The experimental design was a split-plot with three replications. Main plots were assigned to control (i.e., well-watered throughout the entire experiment), drought (i.e., drought until the 12th leaf tip stage, then well-watered throughout the entire experiment) and reduced red to far red ratio (i.e., presence of turfgrass from emergence to the 12th leaf-tip stage, turfgrass then removed). Subplot treatments were genotypes, CG108/CG102,
X58945WP and SK5069WP. Both the drought and reduced red-to-far red ratio were imposed until the 12th leaf-tip stage or V9 (i.e., nine visible collars) to ensure that the end of the floret differentiation period had been reached. Note that the occurrence of the 12th leaf-tip stage corresponded to the stage of development recorded in the control for CG108/CG102.

Main plot (control, drought, reduced red-to-far red ratio) size was 26 22-L pails long by four pails wide for a total of 104 pails and a total area of 9.1 m by 2.8 m (0.35 between the center of the two pails and 0.70 m between rows). Subplots (genotypes) were eight pails long by four pails wide (2.8 m by 2.8 m, 32 pails in total). From the two central rows of pails, six pails from each treatment were used for destructive measurements while four pails were left for measurements and final harvest. The remaining four pails were used to perform non-destructive data collection around silking and destructive sampling at maturity. The outer two rows of pails were used as borders. The purpose of this experiment was to evaluate the effect of single stresses on yield and its components.

3.2.6. Experiment # 2 – Plant Density Stress

This experiment was conducted at the Elora Research Station, Elora, ON (43°38´ N, 80°25´ W, 380 m above sea level). The experimental design was a split plot with four replications. Main plots consisted of plant densities and subplots were assigned to genotypes. The soil type was a London loam soil (Aquic Hapludalf) with tile drainage and soil organic
matter content of 3.8 to 4.0%. Nitrogen, P\textsubscript{2}O\textsubscript{5}, and K\textsubscript{2}O were applied before planting at rates of 150, 85, and 50 kg ha\textsuperscript{-1}, respectively. Soybean was the previous crop during both years.

Weed control was obtained using a pre-plant tank mix of S-metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)]\textsubscript{-},(S)], atrazine [6-chloro-N-ethyl-N’-(1-methylethyl)-1,3,5-triazine- 2,4-diamine] and mesotrione [2-[4-(methylsulfonyl)-2-nitrobenzoxy]-1,3-cyclohexanedione] at rates of 1.6, 1.28, and 0.14 kg a.i. ha\textsuperscript{-1}, respectively. Planting occurred on 10\textsuperscript{th} May 2012 and on 14\textsuperscript{th} May 2013. The seeds were planted with a precision planter ALMACO (Allan Machine Company, Nevada, IA, 50201, USA) resulting in 6 m long by 3.04 m wide (four rows, 0.76 m between rows) plots.

3.2.7. Early high-density stress

Three density treatments were used in this experiment: continuous conventional density of 7.5 plants m\textsuperscript{-2} (i.e., conventional)(Assefa et al., 2018), 15 plants m\textsuperscript{-2}, which at the 12-13\textsuperscript{th} leaf tip stage (ca. V9) was adjusted to 7.5 plants m\textsuperscript{-2} to relax the density stress (i.e., early high-density), and continuous high density of 15 plants m\textsuperscript{-2}. The experimental design consisted of a split-plot with plant density as the main plots the genotypes (CG108/CG102, X58945WP and SK5069WP) assigned to the sub-plots.
3.2.8. Data Collection

Experiments #1 and #2 pre-silking measurements

In Experiment #1 three consecutive destructive samplings (two pails per subplot per sampling date) were made when the first genotype (CG108/CG102) attained seven collars (V7), followed at V8 and completed when the same genotype attained nine collars (V9). Ear length, florets per ear row and plant dry matter accumulation were recorded for three individual plants per plot per sampling date. In Experiment #2 three destructive samplings were carried out at V7, V8 and V10 stages in each density-genotype combination. Each sampling stage corresponded to the phenological stage of the 7.5 plant m$^{-2}$ treatment in the CG108/102 genotype. In contrast with Experiment #1, the third sampling was carried out at V10. In this study only data from the sampling of V9 for Experiment #1 and V10 for Experiment #2 is shown. For dry matter measurements, individual sampled plants were dried at 85°C for at least a week until constant weight was achieved.

Experiments #1 and #2 critical period around silking measurements

Two developmental time points were used to define the critical period bracketing silking, V14 (i.e., pre-tassel emergence) and V14+25 days (i.e., post-silking). In both experiments individual plants were selected to perform non-destructive measurements and estimate dry weights pre-tasseling and post-silking. In Experiment #1, at V14 stage five consecutive plants per plot were selected for destructive sampling and an additional eight plants per plot for
destructive sampling at maturity. Similarly, in Experiment #2, at V14 stage of growth, 20 consecutive plants per plot were selected for destructive sampling and an additional 20 consecutive plants per plot were identified for destructive sampling at maturity. Stem diameter at five cm above ground level (Vega et al., 2001a) and plant height, from ground level to the last stem ligule, were measured on all plants and in both experiments. Once these measurements were completed, plants designated for destructive sampling were manually harvested, bagged and dried at 85°C until constant weight. This measurement and sampling procedure were carried out a second time at V14+25 days in both experiments. This methodology was followed in order estimate individual plant dry matter pre-tasseling and post-silking without requiring destructive sampling. This is a widely used technique (Vega et al., 2001b; Borrás et al., 2009a; Gonzalez et al., 2018) and allowed us to calculate rates of growth around the silking period.

In both, Experiment #1 and #2 flowering parameters such as days to anthesis, silking and anthesis to silking interval were recorded in the individual tagged plants. Plants were examined every day to record days from first appearance of tassel or silk. The anthesis to silking interval was calculated as the difference between days to silking and days to anthesis for each plant. Data is presented as plot averages and at individual plant level.
Experiments #1 and #2 maturity measurements

Following physiological maturity (black layer), the previously identified eight and 20 plants in Experiments #1 and #2 respectively, were destructively sampled in each treatment. Each plant was then separated into stem, leaves and ears. The ear was shelled in order to record kernel number and individual plant grain yield. All plant components, i.e., stem, leaves, ear and kernels were dried at 85°C to a constant weight. Plant kernel number and dry matter were then calculated on a per plant basis. The purpose of this sampling procedure was to relate final kernel number with individual plant growth rates around silking in the first five plants of each set. In addition, plot yield, kernel number, and dry matter were calculated by the sum of each attribute in a total of eight and 20 plants per plant in Experiments #1 and #2 respectively divided by the area collected in each treatment combination.

3.2.9. Statistical analysis – both experiments

Main treatments effects were analyzed using mixed linear models. The SAS PROC MIXED procedure (SAS Institute Inc., 2013, Cary, NC, USA, Version 9.4) was used to generate the ANOVA random and fixed effects coefficients and their p-values for each experiment. Year, replications, and plots were assigned as random effects. Fixed effects were assigned to controls (Stress free control at Arkell and 7.5, 15 plants m⁻² at Elora), and treatments (drought, reduced red-to-far red ratio and 15 thinned to 7.5 plants m⁻²) and genotypes as sub-factors (Eq.[3.1]).
Eq. [3.1] = Y_{gsyrp} = \mu + \alpha_s + \beta_g + Y_y + \rho_r (Y_y) + \sigma_p(\rho_r) + \alpha_s\beta_g + \epsilon_{gsyrp}

Where Y_{gsyrp} represents the phenotype of treatment s (drought, reduced red-to-far red ratio, 15 thinned to 7.5 plants m\(^{-2}\), control, 7.5 and 15 plants m\(^{-2}\)) in the genotype g in year y in plot p and the replicate r. The \(\mu\) term represents the overall mean of the phenotype, \(\alpha_s\) the stress effect (and controls); \(\beta_g\) the genotypic effect; \(Y_y\) is the year effect; \(\rho_r (Y_y)\) is the replicate effect nested within year; \(\sigma_p(\rho_r)\) is the effect of the plot within replicates; \(\alpha_s\beta_g\) is the interaction between the genotype g and the treatment s; and \(\epsilon_{gsyrp}\) is the residual error of the stress s at the genotype g in year y replicate r and plot p. Means were calculated as best linear unbiased estimators and pre-planned contrasts were used to determine significance at the 0.05 level.

To establish the relationship between dry matter and the allometric measurements (stem diameter and plant height) dry matter collected at V14 and V14+25 days was regressed against the product of stem diameter and plant height (Eq. [3.2]). Regressions parameters and their confidence intervals were compared in order to test differences among regressions (Table 3.1).

Eq. [3.2] = Y = \alpha + \beta (X)
Where Y is plant dry matter pre-tasseling or post-silking, $\alpha$ is the intercept value for Y, and $\beta$ is the increase in grams of plant dry weight per unit X which reflects the product of diameter by height per plant. Plant growth rates around silking were calculated as the difference between estimated plant dry matter post-silking and estimated plant dry matter pre-tasseling divided by the number of days between each period. Fitted linear equations, standard error and coefficients of determination were obtained using PROC REG procedure of (SAS © Institute Inc., Cary, NC, USA, Version 9.4).
Table 3.1. Allometric model parameters (\(a, \beta\)) for the plant dry matter vs diameter x height relationship during the pre-tasseling to post-silking period (i.e., V14 and V14+25 days). Parameters correspond to Eq.[3.2]. Experiments conducted at Elora Research Stations, during the 2013-13 growing seasons. Ontario, Canada.

<table>
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<th>Year</th>
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<th>Source of variability</th>
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<th>Coefficient ± standard error</th>
<th>(R^2)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(\alpha)</td>
<td>(\beta)</td>
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<td>2012</td>
<td>Pre-tasseling</td>
<td>Control, Drought, R:FR‡, 7.5, 15-7.5§, 15 plants m(^{-2})</td>
<td>5(^{th}) July</td>
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<td>2013</td>
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<td>23(^{rd}) July</td>
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<td></td>
<td>Post-silking</td>
<td>Control, Drought, R:FR, 7.5, 15-7.5, 15 plants m(^{-2})</td>
<td>17(^{th}) August</td>
<td>221</td>
<td>-33**±10</td>
<td>0.04***±0.002</td>
</tr>
</tbody>
</table>

*\(p<0.05\), **\(p<0.01\), ***\(p<0.001\). †ns: not significant. ‡R:FR: reduced red-to-far red ratio. §15 thinned to 7.5 plants m\(^{-2}\) at the V9 stage of growth. Mid-silking dates were 19\(^{th}\) and 30\(^{th}\) July during the 2012 and 2013 growing seasons respectively.
Two types of non-linear equations were used to study the response variables. The first type described the relationship between grain yield, harvest index, kernel number, plant growth rate around silking (i.e., pre-tasseling to post-silking period), days to flowering, and plant dry matter at maturity (Eq.[3.3]). The second type described the relationship between flowering parameters such as plant growth rates around silking and days to flowering with plant dry matter at V10 (Eq.[3.4]).

\[
Eq\ [3.3] = Y = \begin{cases} \alpha(X - X_0) & [X > X_0] \\ \frac{\alpha(X - X_0)}{1 + \beta(X - X_0)} & [X < X_0] \end{cases}
\]

\[
Eq. [3.4] = Y = \alpha e^{\beta X}
\]

The non-linear model represented in Eq.[3.3] exhibits three coefficients which are biologically meaningful, that is, the \( \alpha \), \( X_0 \) and \( \beta \) parameters (Vega et al., 2000; Sarlangue et al., 2007; Gonzalez et al., 2018). The \( X_0 \) value is a location parameter (Archontoulis and Miguez, 2015) which represents the interception of the Y variable with the X variable if \( X > 0 \). In our study the \( X_0 \) represents the threshold growth rate for kernel establishment, dry matter for yield or the threshold delay in flowering for kernel set. In the kernel number vs days to either anthesis or silking the location parameter be a maximum threshold, i.e., \( X < X_0 \). The \( \alpha \) slope represents the initial response of the dependent value (kernel number, yield). The \( \beta \) value is the degree of curvature of the relationship. In Eq.[3.4] \( \alpha \) represents the intercept and the term \( e^{\beta X} \) estimates the decay of Y per unit X relative to maximum Y. All regressions
were fitted for the whole data set; however, data is shown only for early drought, reduced red to far red and early high density with the corresponding coefficient of determination. In the supplemental section is shown the complete data set figures with the corresponding coefficients of determination (Table A8.2, A8.3, Fig. A8.1, A8.2, A8.3). Each parameter was assessed at a probability level of 0.05 to test the hypothesis of significance within the equation. Non-linear regressions were fitted using PROC NLIN procedure (SAS ® Institute Inc., Cary, NC, USA, Version 9.4).
3.3. Results

3.3.1. Maturity

Grain yield and its components were affected differently by early season stress. Grain yield, dry matter accumulation at maturity and kernel number were reduced by the drought stress in Experiment #1 (Table 3.2). Exposure to a reduced red-to-far red ratio reflected from neighboring weeds from emergence to the V9 stage of growth did not affect grain yield at maturity. In Experiment #2, grain yield, dry matter accumulation, harvest index (HI), and kernel number varied with plant density. Genotypic variation was only observed in Experiment #2, for HI and kernel number (Table B8.4). Across both experiments, there were no significant treatment by genotype interactions, meaning that for all traits genotypes responded to treatments in a similar manner. Therefore, the data presented was pooled across genotypes.

Grain yield was reduced in response to early season stress, with a 46% reduction due to the drought stress and a 27% reduction in early high-density stress (Table 3.2). Similar to grain yield, dry matter at maturity was reduced by 48% in the drought and 27% in the early high-density stress treatment. In addition, the early high-density stress showed 28% less dry matter relative to the continuous high-density. Harvest index (HI), the proportion of dry matter partitioned to the grain, remained unchanged in both the drought and the early high-density stress treatments. In contrast, kernel number, was reduced in both the drought (44% reduction) and early high-density stress (18% reduction). In addition, the early high-density
stress treatment exhibited 22% less kernels than the continuous high-density treatment. The effect on yield of drought and the early high-density stress differed in magnitude. Drought was more severe than the early high-density, however, they both reduced dry matter at maturity and kernel number.
Table 3.2. Mixed model analysis and contrast comparisons for grain yield, dry matter accumulation at maturity, harvest index, and kernel number for control, early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (EHD, 15 thinned to 7.5 plants m$^{-2}$), conventional density (CD, 7.5 plants m$^{-2}$), and high density (HD, 15 plants m$^{-2}$). Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Station during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Contrasts</th>
<th>Grain yield</th>
<th>Dry matter accumulation at maturity</th>
<th>Harvest index</th>
<th>Kernel number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g m$^{-2}$</td>
<td>g g$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contrasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>1266 vs 2422 ***</td>
<td>0.51 vs 0.50ns</td>
<td>2427 vs 4308***</td>
</tr>
<tr>
<td>1</td>
<td>Drought vs Control</td>
<td>655</td>
<td>1266 vs 2422 ***</td>
<td>0.51 vs 0.50ns</td>
<td>2427 vs 4308***</td>
</tr>
<tr>
<td></td>
<td>R:FR vs Control</td>
<td>1212</td>
<td>2348 vs 2422 ns</td>
<td>0.51 vs 0.50ns</td>
<td>4238 vs 4308ns</td>
</tr>
<tr>
<td></td>
<td>Drought vs R:FR</td>
<td>655</td>
<td>1266 vs 2348***</td>
<td>0.51 vs 0.50ns</td>
<td>2427 vs 4238***</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td></td>
<td>102</td>
<td>0.01</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>EHD vs CD</td>
<td>685</td>
<td>1371 vs 1872 ***</td>
<td>0.49 vs 0.50ns</td>
<td>3013 vs 3671***</td>
</tr>
<tr>
<td></td>
<td>EHD vs HD</td>
<td>685</td>
<td>1371 vs 1898 ***</td>
<td>0.49 vs 0.34***</td>
<td>3013 vs 3866***</td>
</tr>
<tr>
<td></td>
<td>HD vs CD</td>
<td>754</td>
<td>1898 vs 1872 ns</td>
<td>0.34 vs 0.50***</td>
<td>3866 vs 3671ns</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td></td>
<td>50</td>
<td>0.02</td>
<td>241</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>ANOVA</th>
<th>$Pr &gt; F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress (S)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
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<tr>
<td>Genotype (G)</td>
<td>0.0762</td>
<td>0.1259</td>
</tr>
<tr>
<td>SxG</td>
<td>0.397</td>
<td>0.2243</td>
</tr>
<tr>
<td>Density (D)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>0.5107</td>
<td>0.9025</td>
</tr>
<tr>
<td>DxG</td>
<td>0.1476</td>
<td>0.3272</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant.
To examine this relationship between stress caused by drought or early high-density on plant dry matter and grain yield, individual plant data was analyzed separately (Fig. 3.1a and 3.1b). Grain yield and HI were positively associated with dry matter (Fig. 3.1a, b), with a dry matter threshold \( X_0 \) for barrenness of 70 g plant\(^{-1} \) (Table 3.3). Plants that attained larger values than this threshold, showed an \( \alpha \) of 1 g g\(^{-1} \) with a curvilinear response at large values of plant dry matter \( (\beta=0.0022) \). Harvest index (HI), exhibited values of 0.51 g g\(^{-1} \) across a wide range of plant dry matter values \( (R^2=0.41, \text{Fig. 3.1b}) \). In plants that exhibited 100 g or less, however, a steep decline in HI was observed \( (\alpha=0.12) \). Reduced yield was explained by reductions in dry matter while HI remained unchanged in response to early season stress.

Individual plant values showed a similar distribution for both grain yield and harvest index. For example, values below the median, which is the central value of plant dry matter values \( (205 \text{ g plant}^{-1}) \) corresponded mostly to the drought and early density treatments. In contrast, dry matter values above the median corresponded to the reduced red to the far-red ratio treatment. Dry matter reductions resulted in less yield in response to drought and early high-density stress.
Figure 3.1. Relationships between (A) grain yield or (B) harvest index with plant dry matter at harvest among the early drought stress (empty circles), early reduced red-to-far red ratio (R:FR) (closed circles) stress, or early high-density stress (15 thinned to 7.5 plants m$^{-2}$) (grey circles) treatments. Non-linear regression parameters are described in table 3.3. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.
Table 3.3. Equation model parameters \((\alpha, X_0, \beta)\) for the relationship between plant dry matter at maturity and either plant grain yield or harvest index for early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (15 thinned to 7.5 plants \(m^{-2}\)). Parameters correspond to Eq.[3.3]. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>(n)</th>
<th>(\alpha) Parameter ± standard error</th>
<th>(X_0) Parameter ± standard error</th>
<th>(\beta) Parameter ± standard error</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield vs plant dry matter at maturity</td>
<td>298</td>
<td>1.02*** ±0.05</td>
<td>70.2***±2.53</td>
<td>0.0022***± 0.0003</td>
<td>0.94</td>
</tr>
<tr>
<td>Harvest index vs plant dry matter at maturity</td>
<td>300</td>
<td>0.12**±0.03</td>
<td>70***±0.66</td>
<td>0.23**± 0.05</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant.
3.3.2. Critical period around silking

Growth rates around silking were reduced and days to flowering (i.e. anthesis and silking) were increased in response to early season stress. In Experiment #1 crop growth rates around silking were reduced by drought and reduced red to far red ratio. In Experiment #2 the early high-density showed also reduced growth rates (Table 3.4). The drought and reduced red-to-far red ratio treatments showed a 25 and 11% decrease in growth rate around silking relative to the non-stressed control. Growth rates around silking of the early high-density treatment were 34% lower than the continuous conventional density treatment and 53% lower than the continuous double density treatment. The magnitude of the reduction in growth rates around silking was larger for the drought followed by the early high-density and reduced red to far red ratio.

Days to anthesis, and days to silking were increased in response to drought and high-density stress but remained unchanged in the reduced red to far red ratio treatment. Anthesis-to-silking interval (ASI) did not vary in Experiment #1; however, in Experiment #2 the early high-density showed a shorter ASI relative to the continuous high-density (2 vs 5.2 days) (Table 3.4). There was no significant stress by genotype interaction in any of the experiments (Table B8.4). Delays in anthesis in the drought and the early high-density were of 5 and 2 days relative to unstressed controls and the conventional density respectively. Similarly, silking was delayed by 5 and 2 days in the drought and the early high-density relative to the control and the conventional density. Early season stress, mainly drought and early high-
density, resulted in reduced growth rates around silking in all three stresses and delayed flowering in drought and early high density; increasing ASI only in the early high-density stress.
Table 3.4. Mixed model analysis and contrast comparisons for crop growth rate at silking, days to anthesis, days to silking, anthesis-to-silking interval for control, early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (EHD, 15 thinned to 7.5 plants m\(^{-2}\)), conventional density (CD, 7.5 plants m\(^{-2}\)), and high density (HD, 15 plants m\(^{-2}\)). Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Station during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Contrasts</th>
<th>Crop growth rate around silking</th>
<th>Days to anthesis</th>
<th>Days to silking</th>
<th>Anthesis to silking interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g m(^{-2}) day(^{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Drought vs Control</td>
<td>28 vs 37***</td>
<td>75 vs 70***</td>
<td>77 vs 72***</td>
<td>2 vs 2.9ns</td>
</tr>
<tr>
<td></td>
<td>R:FR vs Control</td>
<td>33 vs 37**</td>
<td>70 vs 70ns†</td>
<td>72 vs 72ns</td>
<td>2.2 vs 2.9ns</td>
</tr>
<tr>
<td></td>
<td>Drought vs R:FR</td>
<td>28 vs 33***</td>
<td>75 vs 70***</td>
<td>77 vs 72***</td>
<td>2 vs 2.2ns</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td>1.8</td>
<td>0.89</td>
<td>0.82</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>EHD vs CD</td>
<td>24 vs 36***</td>
<td>74 vs 72**</td>
<td>76 vs 74**</td>
<td>2 vs 1.8ns</td>
</tr>
<tr>
<td></td>
<td>EHD vs HD</td>
<td>24 vs 50***</td>
<td>74 vs 74ns</td>
<td>76 vs 79**</td>
<td>2 vs 5.2***</td>
</tr>
<tr>
<td></td>
<td>HD vs CD</td>
<td>50 vs 36***</td>
<td>74 vs 72***</td>
<td>79 vs 74***</td>
<td>5.2 vs 1.8***</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td>1.7</td>
<td>0.25</td>
<td>0.44</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Source of variation

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Pr &gt; F</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Stress (S)</td>
<td>&lt;.0001</td>
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<tr>
<td>Genotype (G)</td>
<td>0.127</td>
</tr>
<tr>
<td>SxG</td>
<td>0.439</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Density (D)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>0.004</td>
</tr>
<tr>
<td>DxG</td>
<td>0.696</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant
3.3.3. **Kernel number, growth rates around silking and flowering dynamics**

Plant kernel number was related positively to growth rates around silking and related negatively to days to flowering. For the kernel number vs growth rate around silking relationship ($R^2=0.61$; Fig. 3.2a) the threshold growth rate ($X_0$) was 1.8 g day$^{-1}$ (Table 3.5). Increases in kernel number occurred from 1.8 up to 4 g day$^{-1}$ as reflected by the steep slope ($\alpha =620$ kernels g$^{-1}$day$^{-1}$). In this initial slope, plant growth rates corresponded mainly to the drought and the early high-density treatments. In contrast, growth rates larger than 3 g day$^{-1}$ were for the reduced red-to-far red ratio treatment. At growth rates of more than 4 g day$^{-1}$ kernel number ranged from 450 to 650 kernels plant$^{-1}$. Drought and early high-density caused a reduction in growth rates around silking that resulted in reduced kernel number.

Delays in anthesis and silking per plant were related negatively to kernel number per plant. Delays from approximately 60 days after planting resulted in less kernels per plant. Rates of decline ($\alpha$) were of -80 and -90 and kernels day$^{-1}$ for either anthesis and silking respectively (Table 3.5). These rates, however, were not linear ($\beta$ term of -0.07 and -0.1 for anthesis and silking respectively). Individual plants that required 80 days or more after planting to reach anthesis (threshold $X_0$), were predicted to be barren ($R^2=0.51$, Fig. 3.2, b). Similarly, individuals that showed days to silking of 85 days after planting or more, were predicted to show an absence of kernels ($R^2=0.64$, Fig. 3.2c). Reduced kernel number in response to drought and early high-density was related to reductions in plant growth rate and delays in flowering.
Figure 3.2. Relationship of kernel number per plant as function of (A) plant growth rate around silking, (B) days to anthesis and (C) days to silking per plant for early drought stress (empty circles), early reduced red-to-far red ratio (closed circles) stress, or early high-density stress (grey circles) treatments. Non-linear regression parameters are described in table 3.5. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.
Table 3.5. Equation model parameters ($\alpha$, $X_0$, $\beta$) for the relationship between kernel number per plant and plant growth rate around silking, days to anthesis, and days to silking for early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (15 thinned to 7.5 plants m$^{-2}$). Parameters correspond to Eq.[3.3]. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>n</th>
<th>Parameter ± standard error</th>
<th>$\alpha$</th>
<th>$X_0$</th>
<th>$\beta$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel number vs plant growth rate around silking‡</td>
<td>296</td>
<td>750***±115</td>
<td>1.8***±0.1</td>
<td>1± 0.24†</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Kernel number vs days to anthesis§</td>
<td>300</td>
<td>-80***±1.6</td>
<td>80***±0.2</td>
<td>-0.07***± 0.004</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Kernel number vs days to silking</td>
<td>300</td>
<td>-90***±9</td>
<td>85***±1.6</td>
<td>-0.1***± 0.02</td>
<td>0.64</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant. ‡ units of g plant$^{-1}$ day$^{-1}$. §Units of days.
3.3.4. Pre-silking stage

Plant dry matter, ear length and the number of florets per ear row were affected in varying degrees by drought and early high-plant density. Plant dry matter at V9-10 stage of growth was reduced by drought and early high-density stress. The interaction of stress by genotype was not significant (Table 3.6). Plant dry matter was reduced 53% in the drought stress and 27% in the early high-density relative to the control and conventional continuous density respectively. The early high-density, however, showed a 12% more plant dry matter than the continuous high-density. Reductions in ear length were only significant in Experiment #2 with the ear length of the early high-density exhibiting 18% less than conventional continuous density. Floret number was only affected in Experiment #1. Floret number per ear row was reduced by 15% in the drought stress relative to the control. Of the components measured at the pre-silking stage the reduction in plant dry matter caused by drought and early high density best explained the reductions in growth rates around silking and delays in flowering.
Table 3.6. Mixed model analysis and contrast comparisons for plant dry matter, ear length, and floret number per ear row at the V9-10 stage of growth for control, early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (EHD, 15 thinned to 7.5 plants m\(^{-2}\)), conventional density (CD, 7.5 plants m\(^{-2}\)), and high density (HD, 15 plants m\(^{-2}\)). Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Station during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Experiment#</th>
<th>Contrasts</th>
<th>Plant dry matter at V9-10</th>
<th>Ear length at V9-10</th>
<th>Floret number at V9-10</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g plant(^{-1})</td>
<td>cm</td>
<td>no row (^{-1})</td>
</tr>
<tr>
<td>1</td>
<td>Drought vs Control</td>
<td>38 vs 82***</td>
<td>3.2 vs 4ns</td>
<td>32 vs 37***</td>
</tr>
<tr>
<td></td>
<td>R:FR vs Control</td>
<td>72 vs 38ns†</td>
<td>3.6 vs 4ns</td>
<td>37 vs 37ns</td>
</tr>
<tr>
<td></td>
<td>Drought vs R:FR</td>
<td>38 vs 72***</td>
<td>3.2 vs 3.6ns</td>
<td>32 vs 37***</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td></td>
<td>2.3</td>
<td>0.31</td>
</tr>
<tr>
<td>2</td>
<td>EHD vs CD</td>
<td>50 vs 69***</td>
<td>2.3 vs 2.8**</td>
<td>35 vs 36ns</td>
</tr>
<tr>
<td></td>
<td>EHD vs HD</td>
<td>50 vs 44***</td>
<td>2.3 vs 2.1ns</td>
<td>35 vs 34ns</td>
</tr>
<tr>
<td></td>
<td>HD vs CD</td>
<td>44 vs 69***</td>
<td>2.1 vs 2.8**</td>
<td>34 vs 36ns</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td>2.9</td>
<td>0.21</td>
<td>1.07</td>
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ANOVA

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<th>P&lt;0.01</th>
<th>P&lt;0.001</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress (S)</td>
<td>&lt;.0001</td>
<td>0.465</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>0.33</td>
<td>0.006</td>
<td>0.904</td>
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<tr>
<td>SxG</td>
<td>0.928</td>
<td>0.128</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density (D)</td>
<td>&lt;.0001</td>
<td>0.003</td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>0.017</td>
<td>&lt;.0001</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>DxG</td>
<td>0.957</td>
<td>0.07</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

*\(p<0.05\), **\(p<0.01\), ***\(p<0.001\). †ns: not significant
Growth rates around silking were related positively to dry matter accumulation during the pre-silking stage; and days to flowering increased with low plant dry matter accumulation. Growth rates around silking increased at rates of 0.11 g day\(^{-1}\) for each increase in 1 g of plant dry matter at V9-10 stage (Fig. 3.3a, Table 3.7). The fitted threshold (\(X_0\)) was not statistically significant nor was the curvilinear term. This would suggest that increases in growth rates around silking caused by enhanced dry matter accumulation during the pre-silking period were best described by a linear relationship.

Plants exhibiting larger cumulative dry matter during the pre-silking phase were more likely to flower early. Days to anthesis were reduced from 82 to 65 days upon increases in dry matter at V9-10 stage (\(R^2=0.60\), Fig. 3.3b). Mean rates of decrease in days to anthesis from its maximum of 82 (\(a\)) were of -0.16 days g\(^{-1}\) from a minimum of approximately 10 g plant\(^{-1}\) (Table 3.7). Similarly, delays in silking were reduced from 84 (\(a\)) to 68 days after planting when mean dry matter increased from 10 to 100 g plant\(^{-1}\) (\(R^2=0.64\), Fig. 3.3c). Mean reductions in days to anthesis were of -0.18 days g\(^{-1}\) of dry matter at V9-10 stage of growth. The median plant dry matter, which represents the central value of the data set, was 49 g plant\(^{-1}\) at the V9 stage of growth. Across traits, mean dry matter values below 49 g plant\(^{-1}\) corresponded mainly to the drought and the early high-density stress. In contrast, mean plant dry matter corresponding to the reduced red-to-far red ratio treatment, were larger than 49 g plant\(^{-1}\) (Fig 3). Cumulative dry plant dry matter during the pre-silking phase, specifically at
the V9-10 stage of growth was related positively with growth around silking and negatively with days to flowering.
Figure 3.3. Plant growth rate around silking (A) days to anthesis (B) and days to silking (C) as a function of plant dry matter at the V9-10 stage for early drought stress (empty circles), early reduced red-to-far red ratio (R:FR) (closed circles), and early high-density stress treatments (closed squares). Each point represents the mean of 5 plants per plot for the independent variable and 3 plants per plot for the dependent variable. Non-linear regression parameters are described in table 3.7. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.
Table 3.7. Equation model parameters ($\alpha$, $X_0$, $\beta$) for the relationship between plant growth rate around silking, days to anthesis, and days to silking with plant dry matter at V9-10 stage of growth for early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (15 thinned to 7.5 plants m$^{-2}$). Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>n</th>
<th>Parameter ± standard error</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant growth rate around silking vs dry matter at V9-10‡</td>
<td>59</td>
<td>$0.11^{**} \pm 0.06$</td>
<td>$0.01ns \pm 0.02$</td>
</tr>
<tr>
<td>Days to anthesis vs dry matter at V9-10§</td>
<td>60</td>
<td>82$^{***} \pm 0.93$</td>
<td>$-0.002^{***} \pm 0.0002$</td>
</tr>
<tr>
<td>Days to silking dry matter at V9-10§</td>
<td>58</td>
<td>84$^{***} \pm 1.1$</td>
<td>$-0.002 \pm 0.0002$</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant. ‡ corresponds to equation 3.3; § corresponds to equation 3.4.
3.4. Discussion

Stress occurring before the critical period around silking reduced grain yield. The impact of stress on physiological mechanisms and yield components underlying grain yield were variable. For example, drought reduced the number of florets per ear row for the period sampled which determined the potential number of kernels. In contrast, in the early high-density and the reduced red to far red stress, floret number was unchanged. Around silking, growth rates were lower for the drought followed by the early high-density and reduced red to far red stress. ASI was lengthened only by the early high-density stress while drought and early high-density showed delays in both anthesis and silking. Therefore, kernel number loss was explained by reduced growth rates around silking along with delayed flowering, particularly in the drought and early high-density stress. Interestingly, despite reduced growth rates and delayed anthesis and silking, the incidence of barrenness was very low (3%) across the data set. This explained, in part, the stability of HI in both the drought and the early high-density treatments when compared to the continuous high-density stress. Furthermore, reduced HI in the continuous high-density stress highlights the different magnitude of early season vs season-long stress. The common yield component that was influenced by all three stresses was dry matter which in turn contributed to reduced grain yield.

This research tested the hypothesis that if yield is reduced in response to early season stress, then resource capture and resource utilization will be reduced proportionally. Specifically, we expected reductions in dry matter accumulation at maturity, growth rates
around silking (resource capture), kernel number, and harvest index (HI) (resource utilization) as well as a lengthening in the anthesis to silking interval (ASI). The severity of the stresses was reflected in the differential reduction in dry matter accumulation and grain yield (Lorens et al., 1987). The severe reduction in dry matter accumulation in the drought stress could be attributed to reductions in leaf area (NeSmith and Ritchie, 1992a) and to reductions in net photosynthesis (Boyer, 1970; Setter et al., 1995). In contrast, the less severe effect of early high density on dry matter could be attributable to reduced leaf area per plant as previously shown by Pagano and Maddonni, (2007). The response of dry matter accumulation to changes in red-to-far red ratio was more variable. The reduced red-to-far red ratio showed no reductions at V9-10 stage of growth (i.e. dry matter). Around silking, growth rates were lower in the reduced red to far red stress, however, no differences were detected at maturity. Similar responses to reduced red-to-far red ratio have been previously reported (Liu et al., 2009; Page et al., 2010b). Full recovery from stress caused by reduced red to far red may be attributed to the continuous supply of water and nutrients provided by our experimental protocol. Throughout the growing season the drought was more severe in terms of reductions in dry matter than the early high-density stress followed by reduced red-to-far red ratio.

Kernel number was reduced while HI remained unchanged in response to early season stress. The stability of HI was attributed, in part, to a low frequency of barren plants and to a proportional reduction of reproductive vs vegetative growth. Few studies have shown this
type of response (Lorens et al., 1987; Moser et al., 2006). In these studies, drought stress applied pre-silking reduced vegetative growth compared to optimal conditions causing HI to remain unchanged or to increase. Similarly, Cerrudo et al., (2012) showed reduced grain yield but no variations or even increases in HI when the crop was exposed to weed competition from emergence to the 10th leaf tip stage of growth. In contrast with season long stresses such as plant density, the balance between supply and demand of assimilates appears to be unaffected by early season stress.

Kernel number related positively with growth rates around silking (Andrade et al., 1999) when stress was applied before silking. Growth rates around silking followed a similar pattern as the dry matter at maturity, that is, lower values for the drought, followed by early density and reduced red-to-far red ratio. In line with HI, drought and the early high-density stresses, did not result in barrenness. Interestingly, growth rates around silking in the early high density were approximately half of the season long high density. This response suggests that reductions in growth rates were a net result of reductions in density and recovery of individual was practically absent after thinning. In addition, the reduction in kernel number caused by early high-density stress did not parallel yield reduction. This suggested, that reduced rates of kernel fill or a shorter duration of grain fill contributed to reduced kernel weight (Johnson and Tanner, 1972) causing yield to be reduced. Apparently, kernel number reductions were attributable, in part, to low growth rates around silking.
Days to anthesis and silking were delayed by early season stress, however, ASI did not vary. In general, ASI is associated with variations in silking (Hall et al., 1982; Bolaños and Edmeades, 1996; DeBruin et al., 2018). In our study, both anthesis and silking were delayed in the same proportion explaining the lack of lengthening in ASI as shown by NeSmith and Ritchie, (1992). Interestingly, ASI was increased in the continuous double density when compared to the early high density and the conventional density. This response suggests that factors driving ASI for early season vs continuous stresses may differ. Furthermore, we found days to silking to be a better predictor of kernel number than ASI. This close association between kernel establishment and time to silking offers support to the notion that late appearing silks are less likely to be fertilized (Bassetti and Westgate, 1993a).

Growth rates around silking and earlier flowering were associated positively to pre-silking dry matter. Plants exhibiting reduced dry matter before silking are likely to have low growth rates throughout the growing season. Similar results were reported by Vega and Sadras, (2003). In their study, however, the magnitude of dry matter and growth rates around silking were lower compared to our study. In addition, our data points represent averages of plant dry matter and not individual data points. In addition, early flowering was related positively to dry matter accumulation. This is similar to reports relating growth rates and rates of silk appearance during later stages of growth (Borrás et al., 2007, 2009a).

In summary, we demonstrated that early season stress affecting yield are chiefly explained by reduced resource capture which negatively impacted growth rates not only
around silking but throughout the growing season. These low growth rates, however, were not accompanied by reductions in resource utilization (HI) or lengthening of the ASI relative to stress free controls. Reduced kernel number in response to growth rates around silking and delayed flowering was explained by irreversible reductions in dry matter accumulation during the pre-silking phase. These results highlight the importance of early season stress on resource capture and utilization in determining yield loss in maize.
Chapter 4: Establishment of Plant Barrenness Under Density Stress in Maize (Zea mays L.)

4.1. Abstract

Plant barrenness in maize (Zea mays L.) has been associated with low plant dry matter accumulation, a condition that arises under high plant population densities. We tested the hypothesis that if an increase in plant density results in a reduction in plant dry matter accumulation leading to barrenness, then, this reduction at maturity will be explained by similar reductions in dry matter accumulation, during the period bracketing silking or the commencement of the grain filling period. Two plant population densities (7.5 and 15 plants m$^{-2}$) were used to create variation in individual plant dry matter accumulation at four stages during the life cycle: vegetative phase, pre-tasseling, post-silking, and at maturity. Higher plant density resulted in reduced plant dry matter, and kernel number, but had no impact on floret number. Plants presenting low dry matter accumulation in each stage of growth exhibited lower floret and kernel number. Barren plants at maturity showed very low or no dry matter accumulation during the grain filling period. In contrast, plants that set more kernels showed an increase in dry matter accumulation during the grain filling, and a subsequent increase in grain yield. These results suggest that the increased frequency of barrenness observed under high-density stress, resulted in reduced dry matter accumulation during the grain filling period on a per plant basis.
4.2. Introduction

Barrenness in maize (*Zea mays* L.) is known to increase when plant population densities go beyond the optimum density (Buren et al., 1974; Tollenaar et al., 1992; Assefa et al., 2018). Reduced barrenness has been one of the most prominent traits in modern density tolerant hybrids (Echarte and Andrade, 2003). The reduction of barrenness at higher plant populations has contributed in part, to a linear increase in grain yield of approximately 149 kg ha\(^{-1}\) year\(^{-1}\) in the last few decades in North America (Duvick, 2005; Assefa et al., 2018). This genetic improvement has also been associated positively with tolerance to stress, yield stability and increased seasonal dry matter accumulation (Tollenaar and Wu, 1999; Tollenaar and Lee, 2002; Di Matteo et al., 2016). The association between crop seasonal dry matter accumulation and the proportion of dry matter allocated to grain yield, i.e., harvest index (HI) underlies the occurrence of barrenness.

Seasonal dry matter accumulation and HI respond differently to increased plant density. Dry matter per unit area increases as plant density increases, however, HI declines. This relationship exists until an optimum density is reached, after which, further increases in dry matter accumulation do not occur and both harvest index and yield are reduced (Hashemi et al., 2005; Assefa et al., 2016). This reduction in crop yield can be accounted for by an increased frequency of individual plants with low or no kernels per plant (i.e., barren plants) (Tollenaar et al., 2006). On a per plant basis, barrenness is known to increase when individual plant dry matter at maturity is reduced. Yield per plant is highly variable or
zero when dry matter at maturity is below approximately 100 g plant\(^{-1}\) (Vega and Sadras, 2003; Andrade and Abbate, 2005; Sarlangue et al., 2007). A primary consequence of crowding stress is the intensification of interplant competition which intensifies the plant to plant variability for several traits such as plant and ear biomass as well as final yield (Borrás et al., 2007; Borrás and Vitantonio-Mazzini, 2018; Gonzalez et al., 2019). Since resources (primarily light) are shared by a larger number of individuals (Tetio-Kagho and Gardner, 1988b) the plant to plant variability associates positively with the establishment of marked competitive hierarchies among plants (Pagano and Maddonni, 2007). As a consequence, less competitive individuals result in poor kernel set causing yield to be reduced (Maddonni and Otegui, 2004). Dry matter in mature barren plants, then, is the result of reduced plant growth rates throughout the growing season (Vega and Sadras, 2003).

Extensive information is available for plant growth rates during the vegetative (Pagano and Maddonni, 2007), and plant and ear growth rates around silking stages under density stress (Andrade et al., 1999; Echarte et al., 2004). The driving mechanism determining kernel set is ear growth and the proportion of this growth relative to overall plant growth (i.e. partitioning). Therefore, increased plant densities reduce ear growth rate and partitioning which in turn impacts negatively the rate of appearance of silks relative to tassel appearance resulting in poor kernel set. This reduced ear and plant growth in less competitive individuals, however, is seldom reported as cumulative growth which makes
difficult to clearly link individual growth rates to final plant dry matter at maturity. It is not clear, whether low dry matter in barren plants is explained by complete arrest in growth during a specific growth stage or occurs as a result of low accumulation of biomass throughout the crop cycle. In addition, despite the importance of ear growth and partitioning to reproductive growth during the critical silking stage, few reports have integrated the progression of the number of developed florets or potential kernel number with plant biomass under density stress during the vegetative phase (Pagano et al., 2007). The assessment of per plant yield potential defined as number of florets per plant and actual yield in relation with the dynamics plant biomass accumulation would provide a practical view of plant community dynamics under density stress.

This research explored the relationship between plant reproductive development (i.e., floret or potential kernel number, and final kernel number) and their association with individual cumulative dry matter during specific stages of the crop cycle of maize grown at two contrasting densities. We aimed to identify, either the progression or the complete arrest of dry matter accumulation in barren plants during the growing season and the gap from individual plant yield potential in barren individuals. Specifically, we tested the hypotheses that i) if an increase in plant density results in a reduction in plant dry matter accumulation leading to barrenness, then the reduction in plant dry matter at maturity will be explained by similar reductions in dry matter accumulation, during the vegetative, bracketing silking or the commencement of the grain filling period and ii) if plant density
reduces cumulative dry matter accumulation early during the growing season then we
would expect a reduced floret development before the critical period.
4.3. Materials and methods

4.3.1. Experimental design

Experiments were conducted at the Elora Research Station, Elora, ON (43°38´ N, 80°25´ W, 380 m above sea level). The soil type was a London loam soil (Aquic Hapludalf) with tile drainage and soil organic matter content of 3.8 to 4.0%. Nitrogen, P, and K were applied prior to planting at rates of 150, 85, and 50 kg ha$^{-1}$, respectively. Soybean was the previous crop during both years. Weed control was obtained using a pre-plant tank mix of S-metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-,-(S)], atrazine [6-chloro-N-ethyl-N’-(1-methylethyl)-1,3,5-triazine- 2,4-diamine] and mesotrione [2-[-4-(methylsulfonyl)-2- nitrobenzoyl]-1,3-cyclohexanedione] at rates of 1.6, 1.28, and 0.14 kg a.i. ha$^{-1}$, respectively. Planting occurred on 10$^{th}$ May 2012 and on 14$^{th}$ May 2013. The seeds were planted with a precision planter ALMACO (Allan Machine Company, Nevada, IA, 50201, USA) resulting in 6 m long by 3.04 m wide (4 rows, 0.76 m between rows) plots. Two densities were used in this experiment: 7.5 plants m$^{-2}$ and 15 plants m$^{-2}$ to create a wide range of individual plant dry weights (Vega et al., 2000). Each density was over planted, and at five visible leaf tips, thinned to final stand.

The experimental design consisted of a split-plot with plant density as the main plot and the genotypes as the subplot. The experimental design had 4 replications. Although two densities were originally planted in these experiments (i.e. 7.5 and 15 plants m$^{-2}$), three densities were used in this experiment, 7.5, 15, and 7.5 thinned to 15 plants m$^{-2}$ at the V9
stage of growth. Data is shown only for the conventional (Assefa et al., 2018) (7.5 plants m\(^{-2}\)) and high density (15 plants m\(^{-2}\)). Three genotypes were used in this study, CG108/CG102 (CG), X58945WP and SK5069WP. CG108/CG102 is a hybrid developed by the University of Guelph’s Corn Breeding Program and has been used in previous studies (Lee et al., 2000, 2001). X58945WP and SK5069WP are isoline hybrids from Syngenta Canada that were classified as either a non-drought tolerant hybrid (NDT; X58945WP) or as a drought tolerant hybrid (DT, SK5069WP) (Agrisure ® Artesian® Syngenta Crop Protection Inc.). For further details on the isoline hybrids, see Reid et al., (2014). Throughout this study the following stages defined as: vegetative = V7, V8, V10 (Hanway, 1966), pre-tasseling=V14, post-silking = V14+25 days, and maturity = following physiological maturity (i.e., appearance of black layer).

4.3.2. Data collection

During the vegetative phase, destructive sampling occurred at the V7, V8, and V10 stages of growth. Within each plot and growth stage, three consecutive plants were individually harvested. The primary ear initial was dissected from each plant and the remaining aboveground plant tissue (stems and leaves) were bagged at 85°C until constant weight was achieved. At the first sampling stage, i.e., V7, ear length was less than 0.5 cm. In order to count accurately the differentiated florets, the ear initials were placed in Karnovsky’s fixative (Karnovsky, 1965) for a maximum of two weeks period. Upon
fixation, each ear initial was dyed with a mixture of ethyl alcohol, glycerine and fucsin in a proportion of 8:1:1 (Smith and Lee, 2016). Each ear was photographed using a zoom trinocular stereo microscope with a 90X magnification capacity (Cyber Scientific Inc, Kitchener, Ontario; model V434B) and a three-megapixel camera (Cyber Scientific Inc, Kitchener, Ontario, model A1530). Floret number was then counted from each photograph. At the V8 and V10 stages of growth, florets were identified visually, thus, no fixation was required.

In order to assess biomass accumulation during the period bracketing silking, we sampled at two developmental stages, V14 (i.e., pre-tasseling, average of 8 days before silking) and V14+25d (i.e., post-silking, average of 17 days post-silking). The purpose of this sampling procedure was to quantify plant cumulative dry weights rather than to calculate growth rates. This approach provided an opportunity to associate floret and kernel number, in retrospect, to cumulative dry weights at specific stages of growth. At each stage of growth, five consecutive plants per plot were selected for destructive sampling and an additional 20 consecutive plants per plot (i.e. treatment combination) were identified for destructive sampling at maturity totalizing 960 plants (20 plants, 3 genotypes, 2 densities, 4 replications, and 2 years). Stem diameter at 5 cm above ground level and plant height, from ground level to the last stem ligule, were measured on all 25 plants. Once these measurements were completed, the five plants per plot designated for destructive sampling were manually harvested, bagged and dried at 85°C until constant weight.
At physiological maturity (i.e., black layer), the previously identified 20 plants were destructively sampled in each treatment. Each plant was then separated into stem, leaves and ears. The primary ear was shelled in order to record kernel and individual plant grain yield. All plant components, i.e., stem, leaves, ear and kernels were dried at 85°C to a constant weight. Plant kernel number and dry matter were then calculated on a per plant basis.

4.3.3. Statistical analysis

Main treatments effects were analyzed using a mixed linear model described in Eq. [4.1]. The SAS PROC MIXED procedure (SAS ® Institute Inc., Cary, NC, USA, Version 9.4) was used to generate the ANOVA random and fixed effects coefficients and their p-values. Year, replications, and plots were assigned as random factors. Fixed effects were 7.5 plants m⁻² and 15 plants m⁻², genotypes and their interactions.

\[
Y_{dgyrp} = \mu + \alpha_d + \beta_g + Y_y + \rho_r (Y_y) + \sigma_p(\rho_r) + \alpha_d\beta_g + \varepsilon_{dgyrp}
\]

Where \(Y_{dgyrp}\) represents the phenotype of density d in the genotype g, year y, plot p and the replicate r. The \(\mu\) term represents the overall mean of the phenotype, \(\alpha_d\) the plant density effect; \(\beta_g\) the genotypic effect; \(Y_y\) is the year effect; \(\rho_r (Y_y)\) is the replicate effect nested
within year; $\sigma_{p(r)}$ is the effect of the plot within replicates; $\alpha_g \beta_t$ is the interaction between the genotype $g$ and the density $d$; and $\varepsilon_{dgyrp}$ is the residual error of the genotype $g$ at the density $d$ in year $y$ replicate $r$ and plot $p$. Means were calculated as best linear unbiased estimators and comparisons were performed using Tukey’s tests at a probability of 0.05.

Plant dry matter collected at V14 (pre-tasseling) and at V14+25 days (post-silking) was regressed against the product of diameter and height. Linear regressions were fitted among treatments since parameters among treatments for the plant dry matter-product (diameter by height) relationship did not differ. Models for years, timing (pre-tasseling and post-silking) (Table 4.1) are described in the Eq. [4.2]:

$$Eq. [4.2] = Y = \alpha + \beta (X)$$

Where $Y$ is plant dry matter pre-tasseling or post-silking, $\alpha$ is the intercept value for $Y$, and $\beta$ is the increase in grams of plant dry weight per unit $X$ which reflects the product of the diameter by height. Fitted linear equations, standard error and coefficients of determination were obtained using PROC REG procedure of (SAS ® Institute Inc., Cary, NC, USA, Version 9.4).
Table 4.1. Allometric model parameters ($\alpha, \beta$), number of observations used to develop each model (n) and the coefficient of determination ($R^2$) for the plant dry matter vs diameter x height relationship across all three single cross hybrids during the V14 for pre-tasseling and V14+25d for post-silking stages. Experiments conducted at Elora Research Stations, during the 2013-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Year</th>
<th>Stage</th>
<th>Source of variability</th>
<th>Date</th>
<th>n</th>
<th>Coefficient ± standard error</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\alpha$</td>
<td>$\beta$</td>
</tr>
<tr>
<td>2012</td>
<td>Pre-tasseling</td>
<td>7.5, 15 plants m$^{-2}$</td>
<td>16$^{th}$ July‡</td>
<td>176</td>
<td>15*** ± 2.8</td>
<td>0.02*** ± 0.0012</td>
</tr>
<tr>
<td></td>
<td>Post-silking</td>
<td>7.5, 15 plants m$^{-2}$</td>
<td>30$^{th}$ August</td>
<td>180</td>
<td>12.6* ± 5.8</td>
<td>0.03*** ± 0.0018</td>
</tr>
<tr>
<td>2013</td>
<td>Pre-tasseling</td>
<td>7.5, 15 plants m$^{-2}$</td>
<td>23$^{rd}$ July</td>
<td>180</td>
<td>-13*** ± 3.6</td>
<td>0.02*** ± 0.00084</td>
</tr>
<tr>
<td></td>
<td>Post-silking</td>
<td>7.5, 15 plants m$^{-2}$</td>
<td>17$^{th}$ August</td>
<td>174</td>
<td>-7.4ns† ± 13.3</td>
<td>0.04*** ± 0.0024</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant ‡Average of two locations. Average mid-silking dates were 26$^{th}$ and 28$^{th}$ July during the 2012 and 2013 growing seasons respectively.
Non-linear equations were used to describe i) the relationship between floret number, kernel number, grain yield, and plant dry matter (vegetative, pre-tasseling, post-silking, grain filling period and maturity). Eq. [4.3] is detailed as follows:

\[
Eq.[4.3] = Y = \left[ \frac{\alpha(X - X_0)}{1 + \beta(X - X_0)} \right] [X > X_0]
\]

In Eq. [3] \(\alpha\) represents the initial slope of \(Y\) (floret or kernel number) in response to \(X\) (plant dry matter), \(X_0\) the threshold or location parameter (Archontoulis and Miguez, 2015) of \(X\) for \(Y > 0\), and \(\beta\) the curvilinear response of the relationship which is inversely related to \(\alpha\) (Sarlangue et al., 2007; Gonzalez et al., 2018). Coefficients calculation, standard errors and coefficients of determination were obtained using the PROC NLIN procedure of (SAS Institute Inc, 2013, Cary, NC, USA, Version 9.4). Calculation of plant dry matter accumulation during the grain filling period is described in Eq. [4.4]. Dry matter accumulation during the grain filling period (\(Y\)) was calculated as the difference between plant dry matter at maturity (\(X\)) and dry matter during the post-silking period (\(Z\)).

\[
Eq. [4.4] = Y = X - Z
\]
4.4. Results

4.4.1. Yield components

Density was a significant source of variation across most developmental timepoints and for most of the traits followed in this study (Table 4.2). The genotype was not a consistent source of significant variation across traits and developmental timepoints. In addition, the density by genotype interaction was not significant across all timepoints and for all traits. As such, the data presented are combined across genotypes. High plant density (15 plants m\(^{-2}\)) reduced dry matter and kernel number but not floret number per plant. Individual plant dry matter was reduced from the V8 stage of growth through to maturity at high density when compared to the conventional density (7.5 plants m\(^{-2}\)). Significant dry matter reductions were observed at V8, V10, pre-tasseling, post-silking, and maturity, in the plants grown at high density relative to plants grown at conventional density (Table 4.2, Fig. 4.1a). While floret number per ear was not affected by plant density, a reduction in kernel number was observed at maturity (Table 4.2, Fig. 4.1b). While plant dry matter was reduced from V8 to physiological maturity by the high density, potential kernel number (i.e., floret number) only varied over phenological stages. Plant density caused grain yield and harvest index per plant to be reduced. An average plant grain yield reduction of 64% was observed in maize grown at high density compared to conventional density (Table 4.3). Harvest index was 0.16 g g\(^{-1}\) lower in the high density when compared with the conventional density.
Kernel number reductions caused by density were associated with an increased frequency of barren and low yielding plants. Kernel number showed a mean reduction of 47% in maize grown at high density relative to conventional density (Tables 4.2 and 4.3, Fig. 4.1b). Accompanying this reduction was an increase in the frequency of plants with low kernel number (i.e., less than 496 kernels per plant) observed at high density (Fig. 4.2). The value of the first quartile (i.e., lowest 25% of the population, cumulative frequency = 0.25) of high-density plants had an upper limit of 28 kernels, compared to 390 kernels for the conventional density. In contrast, the first quartile of both the high density and conventional density populations of plants at V10 stage had an upper limit of 471 florets per plant. The high plant density increased the frequency of low kernel setting plants when compared to the more conventional density.
Table 4.2. Mixed model analysis of treatment effects on plant dry matter, floret number, kernel number, grain yield, and harvest index at V7, V8, V10, pre-tasseling, post-silking and at physiological maturity. Plants grown at conventional (7.5 plant m$^{-2}$) and high density (15 plants m$^{-2}$). Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Plant dry matter</th>
<th>Floret number</th>
<th>Kernel number</th>
<th>Grain yield</th>
<th>Harvest index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V7 V8 V10</td>
<td>Pre-tasseling</td>
<td>Post-silking</td>
<td>Physiological Maturity</td>
<td>V7 V8 V10</td>
</tr>
<tr>
<td>Density (D)</td>
<td>0.08 0.01 &lt;.0001</td>
<td>0.001 &lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.54 0.83 0.26</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>0.02 0.04 0.02</td>
<td>0.001 0.13</td>
<td>0.55</td>
<td>0.0002 0.0002</td>
<td>0.003 0.0009</td>
</tr>
<tr>
<td>D x G</td>
<td>0.62 0.2 0.96</td>
<td>0.95 0.34</td>
<td>0.3</td>
<td>0.95 0.34 0.13</td>
<td>0.18 0.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Num df</th>
<th>Den df</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (D)</td>
<td>1 6</td>
<td></td>
<td>0.08 0.01  &lt;.0001 0.001 &lt;.0001 &lt;.0001 0.54 0.83 0.26 &lt;0.0001 &lt;0.0001 &lt;0.0001</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>2 6</td>
<td></td>
<td>0.02 0.04  0.02 0.001 0.13 0.55 0.0002 0.0002 0.003 0.0009 0.6542 0.0049</td>
</tr>
<tr>
<td>D x G</td>
<td>2 6</td>
<td></td>
<td>0.62 0.2  0.96 0.95 0.34 0.3 0.95 0.34 0.13 0.18 0.11 0.08</td>
</tr>
</tbody>
</table>
Figure 4.1. Plant dry matter (A), floret number or kernel number per plant (B) at the V7, V8, V10, pre-tasseling (V14), post-silking (V14+25 days) and maturity stages for conventional (7.5 plants m$^{-2}$) and high density (15 plants m$^{-2}$). Each bar represents the mean of 72 plants in V7, V8, V10, 480 plants pre-tasseling; post-silking, and maturity stages. *Significant at the 0.05 probability level. * Significant at the 0.01 probability level. *** Significant at the 0.001 probability level. Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.
Table 4.3. Dry matter accumulation at maturity, grain yield, harvest index, and kernel number per plant genotypes grown at conventional (7.5 plants m^{-2}) and high density (15 plants m^{-2}). Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Density</th>
<th>Dry matter accumulation at maturity</th>
<th>Grain yield</th>
<th>Harvest index</th>
<th>Kernel number</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5 plants m^{-2}</td>
<td>253a†</td>
<td>125a</td>
<td>0.50a</td>
<td>496a</td>
</tr>
<tr>
<td>15 plants m^{-2}</td>
<td>127b</td>
<td>45b</td>
<td>0.34b</td>
<td>260b</td>
</tr>
<tr>
<td>Standard error (±)</td>
<td>21</td>
<td>10</td>
<td>0.02</td>
<td>102</td>
</tr>
</tbody>
</table>

† Within columns, means followed by the same letter are not significantly different according to Tukey’s test (0.05).
Figure 4.2. Cumulative frequency of florets at V10 stage of growth and kernel number per plant at maturity for conventional (7.5 plants m$^{-2}$) and high density (15 plants m$^{-2}$). At the V10 stage 72 plants for each density were measured and 480 plants in each density were measured at maturity. Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.
4.4.2. **Barrenness and plant dry matter**

Plants with a low number of florets or kernels, or in some cases barren plants were evident at dry weights of approximately 30, 60, 100 and 100 g plant\(^{-1}\) in the vegetative, pre-tasseling, post-silking, and maturity stages respectively (Fig. 4.3a, b, c, d). The frequency of barren plants at maturity increased with density; 0.04 and 0.14 in the conventional and the high density, respectively (Fig. 4.3). Non-linear equations described the relationship of floret or kernel number with plant dry matter accumulation with \(R^2\) values ranging from 0.68 to 0.84 (Table 4.4). Estimated threshold dry matter values for the vegetative, pre-tasseling, and post-silking stage differed significantly from each other, however, the thresholds values for post-silking and maturity stages were similar (Table 4.4, Fig. 4.4). For example, fitted dry matter thresholds \((X_0)\) to obtain floret or kernel number greater than zero were 20, 45, 75, and 80 g plant\(^{-1}\) for the vegetative, pre-tasseling, post-silking and maturity stages, respectively (Table 4.4). The shape of this curve was defined by coefficients \(\alpha\) (initial slope) and \(\beta\) (curvilinear response), which decreased from the vegetative to the maturity stage and differed with each stage. Values of \(\alpha\), which give an indication of reproductive output in relation to dry matter, corresponded to 130 florets for the vegetative stage and 45, 15 and 6 kernels per g plant\(^{-1}\) for the pre-tasseling, post-silking and maturity stages. Similarly, values of degree of curvature \(\beta\) declined with stage, contributing to a lower slope of the response, with values of 0.18 for the vegetative and 0.06, 0.02 and 0.01 for the pre-tasseling, post-silking and maturity stages (Table 4.4). Dry matter accumulation and floret or kernel number are positively correlated at all stages of development. Plants with the greatest accumulation of dry matter attained the mean 602 florets per plant,
regardless of density. To attain 602 florets required a corresponding dry matter accumulation of 48, 96, 186, and 332 g plant\(^{-1}\) during the end of the floret differentiation period (V10), pre-tasseling, post-silking and at maturity, respectively (Fig. 4.4). In contrast, for values of 260 kernels plant\(^{-1}\) in the high density required dry matter values of 23, 53, 99, 196 g plant\(^{-1}\) for the V10, pre-tasseling, post-silking, and at maturity stages. Plants that accumulated more dry matter in each stage of growth showed more florets and kernels per plant. Those plants that failed to achieve a minimum threshold \((X_0)\) dry matter accumulation became barren at maturity.
Figure 4.3. Floret or kernel number per plant as a function of plant dry matter during (A) the vegetative phase (V7, V8, and V10 stage of growth), (B) pre-tasseling (V14), (C) post-silking (V14+25 days), and (D) physiological maturity for conventional (7.5 plants m$^{-2}$) and high density (15 plants m$^{-2}$). 432 plants were sampled for the vegetative phase (V7, V8, and V10 combined) and 960 plants were sampled for pre-tasseling (V14), post-silking (V14+25 days), and maturity. Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.
Table 4.4. Non-linear model parameters ($\alpha, X_0, \beta$) describing the relationship between floret or kernel number per plant vs plant dry matter at four stages of development. Number of individuals used to generate the models (n) coefficient of determination ($R^2$) for each model are included. Experiments conducted at Elora Research Stations during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>Parameter ± Standard error</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\alpha$</td>
<td>$X_0$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g plant$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Vegetative (V7, V8, V10)</td>
<td>369</td>
<td>130*** ± 3.3 a†</td>
<td>20*** ± 1.1 c</td>
</tr>
<tr>
<td>Pre-tasseling (V14)</td>
<td>960</td>
<td>45*** ± 2.1 b</td>
<td>45*** ± 0.8 b</td>
</tr>
<tr>
<td>Post-silking (V14+25d)</td>
<td>960</td>
<td>15*** ± 1 c</td>
<td>76*** ± 1 a</td>
</tr>
<tr>
<td>Physiological maturity</td>
<td>960</td>
<td>6*** ± 0.4 d</td>
<td>80*** ± 2.8 a</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †Within columns, values followed by the same letter are not significantly different according to Z test at the 0.05 probability level
Figure 4.4. Predicted floret or kernel number per plant as a function of plant dry matter during the vegetative phase (V7, V8, V10 stage of growth), pre-tasseling (V14), post-silking (V14+25 days), and maturity stage for conventional (7.5 plants m\(^{-2}\)) and high (15 plants m\(^{-2}\)) density. Mean floret number (triangle), kernel number conventional density (7.5 plants m\(^{-2}\)) (open circle), kernel number high density (15 plants m\(^{-2}\)) (closed circle). Non-linear regression parameters are indicated in table 4.4. Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.
4.4.3. **Grain filling period**

Dry matter accumulation during the grain fill was lower in barren or low yielding plants. Since threshold dry matter values did not differ between post-silking and maturity, dry matter accumulation during the grain filling period was calculated as the difference between dry matter at maturity and dry matter post-silking. Plant density reduced cumulative dry matter during the grain filling period. Dry matter accumulation during the grain filling period was on average 122 and 40 g plant\(^{-1}\) for the conventional and high densities (Fig. 4.5). The lower dry matter accumulation during the grain filling period in the high density was in part associated with negative values of dry matter accumulation. The cumulative frequency of plants with negative or zero values in grams per plant was 0.13 in the 15 plants m\(^{-2}\) compared to 0.02 in the conventional density (Fig. 4.5). Density stress increased the frequency of plants with low dry matter accumulation during the grain filling period. Barren plants at maturity showed very low or no dry matter accumulation during the grain filling period.

Non-linear regressions adequately explained variations in kernel number and grain yield in response to variations in dry matter accumulation during the grain filling period (Table 4.5). For both traits, the number of barren plants increased when plant dry matter accumulation was very low or clustered near the value zero (see zero value on Fig. 4.6) during the grain filling period. Kernel number increased with successive increases in dry matter accumulation during this same period (Fig. 4.6a). In order to obtain kernel number
greater than zero the predicted threshold \((X_0)\) dry matter during the grain filling period was estimated to be 6 g plant\(^{-1}\) \((p>0.05)\) and the initial slope \(\alpha\) was of 16 kernels g\(^{-1}\). At approximately 50 g per plant, increases in predicted kernels per plant were 50% of the maximum kernel number while at 150 g plant\(^{-1}\), the percentage of maximum kernel number was more than 90%. This positive curvilinear response of kernel number and dry matter accumulation was associated with the \(\beta\) value of 0.02 (Table 4.5). Similarly, grain yield was also related positively to dry matter accumulation during the grain filling period (Table 4.5, Fig.4.6b). Estimated value of dry matter required to obtain grain yield greater than zero \((X_0)\) was 1 g plant\(^{-1}\) during the grain filling period. Across the entire data set illustrated in the figure 4.6b, plant yield increased at a rate \(\alpha\) of 1.3 g g\(^{-1}\) of dry matter during the grain filling period. This response, however, was not linear. As dry matter accumulation increased, the yield response tempered, and it was reflected by the \(\beta\) term of the non-linear regression (see Table 4.5). For example, based on the non-linear regression illustrated in figure 4.6b, if a plant accumulated 50 g of dry matter the predicted yield was 58 g plant\(^{-1}\). In contrast, the predicted yield in plants that had accumulated 200 g of dry matter was 186 g plant\(^{-1}\). These results confirm the positive relationship between kernel number and dry matter accumulation during the grain filling period. Plants that set more kernels showed increased dry matter accumulation during the grain filling period resulting in an increase in grain yield.
Figure 4.5. Cumulative frequency of dry matter during the grain filling period per plant for conventional (7.5 plants m\(^{-2}\)) (empty symbols) and high density (15 plants m\(^{-2}\)) (closed symbols). Dry matter accumulation during the grain filling period is calculated as the difference between dry matter at physiological maturity and dry matter post-silking (V14+25 days). Each frequency represents 480 plants for each density. Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.
Table 4.5. Non-linear model parameters ($\alpha, X_0, \beta$) describing the relationship between kernel number and grain yield per plant vs plant dry matter accumulation during the grain filling period relationship. Experiments conducted at Elora Research Stations during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Model</th>
<th>n</th>
<th>Parameter ± Standard error</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernel number vs. dry matter accumulation during the grain filling period</td>
<td>960</td>
<td>15.8*** ± 1.54 g plant$^{-1}$</td>
<td>6 ns ± 1.6</td>
</tr>
<tr>
<td>Grain yield vs dry matter accumulation during the grain filling period</td>
<td>960</td>
<td>1.3*** ± 0.06</td>
<td>1 ns† ± 2.2</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant
Figure 4.6. Kernel number (A), and grain yield (B) as a function of plant dry matter accumulation during the grain filling period for conventional (7.5 plants m$^{-2}$) (empty symbols) and high density (15 plants m$^{-2}$) (closed symbols). Dry matter accumulation during the grain filling period is calculated as the difference between dry matter at maturity and dry matter post-silking (V14+25 days). Each plot represents 960 plants. Non-linear regression parameters are indicated in table 4.5. Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.
4.5. Discussion

In this study, we tested the hypothesis that i) if an increase in plant density results in a reduction in plant dry matter accumulation leading to barrenness, then the reduction in plant dry matter at maturity will be explained by similar reductions in dry matter accumulation, during the period bracketing silking or the commencement of the grain filling period and ii) if plant density reduces cumulative dry matter accumulation early during the growing season then we would expect a reduced floret development before the critical period.

Plants with increased dry matter accumulation at all stages of growth were most likely to obtain above average kernel set, while low yielding or barren plants were shown to accumulate less dry matter at all stages of growth. While reductions in plant dry matter accumulation in response to high plant density were observed early in development, this did not result in reductions in floret number (i.e., potential kernel number), consistent previous studies (Otegui, 1997; Rossini et al., 2016).

Plant barrenness caused by high plant density was established at the onset of the grain filling period. Dry matter accumulation in barren plants from post-silking to maturity was observed to be very low or non-existent. This response to high density could be explained in part, by a lower sink demand when compared to plants grown at conventional density (Moss,(1962; Ceppi et al.,(1987; Rajcan and Tollenaar, 1999).
Kernel number and yield were positively associated with cumulative plant dry matter during the grain filling period. This increase in dry matter accumulation may be accounted for by either or both, an increase in the rate of growth during this period or a lengthening of the grain filling period in the conventional density compared to the high density (Poneleit and Egli, 1979; Reddy and Daynard, 1983; Hisse et al., 2019). The association of kernel number with dry matter accumulation was observed to be more variable than yield. The larger variability may be associated with the stage at which kernel number was established (Gambín et al., 2006). For example, kernel number is determined during the period bracketing silking (Andrade et al., 1999; Vega et al., 2001a; b; Borrás and Vitantonio-Mazzini, 2018). This is a well-defined period which can be identified clearly across all genotypes. In contrast, the less variable relationship observed between yield and dry matter accumulation during the grain filling period occurred as a result of the active dry matter allocation to individual kernels (Borrás and Gambín, 2010). Increases in yield observed in our study were not proportional to increases in dry matter accumulation during the grain filling, especially, at high values of dry matter. Similar results were shown recently by D’andrea et al., (2016). These results support the notion that temperate corn is presented with a sink limitation during the grain filling period, despite resources being plentiful (Tollenaar and Lee, 2011; Hisse et al., 2019). The results of this study offer support to our hypothesis that if an increase in plant density results in a reduction in plant dry matter accumulation leading to barrenness, then the reduction in plant dry matter at maturity will be explained by reductions in dry matter accumulation that occurred at earlier stages of growth. We identified that plants with very low dry matter accumulation during the pre-tasseling and post-silking stages of growth
exhibited the largest discrepancy between floret number and kernel number per plant. Secondly, barren individual plants accumulated very low dry matter during the grain filling period. This is the first study to clearly link the occurrence of barrenness with before silking, post-silking, and grain filling period dry matter accumulation, in response to density stress on a per plant basis. This new knowledge provides a framework to further understand the physiological mechanisms of density stress in maize.
5. Chapter 5: Conclusions

5.1. Summary and research contributions

The main objectives of this thesis were i) to elucidate the association between ear development and cumulative growth under early season stress, ii) to assess physiological mechanisms underlying yield loss when exposed to three contrasting early season stress, and iii) to understand the dynamics of plant dry matter accumulation in low yielding and barren plants when grown under season-long density stress. The motivations for these objectives after reviewing the available literature in Chapter 1 were i) a lack of an individual growth-based framework to infer ear development, ii) absence of an integrated assessment of common mechanisms in contrasting sources stress during the pre-silking stage, and iii) the poor practical understanding of the link between barrenness and plant cumulative growth under density stress. These motivations resulted in the following contributions.

In Chapter 2, ear development and floret appearance were related positively with individual plant dry matter accumulation across years and locations. This individual plant framework showed a start for ear development when plants attained between 20 and 30 g plant\(^{-1}\). Considerable variability, for the response variables, especially floret number was observed around these values. Collected and predicted plant data, showed that floret number per ear row and its variability remained relatively stable when plants attained around 60 g plant\(^{-1}\) or more. The response, however, changed when stresses were analyzed individually, specifically in the severe drought and the plant density stress. The net result was a reduced minimum dry weight for ear
initiation and an increased slope, that is, either increased ear length or floret number per unit dry matter. This is in clear contrast with observations of reduced growth of the ear relative to the plant in response to stress around silking. This response suggested that ear development, although related with dry matter, is not prevented by restrictions in growth rates during the pre-silking stage. The drought stress, however, showed reduced number of florets at the end of the sampling period. Whether this reductions in floret number was irreversible or not cannot be answered with the data collected at present. Therefore, our results showed that the relationship between ear development and dry matter per plant is narrow and positive under non-stress conditions. This research has addressed two processes that are often studied as independent phenomena during the pre-silking stage, that is, development (Bonnett, 1954) and growth (Tetio-Kagho and Gardner, 1988b). In addition, these results complete the knowledge regarding the relationship between ear development and individual plant growth during the silking phase and grain filling period.

In Chapter 3, physiological mechanisms underlying yield loss varied with early season stress. Overall, both the drought and the early high-density seemed to affect resource capture (i.e. dry matter accumulation) rather than resource utilization (i.e. HI). The severity of stresses was reflected in the reductions in resource capture which were larger for the drought, followed by the early density stress and light quality treatments. Changes in light quality reduced only growth rates around silking. Reduced kernel and reduced yield were related with lower cumulative dry matter in the drought followed by the early high-density. This response shows that drought stresses that occur early during the season (i.e., before silking) as well as early high-density do not disrupt the source to sink balance in the context of HI. More importantly, this is a clear demonstration that a
balanced source to sink ratio does not translate into more yield. In addition, both anthesis and silking dates covaried resulting in no changes in the anthesis-to-silking interval. Although the tassel is a dominant structure relative to the ear in terms of development, our results suggested that this dominance was reduced when exposed to either drought or early high-density. Results of this chapter are unique in terms of comparing, but particularly, showing the effect of early season drought and its impact in kernel set around silking.

In Chapter 4, high plant density caused reductions in individual plant dry matter accumulation, and the proportion of dry matter allocated to yield (i.e., harvest index). Yield potential defined as the number of florets per plant, however, was unaffected by high plant density. Throughout the growing season, reduced dry matter was related to either less florets per plant or reduced kernel number. In the extreme case, barren (non-bearing ear plants) individuals exhibited values of dry matter accumulation that were below 80 g plant$^{-1}$ from pre-tasseling to maturity. This suggested that low yielding individuals were less competitive throughout the growing season. Most importantly, plants that were less competitive also showed no variations from the post-silking stage to physiological maturity. This response, especially in the high-density stress, confirmed that very low yielding and barren plants accumulated practically no dry matter during the grain filling period. The opposite response was true for high yielding plants, that is, they exhibited higher per plant cumulative dry matter from pre-tasseling to maturity, dry matter accumulation during the grain filling period, and overall seasonal dry matter per plant. Clearly, plants that were outcompeted or limited in growth early during the growing season, did not recover resulting in low yielding or barren plants.
5.2. **Research limitations**

This research brings new knowledge regarding ear development, mechanisms of yield loss in response to contrasting early season stress, and on the relationship between barrenness and cumulative growth. Yet, for each of these research components, areas of improvement can be identified clearly. These areas pertain to scope of inferences, methodology, and physical limitations. In terms of the scope of inferences, Chapter 2 results are of practical understanding, however, genotypic variability showed that the conclusions, within a range of confidence, are applicable only to the set of genotypes tested in the study. This was reflected in the larger dry weight threshold for ear development and the larger number of florets in the asymptotic phase of the relationship for the isolines relative to the CG genotype. In addition, we did not assess pistil development per floret which necessarily relates with silk appearance and timing in floret fertilization (Carcova et al., 2003). In terms of methodology, the need to relate development and growth at the ear-plant level in a destructive manner, prevented us from measuring the trajectory of those sampled plants and their fate in terms of flowering dynamics and final yield. The conflict between destructive and non-destructive sampling also arises in the following chapters, for example, in Chapter 3 when early season traits (dry matter, ear length and floret number) are related with around silking traits (growth rates, days to anthesis, and days to silking). In this same chapter, drought and early high-plant density delayed both anthesis and silking which suggested that both tassel and ear initiation were impacted negatively during early stages. Tassel initiation data, however, was not collected which prevent us to explain fully this response. A similar limitation to Chapter 3 is present in Chapter 4 when comparing traits corresponding to the
vegetative stage (V7, V8, and V10) (destructive) with the pre-tasseling, post-silking (non-destructive) and maturity stages (destructive). The potential solution for this challenge could be to convey an experimental design that combine two aspects i) a novel non-destructive method to infer ear development during the vegetative phase and ii) a consideration of the number of sequential sampling replications for the experimental design. Similarly, for Chapter 4, the experimental design and the scope of the inferences altogether could have been improved by creating a high resource per plant scenario. Under field conditions, this could be accomplished by including as a factor, an ultralow plant density of 2 plants m$^{-2}$. The net gain of this new treatment would be to observe the limitations per plant in vegetative and reproductive growth when resources are plentiful. This approach could complete the assessment of the source to sink ratio in a per plant basis depicted in figure 4.7b. In addition, although our study was carried used cumulative growth, a measurement of rates and duration of stages could have been very informative in terms of interpretation of results. For example, the association between ear growth and plant growth is reported for most of the growing season, however, information is lacking for the pre-silking stage. In terms of physical limitations, for the controlled conditions in Experiment #1, there was a clear spatial limitation arising from the experimental platform. Under non-limiting conditions stresses that impact negatively individual plant growth such as shade, and crowding could be included as well as a larger set of genotypes. This limited the number of stresses, therefore affecting the number and combinations of factors tested.
5.3. Future research

This thesis including the review, the results, and the areas of improvement, highlight opportunities for further research. Ear development can be followed using plant cumulative growth, especially, under non-stress conditions. The underlying mechanisms that uncouples ear elongation/floret appearance from cumulative growth should be further investigated. The best approach to do this would be to combine a non-destructive sampling with the calculation of growth rates (i.e. dry matter accumulation per day or thermal time). One of the challenges, if not the main challenge for this calculation, is the assumption that traits are normally distributed in the community of plants at any given point in time. In addition, the distribution of the population can be further skewed (positively or negatively) by the source of variability tested (i.e., stress). The individual plant growth rate framework, however, may solve the observed departure under stress (drought primarily) in the relationship between ear development and plant growth. Provided this approach is successful, then, these rates of ear development would be related to rates of ear growth around silking and potentially with rates of silk appearance. This, on my view is necessary, since results of Chapter 3 showed that silking was delayed which can be a result of early ear development events. In addition, tassel appearance delays mirrored those of silks, which suggested that factors affecting delays in silking also affected tassel development. This response is in clear contrast with the delay only in silking for stresses occurring close to flowering (Hall et al., 1982).

Source to sink relations exhibit an opportunity to be quantified at the individual plant level during the grain filling period as shown in Chapter 4. Two types of limitations with its
quantification arise in the available literature. The first one is associated with the low magnitude in the variability of source to sink ratios and the second relates with the measurements for each component (i.e., the source and the sink). The variability problem was solved in our study by exploiting the naturally occurring interplant variability and its responsivity to crowding stress. For example, crowding stress reduced sink size by reducing one of its components, that is, kernel number causing plant yield to be reduced. The measurement component, that is, differing levels of calculation (average for kernel weight vs individual plant dry weight) was also solved by using yield per plant instead kernel weight averages. Possible further research should involve the response of source to sink relation and its interplant variability to stresses other than density. Two obvious candidates for these hypotheses are whether genotypic variability and variability caused by nitrogen status would affect this relationship considering their impact in yield gains and yield potential respectively. The former associates with an expected balanced source to sink ratio (closer to 1) in modern hybrids relative to older ones. The latter would be related to larger source to sink ratios per plant attributed larger rates of dry matter accumulation and the critical role nitrogen plays on maintenance of active leaf area and photosynthesis during grain fill.

Kernel set around silking is one of the most studied stages in maize and growth rates explain a large variability in flowering dynamics and kernel number. Still it was clear from the review in Chapter 1 and from chapter 4, that this critical stage overlaps temporally with at least the onset of the grain filling period. Although our study accounted for the critical dry weight that may represent this approximate time point (i.e., dry weights of barren plants in the post silking stage),
yet, there are not studies quantifying these two apparently different phases with a common variable which would explain yield variability. Research that could simplify (or unify) these two apparent different points (i.e. around silking and lag phase of grain fill) would be a relevant contribution. A second aspect of kernel set is that, although largely, is not exclusively explained fully by growth rates but also by flowering dynamics. Our results suggest that barren individuals at maturity may have failed into develop reproductive structures as well. Although growth, development and kernel set have been shown to be related (Borrás et al., 2007, 2009a) the net effects of development and growth on yield determination remain to be further investigated.
6. References


Bonnett, O.T. 1954. The Inflorescences of Maize. Science (80-). 120: 77–87. doi: 10.1126/science.120.3107.77.


Borrás, L., M.E. Westgate, J.P. Astini, and L. Echarte. 2007. Coupling time to silking with plant


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Cicchino, M., J.I. Rattalino Edreira, M. Uribelarrea, and M.E. Otegui. 2010. Heat stress in field-


7. Appendix A: Data for Chapter 2
Table A7.1. Variation in of water per pail and % of available water per pail from planting to V9 stages in drought treatments. Experiments conducted at Arkell Research Station, Guelph, Ontario, Canada during 2012 and 2013 growing seasons.

<table>
<thead>
<tr>
<th>Year</th>
<th>Days from planting</th>
<th>n</th>
<th>Water content</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Coefficient of variation (%)</th>
<th>Available water per pail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>---------</td>
<td>---------</td>
<td>----------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>2012</td>
<td>1</td>
<td>6</td>
<td>9±0.54</td>
<td>8</td>
<td>11</td>
<td>14.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6</td>
<td>8±0.45</td>
<td>7</td>
<td>9</td>
<td>13.7</td>
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<td>6</td>
<td>7±0.43</td>
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<td>8</td>
<td>16.1</td>
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<tr>
<td></td>
<td>26</td>
<td>6</td>
<td>7±0.31</td>
<td>6</td>
<td>8</td>
<td>11.0</td>
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<td>27</td>
<td>6</td>
<td>7±0.21</td>
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<td>7.8</td>
<td>73</td>
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<tr>
<td></td>
<td>30</td>
<td>6</td>
<td>6±0.31</td>
<td>5</td>
<td>7</td>
<td>12.9</td>
<td>64</td>
</tr>
<tr>
<td>2013</td>
<td>6</td>
<td>6</td>
<td>9±0.21</td>
<td>9</td>
<td>10</td>
<td>5.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6</td>
<td>10±0.17</td>
<td>9</td>
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<td>4.2</td>
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</tr>
<tr>
<td></td>
<td>18</td>
<td>6</td>
<td>10±0.21</td>
<td>9</td>
<td>10</td>
<td>5.3</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6</td>
<td>9±0.26</td>
<td>8</td>
<td>10</td>
<td>7.0</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>6</td>
<td>8.3±0.31</td>
<td>8</td>
<td>10</td>
<td>8.2</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>6</td>
<td>7.7±0.26</td>
<td>7</td>
<td>9</td>
<td>7.9</td>
<td>85</td>
</tr>
</tbody>
</table>
Table A7.2. Floret number per ear row for CG, DT, and NDT genotypes at V8 stage of growth for control, drought, and reduced red-to far red ratio (Reduced R:FR). Experiment #1 conducted at Arkell Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Floret number per ear row</th>
<th>Cat. Error ±</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>no ear&lt;sup&gt;-1&lt;/sup&gt; row&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>Control</td>
<td>22abc†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drought</td>
<td>23ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduced R:FR</td>
<td>22ab</td>
<td></td>
</tr>
<tr>
<td>DT</td>
<td>Control</td>
<td>15cd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drought</td>
<td>29a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduced R:FR</td>
<td>14d</td>
<td></td>
</tr>
<tr>
<td>NDT</td>
<td>Control</td>
<td>17bcd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drought</td>
<td>20bcd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reduced R:FR</td>
<td>18bcd</td>
<td></td>
</tr>
<tr>
<td>Standard error (±)</td>
<td></td>
<td></td>
<td>2.96</td>
</tr>
</tbody>
</table>

† Within columns, means followed by the same letter are not significantly different according to Tukey’s test (0.05).
Table A7.3. Plant dry matter, ear length, and floret number per ear row CG, DT, and NDT genotypes for three growth stages (V7, V8, and V9). Values averaged across control, drought, reduced red-to far red ratio (Reduced R:FR). Experiment #1 conducted at Arkell Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plant dry matter</th>
<th>Ear length</th>
<th>Floret number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V7   V8 V9</td>
<td>V7 V8 V9</td>
<td>V7 V8 V9</td>
</tr>
<tr>
<td></td>
<td>----g plant⁻¹----</td>
<td>----cm ear⁻¹-----</td>
<td>----no ear⁻¹ row⁻¹-----</td>
</tr>
<tr>
<td>CG</td>
<td>24   38 70</td>
<td>0.4 1 5</td>
<td>11 23 36</td>
</tr>
<tr>
<td>DT</td>
<td>23   31 64</td>
<td>0.3 0.7 2.9</td>
<td>9 19 36</td>
</tr>
<tr>
<td>NDT</td>
<td>24   33 59</td>
<td>0.2 0.7 2.9</td>
<td>8 18 35</td>
</tr>
<tr>
<td>Standard error (±)</td>
<td>3.05 3.95 10.39</td>
<td>0.09 0.24 0.65</td>
<td>1.99 2.22 1.22</td>
</tr>
</tbody>
</table>
Table A7.4. Plant dry matter, ear length, and floret number per ear row CG, DT, and NDT genotypes for three growth stages (V7, V8, and V10). Values averaged across 7.5 and 15 plants m$^{-2}$. Experiment #1 conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plant dry matter</th>
<th>Ear length</th>
<th>Floret number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V7</td>
<td>V8</td>
<td>V10</td>
</tr>
<tr>
<td></td>
<td>-----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>CG</td>
<td>22</td>
<td>35</td>
<td>60</td>
</tr>
<tr>
<td>DT</td>
<td>18</td>
<td>31</td>
<td>52</td>
</tr>
<tr>
<td>NDT</td>
<td>17</td>
<td>30</td>
<td>51</td>
</tr>
<tr>
<td>Standard error (±)</td>
<td>1.5</td>
<td>1.4</td>
<td>2.67</td>
</tr>
</tbody>
</table>
Table A7.5. Models parameters for the ear length and floret number per ear row vs plant dry matter relationship and floret number per ear row vs ear length for three genotypes and two experiments. Data pooled across control, drought, reduced red to far red ratio (R:FR), 7.5 plants m⁻² and 15 plants m⁻². Experiments conducted at Arkell (Experiment # 1) and Elora (Experiment # 2) Research Stations during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Model†</th>
<th>Experiment #</th>
<th>Genotype</th>
<th>n</th>
<th>Parameter ± standard error</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>α</td>
<td>t</td>
</tr>
<tr>
<td>Ear length vs plant dry matter</td>
<td>1,2</td>
<td>CG</td>
<td>327</td>
<td>0.06±0.01a‡</td>
<td>15.02±2.34a</td>
</tr>
<tr>
<td></td>
<td>1,2</td>
<td>DT</td>
<td>284</td>
<td>0.04±0.00360b</td>
<td>18.07±1.20a</td>
</tr>
<tr>
<td></td>
<td>1,2</td>
<td>NDT</td>
<td>345</td>
<td>0.04±0.0044b</td>
<td>18±1.67a</td>
</tr>
<tr>
<td>Floret number per ear row vs plant dry matter</td>
<td>1,2</td>
<td>CG</td>
<td>358</td>
<td>6±0.2a</td>
<td>18±0.87b</td>
</tr>
<tr>
<td></td>
<td>1,2</td>
<td>DT</td>
<td>353</td>
<td>5±0.14b</td>
<td>26±0.70a</td>
</tr>
<tr>
<td></td>
<td>1,2</td>
<td>NDT</td>
<td>345</td>
<td>5±0.12b</td>
<td>25±0.66a</td>
</tr>
<tr>
<td>Floret number per ear row vs ear length</td>
<td>1,2</td>
<td>CG</td>
<td>358</td>
<td>79.10±6.67a</td>
<td>0.03±0.03a</td>
</tr>
<tr>
<td></td>
<td>1,2</td>
<td>DT</td>
<td>353</td>
<td>61.09±5.04b</td>
<td>0.02±0.02a</td>
</tr>
<tr>
<td></td>
<td>1,2</td>
<td>NDT</td>
<td>345</td>
<td>63.56±4.26b</td>
<td>0.04±0.01 a</td>
</tr>
</tbody>
</table>

†Equation= Y= [α (X- t)/1 + β(X- t)]( X>t)

‡Different letters among treatments for each model parameter indicate significant differences using Z test (p<0.05).
8. Appendix B: Data for Chapter 3
Table B8.1. Variation in of water per pail and % of available water per pail from planting to V9 stages in drought treatments. Experiments conducted at Arkell Research Station, Guelph, Ontario, Canada during 2012 and 2013 growing seasons.

<table>
<thead>
<tr>
<th>Year</th>
<th>Days from planting</th>
<th>n</th>
<th>Available water Kg pail$^{-1}$</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Coefficient of variation (%)</th>
<th>Available water per pail %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>1</td>
<td>6</td>
<td>9±0.54</td>
<td>8</td>
<td>11</td>
<td>14.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6</td>
<td>8±0.45</td>
<td>7</td>
<td>9</td>
<td>13.7</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>6</td>
<td>7±0.43</td>
<td>5</td>
<td>8</td>
<td>16.1</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>6</td>
<td>7±0.31</td>
<td>6</td>
<td>8</td>
<td>11.0</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>6</td>
<td>7±0.21</td>
<td>6</td>
<td>7</td>
<td>7.8</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>6</td>
<td>6±0.31</td>
<td>5</td>
<td>7</td>
<td>12.9</td>
<td>64</td>
</tr>
<tr>
<td>2013</td>
<td>6</td>
<td>6</td>
<td>9±0.21</td>
<td>9</td>
<td>10</td>
<td>5.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6</td>
<td>10±0.17</td>
<td>9</td>
<td>10</td>
<td>4.2</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>6</td>
<td>10±0.21</td>
<td>9</td>
<td>10</td>
<td>5.3</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>6</td>
<td>9±0.26</td>
<td>8</td>
<td>10</td>
<td>7.0</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>6</td>
<td>8.3±0.31</td>
<td>8</td>
<td>10</td>
<td>8.2</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>6</td>
<td>7.7±0.26</td>
<td>7</td>
<td>9</td>
<td>7.9</td>
<td>85</td>
</tr>
</tbody>
</table>
Table B8.2. Equation model parameters ($\alpha$, $X_0$, $\beta$) for the relationship between grain yield, harvest index, kernel number vs plant dry matter at maturity, growth rates at silking, days to anthesis, days to silking, and anthesis-to-silking interval for early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (15 thinned to 7.5 plants m$^{-2}$) and controls (Control, 7.5 and 15 plants m$^{-2}$). Parameters correspond to Eq.[3.3]. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>n</th>
<th>Parameter ± Standard error</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain yield</td>
<td>Plant dry matter at maturity</td>
<td>628</td>
<td>$1.02^{***} \pm 0.05$</td>
<td>$70.2^{***} \pm 2.53$</td>
</tr>
<tr>
<td></td>
<td>Plant growth rate at silking</td>
<td>630</td>
<td>$69^{***} \pm 5$</td>
<td>$1.2^{***} \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>Days to anthesis</td>
<td>622</td>
<td>$-22^{***} \pm 0.4$</td>
<td>$81^{***} \pm 0.2$</td>
</tr>
<tr>
<td></td>
<td>Days to silking</td>
<td>630</td>
<td>$-22^{***} \pm 1.2$</td>
<td>$85^{***} \pm 0.9$</td>
</tr>
<tr>
<td></td>
<td>Anthesis-to-silking interval</td>
<td>630</td>
<td>$-150^{***} \pm 16$</td>
<td>$5^{***} \pm 0.1$</td>
</tr>
<tr>
<td>Harvest index</td>
<td>Plant dry matter at maturity</td>
<td>630</td>
<td>$0.12^{**} \pm 0.03$</td>
<td>$70^{***} \pm 0.66$</td>
</tr>
<tr>
<td></td>
<td>Plant growth rate at silking</td>
<td>628</td>
<td>$1.2^{***} \pm 0.1$</td>
<td>$1.5^{***} \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>Days to anthesis</td>
<td>615</td>
<td>$-0.3^{***} \pm 0.03$</td>
<td>$82^{***} \pm 0.2$</td>
</tr>
<tr>
<td></td>
<td>Days to silking</td>
<td>630</td>
<td>$-0.3^{***} \pm 0.03$</td>
<td>$85^{***} \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>Anthesis-to-silking interval</td>
<td>630</td>
<td>$-1^{***} \pm 0.1$</td>
<td>$5^{***} \pm 0.1$</td>
</tr>
<tr>
<td>Kernel number</td>
<td>Plant dry matter at maturity</td>
<td>617</td>
<td>$7.1^{***} \pm 0.7$</td>
<td>$69^{***} \pm 4.1$</td>
</tr>
<tr>
<td></td>
<td>Plant growth rate at silking</td>
<td>626</td>
<td>$750^{***} \pm 115$</td>
<td>$1.8^{***} \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>Days to anthesis</td>
<td>630</td>
<td>$-80^{***} \pm 1.6$</td>
<td>$80^{***} \pm 0.2$</td>
</tr>
<tr>
<td></td>
<td>Days to silking</td>
<td>630</td>
<td>$-90^{***} \pm 9$</td>
<td>$85^{***} \pm 1.6$</td>
</tr>
<tr>
<td></td>
<td>Anthesis-to-silking interval</td>
<td>630</td>
<td>$-350^{***} \pm 78$</td>
<td>$5^{***} \pm 0.2$</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant. All parameters correspond to equation 3.3.
Table B8.3. Equation model parameters ($\alpha, X_0, \beta$) for the relationship between plant growth rate around silking, days to anthesis, days to silking, and anthesis-to-silking interval with plant dry matter, ear length, and floret number at V9-10 stage of growth for early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (15 thinned to 7.5 plants m$^{-2}$) and controls (Control, 7.5 and 15 plants m$^{-2}$). Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>n</th>
<th>Parameter ± standard error</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\alpha$</td>
<td>$X_0$</td>
</tr>
<tr>
<td>Plant growth rate around silking‡</td>
<td>Dry matter at V9-10</td>
<td>126</td>
<td>0.11***±0.06</td>
<td>0.1ns†±0.06</td>
</tr>
<tr>
<td></td>
<td>Earl length</td>
<td>126</td>
<td>16**±7.1</td>
<td>1***±0.07</td>
</tr>
<tr>
<td></td>
<td>Floret number</td>
<td>126</td>
<td>3.6±15.2</td>
<td>15±79</td>
</tr>
<tr>
<td>Days to anthesis§</td>
<td>Dry matter at V9-10</td>
<td>126</td>
<td>82***±0.93</td>
<td>0.002***±0.0002</td>
</tr>
<tr>
<td></td>
<td>Earl length</td>
<td>126</td>
<td>76***±0.77</td>
<td>-0.02***±0.003</td>
</tr>
<tr>
<td></td>
<td>Floret number</td>
<td>126</td>
<td>75***±3.3</td>
<td>-0.002±0.001</td>
</tr>
<tr>
<td>Days to silking§</td>
<td>Dry matter at V9-10</td>
<td>124</td>
<td>84***±1.1</td>
<td>-0.002±0.0002</td>
</tr>
<tr>
<td></td>
<td>Earl length</td>
<td>126</td>
<td>80***±0.8</td>
<td>-0.02***±0.003</td>
</tr>
<tr>
<td></td>
<td>Floret number</td>
<td>126</td>
<td>87***±4.3</td>
<td>-0.004±0.001</td>
</tr>
<tr>
<td>Anthesis-to-silking interval§</td>
<td>Dry matter at V9-10</td>
<td>126</td>
<td>4.7***±0.69</td>
<td>-0.01**±0.003</td>
</tr>
<tr>
<td></td>
<td>Earl length</td>
<td>126</td>
<td>4.4***±0.54</td>
<td>-0.2***±0.05</td>
</tr>
<tr>
<td></td>
<td>Floret number</td>
<td>126</td>
<td>4±2</td>
<td>-0.01±0.01</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01, ***p<0.001. †ns: not significant. ‡ corresponds to equation 3.3; § corresponds to equation 3.4.
Table B8.4. Crop growth rate around silking, days to anthesis, days to silking, anthesis-to-silking interval, grain yield, dry matter accumulation, harvest index and kernel number for CG, DT, and NDT genotypes. Values are means across early season drought stress, early season reduced red-to-far red stress (R:FR), early high-density stress (15 thinned to 7.5 plants m\(^{-2}\)) and controls (Control, 7.5 and 15 plants m\(^{-2}\)). Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Genotype</th>
<th>Crop growth rate around silking g m(^{-2}) day(^{-1})</th>
<th>Days to anthesis Days to silking Anthesis-to-silking interval</th>
<th>Grain yield g m(^{-2})</th>
<th>Dry matter accumulation g g(^{-1})</th>
<th>Harvest index</th>
<th>Kernel number no m(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CG</td>
<td>31</td>
<td>68</td>
<td>70</td>
<td>2.2</td>
<td>944</td>
<td>1884</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>34</td>
<td>73</td>
<td>75</td>
<td>2.6</td>
<td>1078</td>
<td>2064</td>
</tr>
<tr>
<td></td>
<td>NDT</td>
<td>34</td>
<td>74</td>
<td>76</td>
<td>2.3</td>
<td>1067</td>
<td>2087</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td>2.66</td>
<td>1.05</td>
<td>1.01</td>
<td>0.31</td>
<td>116</td>
<td>229</td>
</tr>
<tr>
<td>2</td>
<td>CG</td>
<td>31.4</td>
<td>69.2</td>
<td>71.7</td>
<td>2.3</td>
<td>825</td>
<td>1688</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>39.1</td>
<td>75.1</td>
<td>78.2</td>
<td>3.3</td>
<td>785</td>
<td>1717</td>
</tr>
<tr>
<td></td>
<td>NDT</td>
<td>38.7</td>
<td>76.0</td>
<td>79.1</td>
<td>3.5</td>
<td>768</td>
<td>1710</td>
</tr>
<tr>
<td></td>
<td>Standard error (±)</td>
<td>3.34</td>
<td>0.27</td>
<td>0.59</td>
<td>0.37</td>
<td>96</td>
<td>196</td>
</tr>
</tbody>
</table>
Figure B8.1. Relationships between (A) grain yield or (B) harvest index with plant dry matter at harvest among the early drought stress (empty circles), early reduced red-to-far red ratio (R:FR) (closed circles) stress, or early high-density stress (15 thinned to 7.5 plants m$^{-2}$) (grey circles), control (grey triangle), high density (15 plants m$^{-2}$)(full triangles) and conventional density (7.5 plants m$^{-2}$)(empty triangle) treatments. Non-linear regression parameters are described in table 3. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.
Figure B8.2. Relationship of kernel number per plant as function of (A) plant growth rate around silking, (B) days to anthesis and (C) days to silking per plant for early drought stress (empty circles), early reduced red-to-far red ratio (R:FR) (closed circles) stress, or early high-density stress (15 thinned to 7.5 plants m$^{-2}$) (grey circles), control (grey triangle), high density (15 plants m$^{-2}$)(full triangles) and conventional density (7.5 plants m$^{-2}$)(empty triangle) treatments. Non-linear regression parameters are described in table 3. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.
Figure B8.3. Plant growth rate around silking (A) days to anthesis (B) and days to silking (C) as a function of plant dry matter at the V9-10 stage for early drought stress (empty circles), early reduced red-to-far red ratio (R:FR) (closed circles) stress, or early high-density stress (15 thinned to 7.5 plants m$^{-2}$) (grey circles), control (grey triangle), high density (15 plants m$^{-2}$)(full triangles) and conventional density (7.5 plants m$^{-2}$)(empty triangle) treatments. Non-linear regression parameters are described in table 3. Experiments conducted at Arkell (Experiment #1) and Elora (Experiment #2) Research Stations, during 2012-13 growing seasons. Ontario, Canada.
9. Appendix C: Data for Chapter 4
Table C9.1 Dry matter accumulation at maturity, grain yield, harvest index, and kernel number per plant for CG, DT, and NDT. Values are averages of plants grown at conventional (7.5 plants m$^{-2}$) and high density (15 plants m$^{-2}$). Experiments conducted at Elora Research Station during the 2012-13 growing seasons. Ontario, Canada.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Dry matter accumulation at maturity g plant$^{-1}$</th>
<th>Grain yield g g$^{-1}$</th>
<th>Harvest index</th>
<th>Kernel number no plant$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>189</td>
<td>92</td>
<td>0.45</td>
<td>416</td>
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<tr>
<td>DT</td>
<td>190</td>
<td>88</td>
<td>0.41</td>
<td>350</td>
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<tr>
<td>NDT</td>
<td>192</td>
<td>88</td>
<td>0.39</td>
<td>371</td>
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<tr>
<td>Standard error (±)</td>
<td>4.75</td>
<td>3.17</td>
<td>0.01</td>
<td>13</td>
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