MAIZE (*ZEA MAYS* L.) YIELD IN RESPONSE

TO LIGHT QUALITY AND DROUGHT

STRESS

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ABSTRACT

MAIZE (*ZEA MAYS* L.) YIELD IN RESPONSE

TO LIGHT QUALITY AND DROUGHT

STRESS

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University of Guelph, 2011   Professor C.J. Swanton

Shade avoidance triggered by light with altered red to far-red ratio (R:FR) reflected from neighbouring weed species may reduce the tolerance of maize to subsequent drought stress. This study was conducted to test the hypothesis that a reduction in root biomass caused by low R:FR will reduce the ability of maize to recover from subsequent drought stress. Field studies were conducted under non-limiting resource conditions. Maize seedlings were exposed to low R:FR reflected from ⁻neighbouring weeds until the 6 leaf tip stage. Subsequently, drought stress was imposed until leaf rolling of the youngest leaf was observed in all plants, after which water was provided until harvest. Drought stress reduced yield components consistently. The interaction, however, of light quality effects on maize root biomass and drought stress was observed only with rate of leaf appearance in one year of the study. This lack of interaction may be attributed to severity and duration of the drought stress.

Acknowledgements

Table of Contents

List of Tables

List of Figures

List of abbreviation

bHLH - basic helix-loop-helix

CPWC - critical period for weed control

CRY - cryptochromes

DAE - days after emergence

DAP - days after planting

GA – gibberellins

HI – harvest index

M_{BIO} - mean for aboveground biomass

M_{HI} - mean for harvest index

M_K - mean for kernel number

M_Y - mean for yield

P - phytochrome

Pfr – phytochrome far- red

Pfr:P - phytochrome equilibrium

PHOT - phototropins

PHYA - Phytochrome A

PHYB - Phytochrome B

PHYC - Phytochrome C

PHYD - Phytochrome D

PHYE - Phytochrome E

PIF3 - phytochrome interacting factor3

Pr – phytochrome red

R:FR - red to far red ratio

R:S - root to shoot ratio

WS - weedy + stress

 WFS - weed-free + stress

V1 - development stage with one fully expanded leaf (leaves with one visible collar)

V3 - development stage with three fully expanded leaves (leaves with three visible

collars)

W – weedy   .

WF - weed-free

Chapter 1: Literature review

Crop-weed competition

Weeds limit crop yield through direct competition for resources such as light, water, nutrients and space. The magnitude of grain yield loss depends on the weed density, weed species and the time of weed emergence relative to the crop; weeds that emerge later in crop development do not cause as great a yield loss as weeds that emerge with or shortly after the crop (Dew 1972; Knezevic *et al.* 1994; Swanton *et al.* 1999).

In order to minimize crop losses caused by early weed competition, recent research has highlighted the need to understand how changes in light quality, specifically R:FR (red to far red ratio) can influence crop physiology and development. Light quality signals such as R:FR reflected from the surface of neighbouring plants may be an early signal of oncoming competition (Ballaré *et al.* 1992). Plants have the ability to detect neighbouring plants by perceiving changes in light quality. Once plants detect this change, they may respond by developing shade avoidance characteristics such as thin leaves, elongated internodes, thinner stems, and a reduction in the root to shoot ratio (R:S) (Rajcan and Swanton 2004; Ballaré *et al.* 1987, 1990; Casal and Smith 1989; Ballaré and Casal 2000). In addition to signalling the onset of competition, recent research has suggested that R:FR should be viewed as a potential mechanism of competition (Liu *et al.* 2009; Page *et al.* 2010; Rajcan *et al.* 2004).

Rajcan *et al.* (2004) and Liu *et al.* (2009) demonstrated that reflected FR light from weed competitors during the early stage of maize growth and development was sufficient to induce modification in biomass partitioning, leaf orientation and leaf area

1

development. Under conditions of non limiting resources, Page *et al.* (2010) and Green-Tracewicz *et al.* (2011) found that neighbouring weeds induced typical shade avoidance characteristics in maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). In this recent research, shade avoidance was examined in response to early season weeds. Page *et al.* (2010) reported that plants exposed to light quality signals from neighbouring weeds set fewer kernels per plant and partitioned less biomass to the developing ear. Similarly, Green-Tracewicz *et al.* (2011) reported that soybean plants had fewer branches, pods and seeds per plant when grown near neighbouring weeds. More importantly, these studies also documented an increase in plant to plant variability in both crops. For example in maize, shade avoidance increased plant to plant variability in silking date, harvest index and kernel number per plant without affecting the mean or frequency distribution of plant size at maturity or yield loss for the whole plant population (Page *et al.* 2010).

In addition, crop sensitivity to changes in the R:FR ratio has been implicated in defining the critical period for weed control (CPWC). The CPWC is an interval of time in the life cycle of the crop that must be kept weed free to prevent unacceptable yield loss (Zimdahl 1980). Timing of weed emergence relative to crop emergence is an important parameter in estimating yield losses caused by weeds (Kropff *et al.* 1992). Weed control for maize must be effective from the 4 to the 10 leaf tip stage of growth in order to avoid significant yield losses (Hall *et al.* 1992). Green-Tracewicz *et al.* (2011) and Page *et al.* (2009) explored the role of R:FR as a possible mechanism by which the critical period could be explained. For example, Green-Tracewicz *et al.* (2011) determined that the critical period in soybean occurred between V1 and V3 by determining difference in

2

sensitivity to R:FR at different stages of soybean development. This research and the research of Page *et al.* (2010) has provided additional evidence in support of the R:FR ratio in defining the concept of the critical period.

The physiology of shade avoidance

The presence of neighbouring weeds can alter light quality signals being received by the crop plant. Since plants are stationary organisms, they are incapable of choosing their environment. As a result, the ability to sense changes in their surrounding environment is important for growth and survival (Lambers *et al.* 2008). The ability to detect changes in light for example, is crucial to photosynthesis. The light spectrum is a complex environmental factor composed of two components: light quantity and light quality. Light quantity refers to the number of photons; a photon is a quantum or a discrete bundle of light or electromagnetic energy (Lambers *et al.* 2008).

Light quality refers to wavelength composition; the range of 400-730 nm can be used by plants for photosynthesis. Red light and far red light are within the ranges of 660-730 nm and 730-760 nm, respectively (Holmes and Smith 1977b; Rivadossi *et al.* 2008). Green leaves absorb approximately 90% of violet and red light, whereas they reflect and transmit 30% of green and 90% of far-red light (Kasperbauer 1987). Because of the selective absorption of red light, light reflected from or passing through leaves is enriched in far-red wavelengths (730-760 nm) relative to red (Kasperbauer 1971, 1987).

Changes in light quality are detected by phytochrome which is a photoreceptor, consisting of a low molecular weight protein covalently attached to a photoreversible pigment (Schmitt and Wulff 1993). Phytochromes exist in two different photoconvertible forms: Pr or Pfr. Phytochrome acts as a sensor of space and time. The main function of

3

phytochrome is the detection of day-length. In addition to phytochromes, there are two

other plant signal transducing photoreceptors, cryptochromes (CRY), and the

phototropins (PHOT) (Gyula *et al.* 2003; Jiao *et al.* 2007; Schmitt and Wulff 1993; Smith

2000). Five different phytochromes have been identified. (Ballaré and Casal 2000;

Lambers *et al.* 2008; Moller *et al.* 2002). Phytochrome A (PHYA) is considered light-

labile (i.e., easily degraded in the Pfr form), whereas PHYB, PHYC, PHYD and PHYE

are more light-stable (Lambers *et al.* 2008; Moller *et al.* 2002). The Pfr is relatively

stable, requiring prolonged periods of exposure to far-red light in order to be converted to

Pr form. PHYA is implicated in the regulation of seedling germination, inhibition of

hypocotyl elongation, cotyledon expansion and anthocyanin synthesis (Ballaré and

Scopel 1997; Jiao *et al.* 2007; Moller *et al.* 2002). PHYB is described as the major

regulator of shade avoidance responses and appears to be responsible for sensing red

light; it plays a fundamental role in stem and leaf elongation, in early flowering and in

reduction of leaf area. PHYB plays a prominent role during all stages of plant

development (Ballaré *et al.* 1992; Franklin and Whitelam 2005; Jiao *et al.* 2007; Moller

et al. 2002). PHYC may play a role in detecting day length and regulating leaf extension

(Halliday *et al.* 1997; Qin *et al.* 1997). The remaining phytochromes PHYD and PHYE

are considered relevant in regulating seedling establishment, plant architecture, flower

induction and seed dormancy (Smith 2000). The functions of PHYA and PHYB are the

most well known, but additional research is needed to better understand their mechanisms

of action. The overall picture is that PHYB has a function at all stages of plant growth

and development, whereas PHYA, PHYD and PHYE play principal roles at particular

stages in the development of the plant (Smith 2000).

4

PHYB is the primary receptor of changes in the R:FR ratio. In the absence of light, PHYB remains in the inactive form (Pr). Under day light conditions this phytochrome absorbs higher levels of red light (R), which triggers the conversion of Pr to Pfr. This conversion to Pfr allows for the absorption of R wavelengths in the 665 nm to 730 nm range. In day light most phytochrome exists as Pfr; conversion of Pfr to Pr occurs slowly during darkness. This is the basis of the ability of the phytochrome to detect day length or, more exactly, length of the dark period (Holmes and Smith 1977b, 1977a; Jiao et al. 2007). Thus, the ratio of Pfr to total phytochrome (P) is called the phytochrome equilibrium (Pfr:P). Under day light conditions, Pfr:P has been reported to range from 0.54 (Holmes and Smith 1977b) to 0.6 (Smith 2000). This Pfr:P is very sensitive to changes in light quality and has been reported be as low as 0.1 (Smith 2000) or 0.04 (Holmes and Smith 1977b) under shaded canopy conditions.

At the cellular level, phytochromes are considered to be in the cytosol, regulating both the cellular and ionic balances within the cytoplasm and modulating cell extension (Smith 2000). Pfr within the cytoplasm can migrate into the nucleus. Once within the nucleus, this active form of the phytochrome will regulate genes by interacting with transcription factors such as phytochrome interacting factor3 (PIF3). PIF3 is a basic helix-loop-helix (bHLH) protein located in the nucleus. PIF3 acts as both a positive and negative regulator, mediating change in gene transcription (Bauer et al. 2004; Dubois and Brutnell 2009; Vandenbussche et al. 2005). This change in gene transcription influences the synthesis of auxins, gibberellins (GA), ethylene, brassinosteroid, and cytokinins. These hormones affect cell development and are linked with phytochrome signalling pathways (Von Arnim and Deng 1996; Jensen et al. 1998).

The lateral distribution of auxin within the plant occurs as FR light increases. An increase in auxin concentration in the stem causes stem elongation, and a reduction of auxin content within the roots results in delayed root development (Vandenbussche et al. 2005). Ethylene has been reported to accumulate under conditions of low R:FR . Ethylene is known to increase stem elongation and change leaf orientation in response to low R:FR light (Pierik et al. 2006). Moreover, ethylene also may act directly with GA. An increase in GA concentration within the cell caused by low R:FR light has been reported to enhance cell elongation by increasing the elasticity of cell walls (Lo'pez-Juez et al. 1995; Weller et al. 1994). In addition, cytokinins have been observed to increase in response to low R:FR light; however, little is known about how R:FR may influence cytokinin activity (Kurepin et al. 2007). It is clear from these studies that several factors including external signals and internal hormonal control influence plant morphological development.

Morphological responses of plants to low R:FR and plant plasticity

Plants are able to respond to changes in the R:FR ratio through a series of morphological and physiological processes designed to re-direct available resources (Ballaré et al. 1990; Weiner and Thomas 1992; Salter et al. 2003; Hotta et al. 2007; Liu et al. 2009). In maize, the R:FR ratio triggered typical shade avoidance responses such as increased plant height and leaf area, the re-orientation of leaves away from neighbouring plants and changes in biomass (Ballaré et al. 1990; Ballaré et al. 1987; Page et al. 2010; Rajcan et al. 2004). Liu et al. (2009) found that the presence of weeds from germination until silking reduced total dry matter accumulation in the stem and ear. The shade avoidance response in soybean reduced branches, pods per plant and seed number

(Green-Tracewicz *et al.* 2011). Similarly, in sunflower (*Helianthus annuus* L.) low R:FR light ratio reaching the sunflower stem at low densities reduced grain number and yield up to 20% in comparison with the sunlit control (Libenson *et al.* 2002). Weining and Delph (2001) found that in velvetleaf (*Abutilon theophrasti* Medic.) the early expression of shade avoidance greatly reduced the ability of the plant to make the same response at later stages in development. In this study, seedlings of velvetleaf once exposed to low R:FR were less responsive to a second treatment of low R:FR compared to seedlings grown under high R:FR. This evidence would suggest that the ability to express phenotypic plasticity may be limited.

Phenotypic plasticity is defined as an adaptive strategy that allows a genotype or individual organism to alter development in order to respond to variation in abiotic and biotic stresses (Bradshaw 1965; Sultan 2000). There are two types of plasticity. The first is long-term physiological plasticity, such as reduction in plant growth and total biomass under resource shortages or an increase in root biomass in response to low nutrients availability. Short term physiological plasticity refers to changes such as leaf orientation, adjustment of stomatal aperture and reduction in photosynthetic rate under hot weather conditions (Gedroc *et al.* 1996; Schlichting 1986; Sultan *et al.* 1998). The typical shade avoidance response in morphology and physiology triggered by a low R:FR ratio is one of the most fundamental examples of the importance of phenotypic plasticity (Smith 1982; Smith 2000).

Several studies have investigated the potential cost associated with the expression of phenotypic plasticity caused by shade avoidance in natural and agricultural systems (Ballaré and Scopel 1997; Dudley and Schmitt 1996; Kasperbauer *et al.* 1994; Schmitt *et*

7

al. 1995; Weijschedé *et al.* 2006; Weinig 2000) . In agriculture, crops such as maize are known to respond to biotic and abiotic stresses by expressing phenotypic plasticity in an effort to reduce potential yield loss (Thompson 1991). It is unknown whether the expression of phenotypic plasticity caused by changes in the R:FR ratio during early maize seedling development will alter the ability of maize to respond to subsequent drought stress. Several studies, however, have suggested that changes in plant partitioning such as an increase in the shoot: root ratio will reduce the tolerance of maize to subsequent moisture or nutrient limitation (Rajcan *et al.* 2004; Rajcan and Swanton 2001) Therefore, in this research experiments were conducted to test the hypothesis that a reduction in root biomass caused by low R:FR will reduce the ability of maize to recover from subsequent drought stress.

Chapter 2: Experiment

Introduction

Maize (*Zea mays* L.) is one of the most important cash crops in North America. Grain yield has increased approximately 100 kg ha^{-1} yr^{-1} in the United states and 80 kg ha^{-1} yr^{-1} in Canada since 1939 (Lee and Tollenaar 2007). The improvement and increase in grain yield was attributed to genetic gain, advances in agronomy and greater stress tolerance (Duvick 1977, 1984, 1992; Tollenaar *et al*. 1994; Tollenaar and Lee 2002). Despite these advances, stress caused by weed competition remains an important variable which may limit the yield potential of maize. The traditional view of crop-weed competition has focused on interspecific competition between crop and weed and capturing resources (Merotto Jr *et al*. 2009; Rajcan and Swanton 2001). Weeds limit crop yield by direct competition for light, water and nutrients. Weeds emerging with the crop or shortly thereafter have been found to cause greater yield loss than weeds emerging at later developmental stages (Dew 1972; Kropff 1988; Knezevic *et al*. 1994; Swanton *et al*. 1999). For example, Bosnic and Swanton (1997) reported that, in maize, yield loss from competition with barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) ranged from 6-35% depending on the time of weed emergence. Hall *et al*. in 1992 reported a rapid yield loss in maize if weed emergence occurred without control prior to the 3- to 4-leaf tip stage of maize; uncontrolled weeds during this critical period of maize caused 0.3-2.2% yield loss per day.

Weed competition in crops has been evaluated through the critical period for weed control and threshold studies. The critical period of weed control refers to an interval in the life cycle of the crop when it must be kept weed free to prevent yield loss

of the crop (Zimdahl 1980). The time of weed emergence relative to the crop, weed density and weed species are important variables influencing the duration of the critical period for weed control and the outcome of threshold studies (Hall *et al*. 1992; Bosnic and Swanton 1997; Kropff *et al*. 1992).

Weed competition can also be viewed as a resource independent process (Harper 1977). Independent factors such as light quality and hormonal signalling can contribute to the outcome of weed-crop competition (Aphalo *et al*. 1999; Rajcan and Swanton 2001). To date, limited attention has been paid to the role of resource independent variables such as the R:FR light ratio (Merotto Jr *et al*. 2009). Changes in light quality such as a lowering of the R:FR ratio have been suggested to be an early signal of impending competition during canopy growth (Ballare *et al*. 1992). Upon the detection of neighbouring weeds, crop plants respond by developing shade avoidance characteristics such as thin leaves, elongated internodes, and a low root: shoot dry matter ratio (Rajcan and Swanton 2004). These alterations in plant morphology may reduce the ability of the plant to respond to subsequent stress. Weining and Delph (2001) reported that seedlings of velvetleaf once exposed to low R:FR were less responsive to a second treatment of low R:FR compared to seedlings grown under high R:FR. This evidence would suggest that the ability to express phenotypic plasticity may be limited. Thus, it is possible to consider the potential for an interaction between low R:FR and subsequent abiotic stress such as drought.

To date, no study has examined the potential for this interaction. This study was conducted to test the hypothesis that a reduction in root biomass caused by low R:FR will reduce the ability of maize to recover from subsequent drought stress.

Materials and Methods

Plant material and growth condition

Field experiments were conducted in 2009 and 2010 at the Arkell Research

Station, University of Guelph, Guelph, ON, Canada (43°31'30"N and 80°10'50"W and

325 m above sea level). A University of Guelph maize hybrid (CG108×CG102, (Lee *et*

al. 2000; Lee *et al*. 2001) was selected for this experiment. An experimental unit

consisted of two maize seedlings planted in one 28-cm, 22-L plastic pail with three

drainage holes on the bottom. Pails were filled to the rim with Turface, a baked

montmorillonite clay growth medium (Turface MVP®; Profile Products LLC, Buffalo

Grove, IL, USA). These units formed part of semi-hydroponic system that has been used

for two decades for maize research under controlled water and nutrient conditions (e.g.,

Tollenaar and Migus 1984; Ying *et al*. 2000; Liu *et al*. 2009). Maize plants were irrigated

three times per day for 10 minutes each time. This frequency and duration of irrigation

was controlled by a timer clock connected with a 5-cm type 2-way PVC electric solenoid

valve that opened and closed the water supply. Each experimental unit received a

sufficient amount of nutrient solution as described by Tollenaar (1989). Maize seedlings

and turf grass were fed by four vinyl supply tubes (160-220 vinyl tubing), two vinyl pipes

for maize and two for turf grass with one 12.5-cm slotted drip spike in each pipe, thus

ensuring no water movement between root systems. Supply tubes were connected to

12.5cm poly pipes by inserted 3/4" GPH emitters. These emitters received the nutrient

solution through liquid fertilizer injectors (Advantage A40-2.5%, Dosmatic Inc.

California). Each pail received 2.25 L of nutrient solution per day.

Two light quality environments were established: (1) High R:FR (i.e., weed-free (ambient R:FR)) by filling the area surrounding the maize seedling with Turface MVP® in each experimental unit (weed-free treatment, R:FR=0.85) and (2) Low R:FR (i.e., weed) by filling the area surrounding the maize seedling with Turface plus perennial ryegrass grass (*Lolium perenne* L.) on the surface (weedy treatment, R:FR=0.45). The root system of the perennial ryegrass was separated from physical contact with the maize seedling by a clear plastic sheet barrier placed on the surface of each pail prior to the establishment of the treatments. A 10 by 15 cm slit was cut in the centre of the plastic where the two maize seeds were planted. At the time of seedlings appearance, the only difference between weed-free and weedy experimental units was the arrangement of the area surrounding the maize seedlings. During this time, the turf grass was maintained by manual clipping to prevent shading of maize seedlings. The light quality environment provided by the two treatments was characterized several times from emergence to the time of weed removal. The R:FR reflected light was measured 10 cm above the ring of ryegrass or Turface bordering the maize seedling centred within the pail using an inverted R:FR Skye sensor (SKR110/100 660/730 Skye Instruments Ltd. Llandrindod Wells, Powys, UK). These measurements were recorded at midday under conditions of full sunlight.

Experiment I. Effect of R:FR ratio on tolerance of maize to drought stress

In 2009, light quality treatments were isolated from one another in eight rows of 25 pails with 1.4 m between rows for a plant density 40×10^3 plant ha^{-1}. In each row three pails served as borders (i.e., one in the centre of the row separating weed-free and weedy treatments and one on either end of the row). The entire experimental area was surrounded by two border rows. Each experimental unit (defined as single pail) contained

two maize plants. The root systems of the two plants within the pail were not separated. To study the effects of R:FR and drought stress this experiment was set up as a split-plot design with four replications from germination until crop maturity. The main factor was drought stress and the sub plot factor light quality. The low R:FR (turfgrass) treatment was maintained from planting until weed removal at 6-leaf tip stage of maize development, approximately 30 days after planting (DAP) (Fig.1).

In 2010, the same experimental design (split plot) consisting of four replications was used. Each replication consisted of four rows of 100 pails. The entire experimental area was surrounded by four border rows. Once the drought stress was completed, as described below, the experimental units were re-arranged into a Latin square design at a final density of 80×10^3 plants ha^{-1}. A 24-row by 22-column matrix was established with 0.5 m between rows. The entire matrix consisted of 528 plastic pails. The effect of the low R:FR on maize seedlings was maintained from planting until weed removal at the 6th leaf tip stage of maize development, approximately 21 DAP. At this time four pails were harvested in each replication (i.e., 16 experimental units per treatment with a total of 32 plants sampled). Drought stress was initiated from the 6- to 12-leaf tip stage of maize, approximately 37 DAP. At this leaf stage, three pails per replication were harvested per treatment, n=24.

In both years, at the time of weed removal, plastic sheets that were used to separate maize and grass roots were removed from each pail. All pails involved in the drought stress and control treatments were covered with new plastic bags, wrapped around the base of maize stems in order to exclude precipitation from the pails. This

drought stress treatment was designed such that the treated plants experienced moderate stress but did not suffer severe stress overriding the differential response generated from the previous light quality treatments. To accomplish this goal, both drip line irrigation tubes per pail were removed and plants in the drought stress treatment were allowed to go without water and nutrients until leaf rolling was observed in >95% of all leaves per plant. In 2009, drought stress was initiated on June 23rd and lasted 14 days until July 7th, at which time >95% leaf rolling was observed. In 2010, drought stress was imposed from June 5th to June 23rd, a period of 17 days. Once >95% of leaf rolling occurred (i.e. July 7th 2009 and June 23rd 2010) drought stress was terminated 24 hours later (2009) or 48 hours later (2010) by restoring the full fertigation supply (i.e., 2 drip lines per pail).

Biomass measurements prior to and after drought stress

In both years, maize plants were measured for above and below ground biomass. Plants were harvested at the 6 and 12 leaf tip stages in both years. These leaf stages represented the beginning and the end of the water withdrawal period. Height and leaf tips number were recorded prior to the harvest. Leaf area was determined using a leaf area meter (LI-COR 3100 Area Meter, LI-COR Biosciences Lincoln, NE, USA). At harvest, individual plants were separated into leaves, stems and roots. Each component was bagged separately and dried to constant weight at 80° C prior to weighing.

Measurements at silking and at maturity

Each individual plant was monitored daily for the occurrence of tasseling emergence and silking on the remaining plants, n=640 and 256 in 2009 and 2010, respectively. At physiological maturity, the above ground biomass of these plants was

harvested. Each individual plant was separated into ears and stover (i.e., stem and leaves), and then dried to constant weight at 80° C prior to weighing. Ears were then shelled and kernel number and weight per plant were determined.

Experiment II. Root morphology

This study was conducted to determine the influence of the R:FR ratio on root morphology. In 2010, prior to the start of the experiment, a physical white plastic divider was installed in 32 pails. Each pail was filled with Turface MVP®. Two seeds were placed in each pail as described in experiment I. By placing the divider within the pail, the root system of each maize seedling was separated.

At the initiation of the drought stress, each root system was harvested, separated from the Turface, and washed. Once all roots were free of any Turface particles the root system for each plant was placed into a 30×42 cm transparent plastic tray containing water. The roots while floating in this water were carefully separated using a fine plastic needle and scanned using a large area scanner (LA 2400, Hewlett Packard, USA). Root traits were measured using WinRhizo software (Version PRO 2007, Regent Instruments Inc, Canada). Images of harvested roots were analyzed for total root length, root volume and root surface area. In addition, the image analyzer was set up to analyse root diameters ranging from <0.25 up to a maximum diameter of >6.5 mm.

Data Analysis

Statistical analyses were conducted using the PROC MIXED procedure of SAS 9.1 (SAS Institute, Cary, North Carolina, USA). Analyses were conducted separately for each year. F-tests were used to identify significant sources of variances within the model.

15

Standard split plot analysis was used in both years, with four replications and two factors. The whole plot factor was drought stress and the subplot factor was low R:FR. The low R:FR and drought stress as well as their interaction were considered as fixed effects. The effect of block, block by drought stress, row and each pail were treated as random effects.

Type I error of $P=0.05$ was established for all comparisons. Residuals of plant height, leaf tips, leaf area, plant biomass above and belowground, and maturity grain yield and kernel number, were plotted against predicted values to examine the homogeneity of variance, and the independence of error. The Shapiro-Wilk statistic was used to test normality of the error distributions. Outliers within the data set were identified using Lund's test of studentized residuals (Bowley 2008). Heterogeneity of error variance was corrected by square root transformation where appropriate. The population frequency distributions for silking date, biomass and yield components at maturity were compared using the non-parametric Kolmogorov-Smirnov two-sample test (Massey 1951; Young 1977). Differences in the shape of the distributions among treatments were described in terms of skewness and kurtosis, as described by Edmeades (1976). Plant to plant variability was described using coefficients of variation (CV).

Results

Shade Avoidance and drought stress

Maize seedlings responded to the presence of the weedy competitor by inducing a typical shade avoidance response in both years 2009 and 2010. The most immediate consequence of this was an increase in plant height in the weedy treatment (i.e., low R:FR ratio), documented in 2009 beginning at 13 DAP and at weed removal time (i.e., 30 DAP). In 2009 at the end of the drought stress, maize seedlings in the weed-free treatment were taller compared to those in the weedy treatment.

The implementation of drought stress reduced maize seedling height significantly in both the weed-free + stress (WFS) and weedy + stress (WS) treatments compared to the no stressed treatments (weed-free (WF) and weedy (W)). For example, plant height at the end of the drought stress in the WFS treatment was 55.3 cm, 55.6 cm in the WS, 65.8 cm in the W and 73.9 cm in WF (see Table 1, Fig. 2). Similarly in 2010 the difference in plant height remained from 11 DAP until weed removal time (i.e., 21 DAP) in the low R:FR light treatments, and at the end of the drought stress (i.e., 37 DAP). At the end of the drought stress period, no differences in plant height were detected between WF and W. Drought stress, however, resulted in a reduction in maize height in both drought stress treatments. For example, plant height in the WFS treatment was 52.6 cm, 52.8 cm in the WS, 65.2 cm in the W and 63.9 cm in WF (see Table 2, Fig. 3).

In 2009 leaf tips number did not differ among treatments when sampled at the 3- and 6-leaf tip stage of maize. Maize seedlings grown within the WF treatment had on average only 0.2 leaf tips more than those in the W treatment at the 6-leaf tip stage (Table 1, Fig 2). Once the drought stress was implemented from the 6- to 12-leaf tip stage, there

was a noticeable reduction in the rate of leaf appearance. By the 12-leaf tip stage, plants in both stressed treatments had an average of 1.7 fewer leaves than the control treatment WF. Similarly, both stress treatments differed in leaf number (1.3 leaves less) compared to the W treatment. No difference, however, was detected in leaf number between the WF and WS treatments.

In 2010, differences were detected in leaf tip number among treatments by the 5.6 leaf tip stage, 21 DAP and at the end of the stress period 37 DAP. Maize seedlings grown under WF, no stress conditions had on average 0.3 leaves more than those in the W, no stress treatment at the 5.6 leaf tip stage (Table 2, Fig. 3). This difference in leaf number was still observed at the end of the drought stress period (i.e., 37 DAP); the WF no stress treatment had an average of 0.2 leaves more than those in the W no stress. In those treatments in which drought stress was applied, there were 0.5 leaves fewer in the WS treatment compared to the WFS.

Leaf surface area, stem diameter, and aboveground biomass differed in response to the presence of aboveground weeds in 2009 and 2010. In 2009, leaf surface area at the 6-leaf tip stage was 247 cm^2 in the W treatment compared to 187 cm^2 in the WF treatment ($P < 0.007$, Table 1). In 2010, however, this difference was not detected at 6leaf tip stage (Table 2). In both years, maize seedlings receiving the drought stress treatment showed a clear reduction in leaf surface area when compared to the control no stress treatments. Leaf surface area, however, did not differ between drought stressed treatments in either year. Under the cool growing conditions (Appendix I) of 2009, the low R:FR did not reduce stem diameter, stem biomass, leaf biomass or total plant

biomass (Table 1, Fig 4). In 2010, low R:FR did reduce stem diameter and biomass, although leaf and total plant biomass were not reduced.

The interaction of light quality × drought stress varied between years. The interaction of these two variables was detected only in 2010 in leaf tip number (Fig. 3). In neither year, was an interaction was detected for stem diameter, leaf area, stem biomass, leaf biomass or total plant biomass (Table 4 and Table 5). This lack of interaction was likely accounted for by the magnitude of the drought stress. Both treatments receiving the drought stress did not differ, however, when compared to the control treatments (WF and W) both treatments differed significantly.

Root morphology at the time of weed removal, prior to initiation of drought tolerance

While the response of total root biomass to low R:FR differed between years, the effect on R:S was consistent across years. In 2009, no reduction in total root biomass was observed whereas in 2010, a reduction in root biomass of 21% in the W compared to the WF treatment was detected (Table 2, Fig. 4). In both years, the R:S was reduced in the W compared to the WF treatment. The presence of the aboveground weeds resulted in R:S ratio of 0.4 in W vs. 0.48 in WF treatment in 2009, and 0.46 in W vs. 0.52 in WF treatment in 2010 (Table 1 and Table 2).

In 2010, the presence of above ground neighbouring weeds until the 6 leaf tip stage reduced root surface area, volume and root diameter compared to roots grown under WF conditions. No differences were detected in root length (Fig. 5). Low R:FR reduced root surface area, root volume and root diameter by 13%, 19% and 4%, respectively

compared to the WF treatment. At the end of the drought stress period, no interaction was detected with exposure to low R:FR and drought stress (Table 3, Fig. 4).

Silking date

Drought altered the silking date in both WF and W treatments in 2009. Mean silking date and distribution of silking dates in both the WFS and WS treatments differed (P <0.05) from those in the WF and W no stress treatments. Silking was delayed 0.75 and 0.43 days in the WFS and the WS treatments relative the respective no stress treatments (P < 0.05; Table 6, Fig. 6). This effect of drought stress on silking date was not evident in 2010. In 2010 only the decreased R:FR light delayed silking (P < 0.057). No interaction with drought was detected (P < 0.79). Thus, a delay of 0.34 and 0.79 days in the W and WS treatment, respectively compared to the respective WF treatment could only be attributed to the effect of low R:FR (Table 7, Fig. 7). In addition, the distributions of silking dates between the treatments did not differ in 2010.

Maturity harvest

Plant to plant variability measured using the coefficient of variation (CV) for aboveground biomass at maturity, increased in the W 21%, the WFS 23% and WS 25% treatments compared with WF 17% in 2009 (Fig. 8). The frequency distribution of aboveground biomass showed a difference between WF vs W (P < 0.0001), WF vs WFS (P < 0.0001), WF vs WS (P < 0.0001) and WFS vs WS (P < 0.002). In 2010, the CV for aboveground biomass in the W was 21%, the WFS stress 20% and WS 21% treatments compared with WF 19%. The frequency distribution of aboveground biomass showed a difference from the WFS and WS treatments compared to the WF and W no stress

treatments ($P < 0.0001$) (Fig. 9). This result can be attributed to the higher magnitude of the drought stress in the second year.

At harvest, above ground biomass and harvest index (HI) were influenced in an additive manner by the low R:FR ratio plus drought stress. In 2009, the reductions in aboveground biomass compared to the WF control were 9%, 12% and 21% in the W, WFS and WS treatments, respectively (Fig. 8). In 2010, a reduction in aboveground biomass of 18% and 17% occurred in the WFS and WS treatments, respectively (Fig. 9). No differences were detected between the control treatments where no stress was applied.

In 2009, harvest index remained relatively stable, at 0.54, 0.55 and 0.55 in the control WF, the W and WFS treatments. A notable increase in HI (0.57) was observed only for the WS treatment (Fig.8). In 2010, harvest index increased to 0.53 in both WFS and WS treatments compared to 0.5 in WF and 0.51 in W no stress treatments (Fig. 9).

The CV for kernel number per plant (KNP) increased in 2009 in the WS 22%, WFS 20% compared with W 14% and WF 15% no stress treatments (see Fig. 10). This reduction and the notable shift in skewness indicated greater plant-to-plant variability in KNP when compared to the WF and W no stress treatments. In 2010, the CV of KNP in the WS and W no stress treatments increased relative to the WF (Fig. 11). This increase in variation in kernel number did not result in significant yield loss in either year. There were differences in the frequency and the distribution of KNP in 2009 at maturity in the WF vs W ($P < 0.003$), WF vs WFS ($P < 0.03$), WF vs WS ($P < 0.0001$) and WFS vs WS ($P < 0.04$) (Fig. 10). In 2010, no differences were detected between the control treatments however; both WFS and WS treatments differed in terms of frequency and distribution from the control treatments.

In 2009, maize KNP and yield at maturity were influenced by the low R:FR ratio. Drought stress did not result in an additional reduction in kernel number. For example, low R:FR reduced kernel number (as a percentage of the WF control) in the W, and WS treatments by 6% and only 2% in the WFS treatment (Fig. 10) . Moreover, the relative reductions in grain yield were 7%, 8% and 13% in the W, WFS and WS treatments, respectively. In 2010, the intensity of the drought stress was increased relative to 2009; as a result, drought stress reduced kernel number and yield. For example, drought stress reduced kernel number in the WFS and WS treatments by 6% and 7%, respectively (Fig. 11). The relative reduction in yield by drought stress was very similar to that in KNP the reduction in grain yield averaging 16% in both WFS and WS plants.

Table 1. Morphological features of maize seedlings at the 6 leaf tip grown under weed-free and weedy conditions prior to the onset of drought in 2009.

Response variable	Treatment		
	Weed-free	Weedy	P- value
Leaf tip number	6.5(0.15)	6.3(0.15)	0.45
Plant height (cm)	24.5 (0.69)	33.6 (0.71)	<.0001
Stem diameter (cm)	0.77 (0.05)	0.75 (0.05)	0.86
Root biomass (g plant^{-1})	0.4 (0.02)	0.38 (0.02)	0.4
Leaf surface area (cm^2 plant^{-1})	187 (15)	247 (16)	0.007
Stem biomass (g plant^{-1})	0.29 (0.03)	0.36 (0.03)	0.1
Leaf biomass (g plant^{-1})	0.56 (0.05)	0.67 (0.06)	0.27
Total biomass (g plant^{-1})	1.3 (0.09)	1.4 (0.09)	0.55
Root : Shoot	0.48 (0.03)	0.40 (0.03)	0.03

Values in treatment columns represent the mean and the standard error (±SE). P-values were determined by ANOVA and represent significance between treatments at $P \leq 0.05$.

Table 2. Morphological features of maize seedlings at the 6 leaf tip grown under weed-free and weedy conditions prior to the onset of drought in 2010.

Response variable	Treatment		P- value
	Weed-free	Weedy	
Leaf tip number	5.6 (0.09)	5.3 (0.09)	0.001
Plant height (cm)	23.3 (1.03)	26.1 (1.03)	0.0006
Root biomass (g plant $^{-1}$)	0.52 (0.03)	0.41 (0.02)	0.006
Stem diameter (cm)	0.89 (0.04)	0.85 (0.04)	0.009
Leaf surface area (cm^2 plant^{-1})	176.46 (7.53)	171.08 (7.53)	0.291
Stem biomass (g plant^{-1})	0.31 (0.01)	0.35 (0.01)	0.013
Leaf biomass (g plant^{-1})	0.61 (0.02)	0.58 (0.02)	0.227
Total biomass (g plant^{-1})	1.4 (0.06)	1.3 (0.06)	0.164
Root: Shoot	0.52 (0.03)	0.46 (0.03)	0.03

Values in treatment columns represent the mean and the standard error (±SE). P-values were determined by ANOVA and represent significance between treatments at $P \leq 0.05$.

Table 3. Morphological features of maize seedling roots at the 6 leaf tip grown under weed-free and weedy conditions prior to the onset of drought in 2010.

Response variable	Treatment		P- value
	Weed-free	Weedy	
Root length (m)	3921 (223.23)	3559 (223.23)	0.278
Root surface area (cm^2)	546.7 (27.69)	478 (27.69)	0.025
Root volume (cm^3)	6.2 (0.3)	5.04 (0.3)	0.002
Root diameter(mm)	0.45 (0.006)	0.43 (0.006)	0.007

Values in treatment columns represent the mean and the standard error (±SE). P-values were determined by ANOVA and represent significance between treatments at P≤ 0.05.

Table 4. Analysis of variance for maize harvested at the end of drought stress (12 leaf tip) from seedlings initially grown continuously under weedy or weed-free conditions and subsequently exposed to drought stress in 2009.

Covariance parameters	df	Leaf Tips	Height	Stem Diameter	Leaf Area	Stem Biomass	Leaf Biomass	Root Biomass	Root: Shoot Biomass	Total Biomass
						PrZ				
Block		0.2812	-[b]	0.4149	-	0.4149	0.4163	-	-	0.4355
Block*stress		-	0.0991	0.1982	-	0.1982	-	0.1027	0.4865	0.2135
Row		-	-	0.4396	-	0.4396	-	-	0.4026	-
Pail		-	0.1695	0.3242	0.2163	0.3242	0.2687	-	-	-
Residual		<.0001	0.0013	0.0023	0.0002	0.0023	0.0008	0.0416	0.0030	0.0008
Fixed Effects	ndf/ddf									
						P-values				
Light quality	1/16	0.0209	0.0187	0.3481	0.7002	0.3481	0.8156	0.6245	0.8861	0.8331
Drought stress	1/3	0.0007	0.0217	0.0527	0.0057	0.0527	0.0147	0.6782	0.0160	0.0327
Light quality × drought	1/16	0.1100	0.0777	0.1940	0.6363	0.1940	0.7007	0.7144	0.3628	0.2658

[b] Covariance parameter estimate= 0.

Table 5. Analysis of variance for maize harvested at the end of drought stress (12 leaf tip) from seedlings initially grown continuously under weedy and weed-free conditions and subsequently exposed to drought stress in 2010.

Covariance parameters	df	Leaf Tips	Height	Stem Diameter	Leaf Area	Stem Biomass	Leaf Biomass	Root Biomass	Root: Shoot	Total Biomass
						PrZ				
Block	-[b]	-	-	-	-	-	-	0.3484	-	
Block*stress	-	0.2879	-	-	-	0.4298	-	0.3679	-	
Row	0.4063	0.2074	-	0.1652	0.4623	0.3177	-	0.2119	-	
Pail	0.0704	0.1236	-	-	0.1885	0.2215	-	-	0.4601	
Residual	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	
Fixed Effects	ndf/ddf					P-values				
Light quality	1/60	0.0002	0.8248	0.3904	0.1201	0.7165	0.8371	0.4044	0.4138	0.9842
Drought stress	1/3	0.0021	0.0065	0.0032	0.0042	0.0028	0.0054	0.1211	0.0021	0.0463
Light quality × drought	1/60	0.0405	0.5276	0.4421	0.8296	0.8673	0.5067	0.6152	0.6684	0.9844

[b] Covariance parameter estimate= 0.

Table 6. Analysis of variance for maize harvested at maturity from seedlings initially grown under weedy and weed-free conditions and subsequently exposed to drought stress treatments in 2009.

Covariance parameters	df	Days to Silking	Grain	KNP	Harvest index[a]	Aboveground biomass
				PrZ		
Block	-[b]	-	-	-	-
Block*stress		0.13	-	-	0.17	-
Row		0.26	0.42	-	-	0.24
Pail		-	0.11	0.22	0.17	0.06
Residual		<.0001	<.0001	<.0001	<.0001	<.0001
Fixed Effects	ndf/ddf					
				P-Values		
Light quality	1/557	0.12	<.0001	<.0001	0.0020	<.0001
Drought stress	1/3	0.05	0.012	0.29	0.03	0.005
Light quality× drought	1/557	0.22	0.48	0.38	0.23	0.99

[a] Transformed as $Y=Y^2$

[b] Covariance parameter estimate= 0.

Table 7. Analysis of variance for maize harvested at maturity from seedlings initially grown under weedy and weed-free conditions and subsequently exposed to drought stress treatments in 2010.

Covariance parameters		Days to Silking	Grain	KNP	Harvest index[a]	Aboveground biomass
				PrZ		
Block		$-^b$	-	-	-	-
Block*stress		0.1946	0.11	0.37	-	0.12
Row		-	0.19	0.2	0.33	0.42
Pail		0.33	-	-	-	-
Residual		<.0001	<.0001	<.0001	<.0001	<.0001
Fixed Effects	ndf/ddf					
				P-Values		
Light quality	1/209	0.057	0.65	0.73	0.78	0.72
Drought stress	1/3	0.26	0.04	0.03	0.05	0.03
Light quality× drought	1/209	0.79	0.34	0.56	0.98	0.47

[a] Transformed as $Y=Y^2$
[b] Covariance parameter estimate= 0.
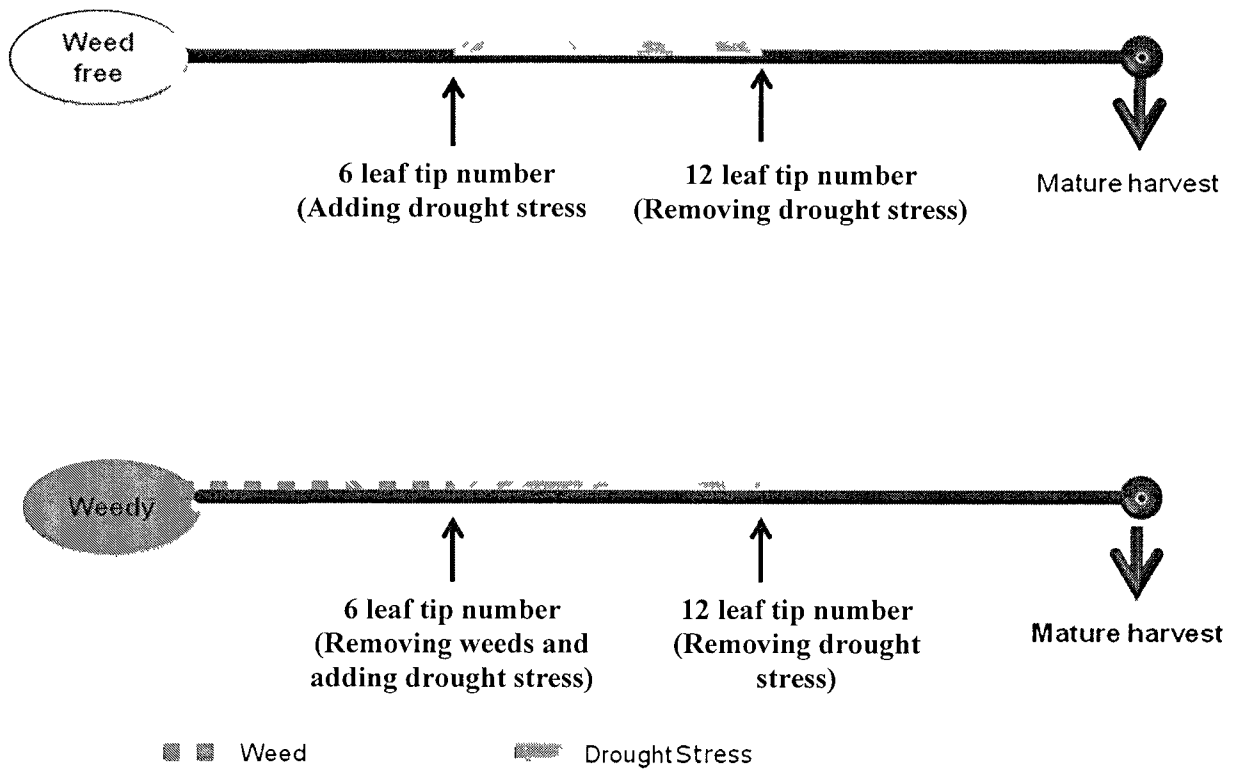
Figure 1. Stress stage for weed-free and weedy treatments
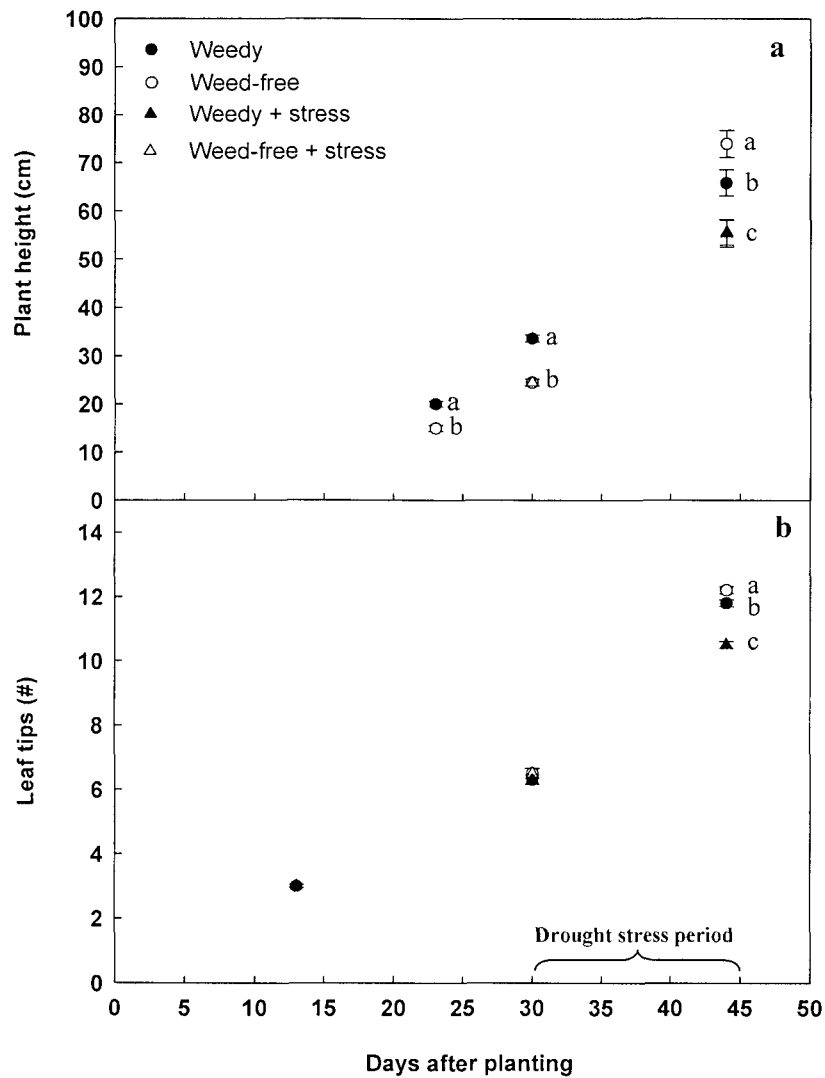
Figure 2. Repeated measures of plant height (a) and the number of visible of leaf tips (b) from planting to the removal of drought stress at the 12 leaf tip in 2009. ANOVA for both plant height and leaf tips number. Values with different letter indicate there was a significant difference by treatment ($P < 0.05$). Refer to Tables 1and 4 for associated P-values.
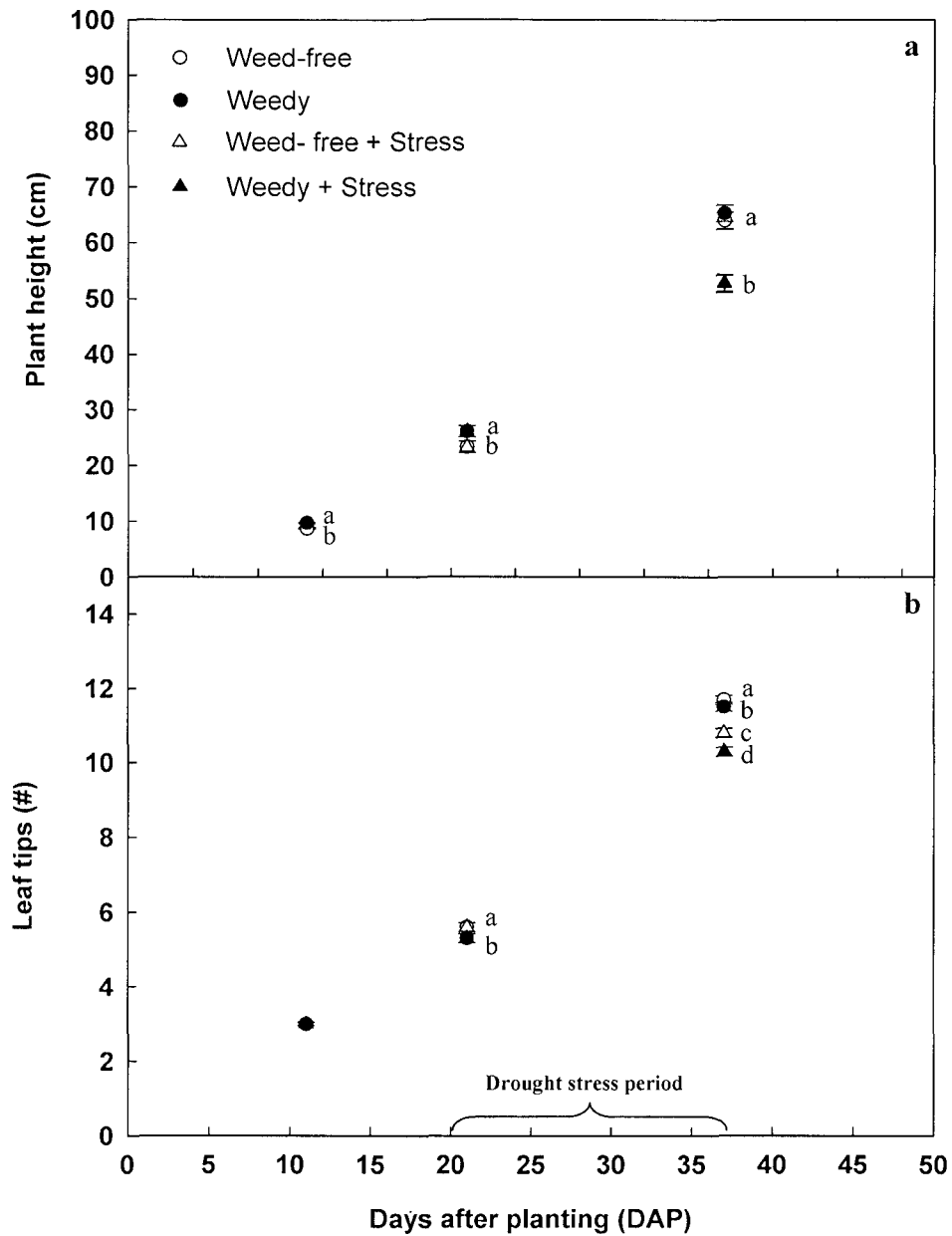
Figure 3. Repeated measures of plant height (a) and the number of visible of leaf tips (b) from planting to the removal of drought stress at the 12 leaf tip in 2010. ANOVA for both plant height and leaf tip number. Values with different letter indicate there was a significant difference by treatment ($P < 0.05$). Refer to Tables 2 and 5 for associated P-values.
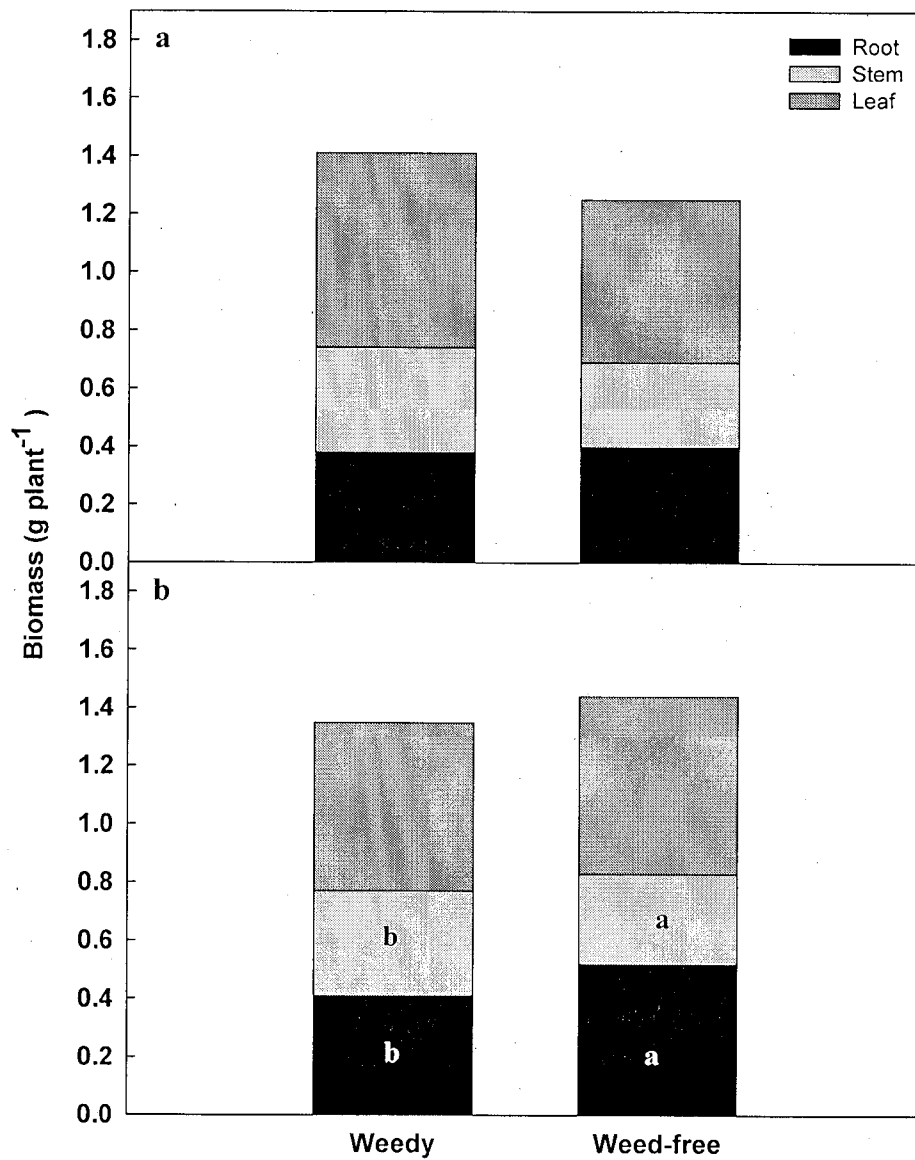
Figure 4. Biomass distribution in root, stem and leaf of maize seedlings grown under continuously weedy or weed-free conditions prior to the onset of drought at (a) 30 DAP in 2009 and (b) 21 DAP in 2010. Values with different letter indicate there was a significant difference by treatment. Refer to Tables 1 and 2.
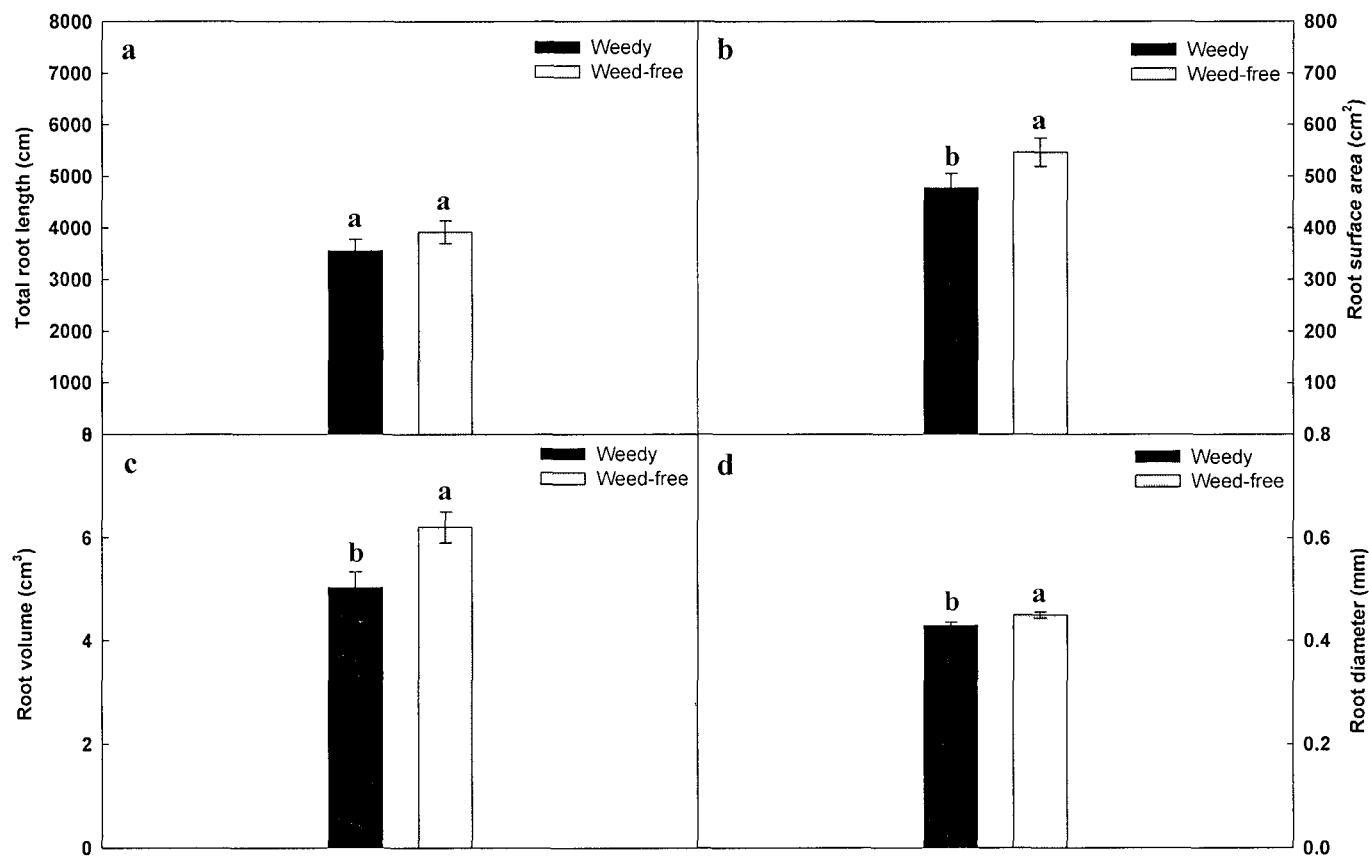
Figure 5. Morphological changes in maize seedling roots grown under weed-free and weedy conditions prior to the onset of drought in 2010. (a) Total length of root system; (b) root surface area; (c) root volume and (d) root diameter. Values with different letter indicate there was a significant difference by treatment.

Frequency %

WF
M=70.1
CV=1.8
K= 0.3
S= 0.3

W
M=70.1
CV=2
K= - 0.3
S= 0.21

M=70.9
CV=2.1
K= -0.21
S= 0.611

WS
M=70.6
CV=2.1
K= 0.51
S= 0.676

Days after planting (DAP)
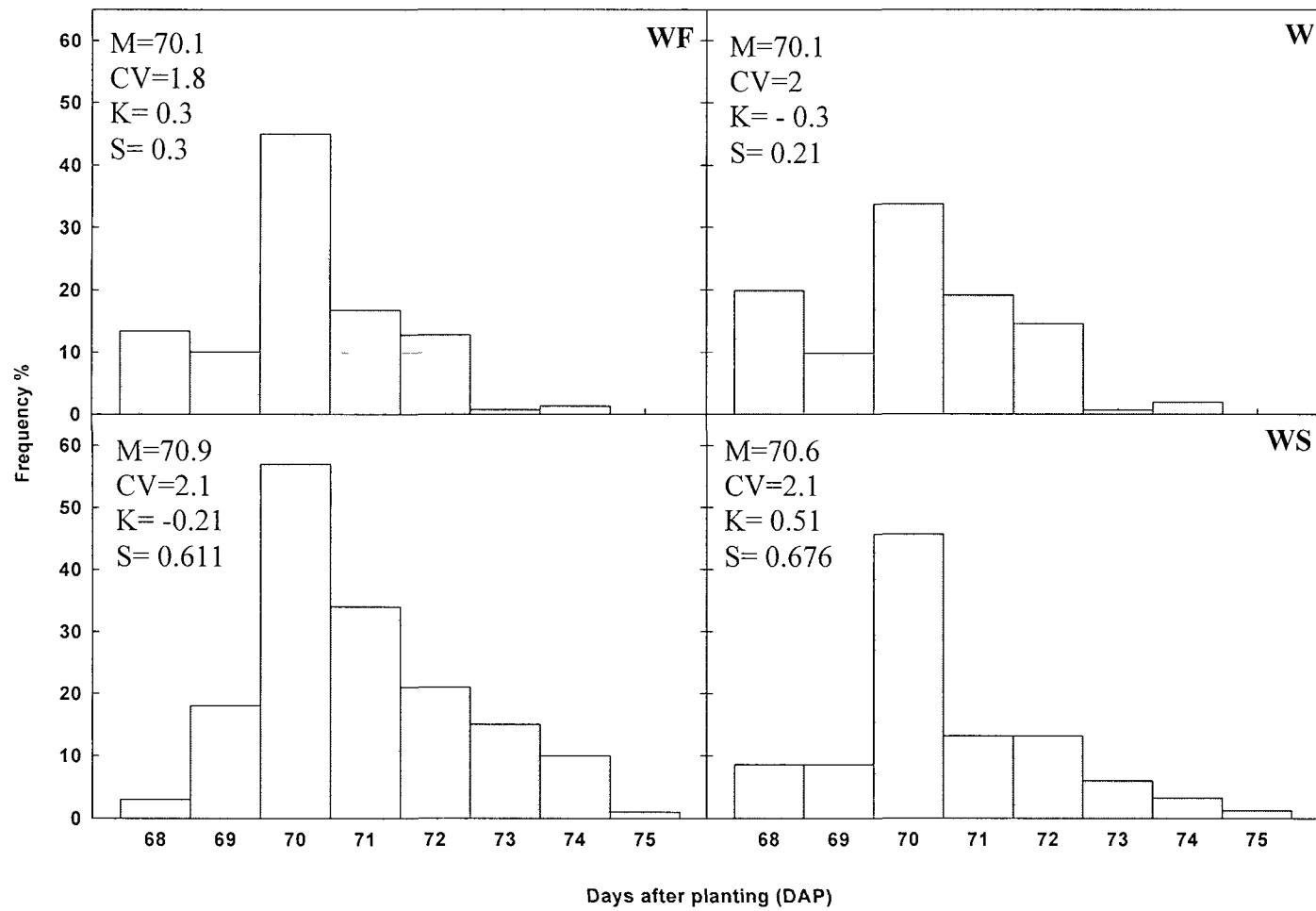
Figure 6. Maize silking dates for the (WF) weed-free, (W) weedy, (WFS) weed-free + stress and (WS) weedy + stress conditions in 2009. Stress treatments differed in the mean silking date (ANOVA, $P < 0.05$). The Kolmogorov-Smirnov two-sample test also indicated that the frequency distribution of the individual plants differed for stress treatments (weed-free + stress and weedy + stress) ($P < 0.0001$ and $P < 0.02$, respectively), compared to no stress treatments. The coefficients of variation (CV), skewness (S) and kurtosis (K) of the frequency distribution are presented.

Days after planting (DAP)
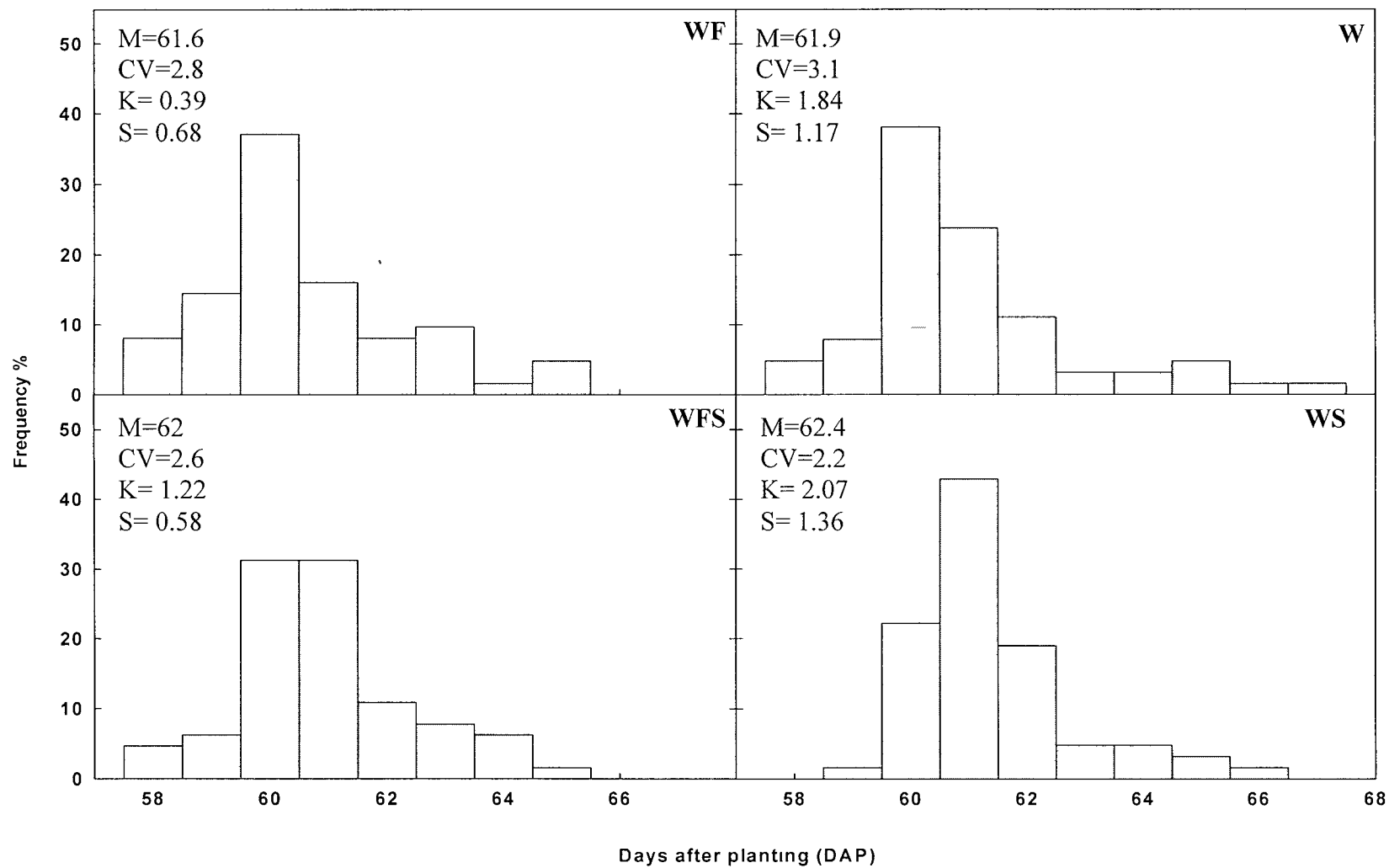
Figure 7. Maize silking dates for (WF) weed-free, (W) weedy, (WFS) weed-free +stress and (WS) weedy + stress conditions in 2010. Light quality, stress treatments and their interaction did not differ in the mean silking date ($P < 0.057$, $P < 0.26$ and $P < 0.79$) respectively. The Kolmogorov-Smirnov two-sample test also indicated that the frequency distribution of the individual plants did not differ among weedy, weed-free + stress and weedy + stress treatments ($P < 0.298$, $P < 0.253$ and $P < 0.237$, respectively) compared to weed-free. The coefficients of variation (CV), skewness (S), and kurtosis (K) of the frequency distribution are also presented.

Figure with four panels (WF, W, WFS, WS) showing Harvest index (g g^{-1}) versus Aboveground biomass (g plant^{-1}) with Frequency %.

WF
$M_{BIO}= 253$
$M_{HI}= 0.54$
$CV_{HI}= 11$
$K= 0.7$
$S=-0.3$
$CV_{BIO}=17$

W
$M_{BIO}= 232$
$M_{HI}= 0.55$
$CV_{HI}= 12$
$K=- 0.05$
$S=-0.4$
$CV_{BIO}=21$

WFS
$M_{BIO}\ 218$
$M_{HI}= 0.55$
$CV_{HI}= 17$
$K= 0.5$
$S=-1$
$CV_{BIO}=23$

WS
$M_{BIO}= 196$
$M_{HI}= 0.57$
$CV_{HI}= 19$
$K= 1$
$S=-0.7$
$CV_{BIO}=25$

39
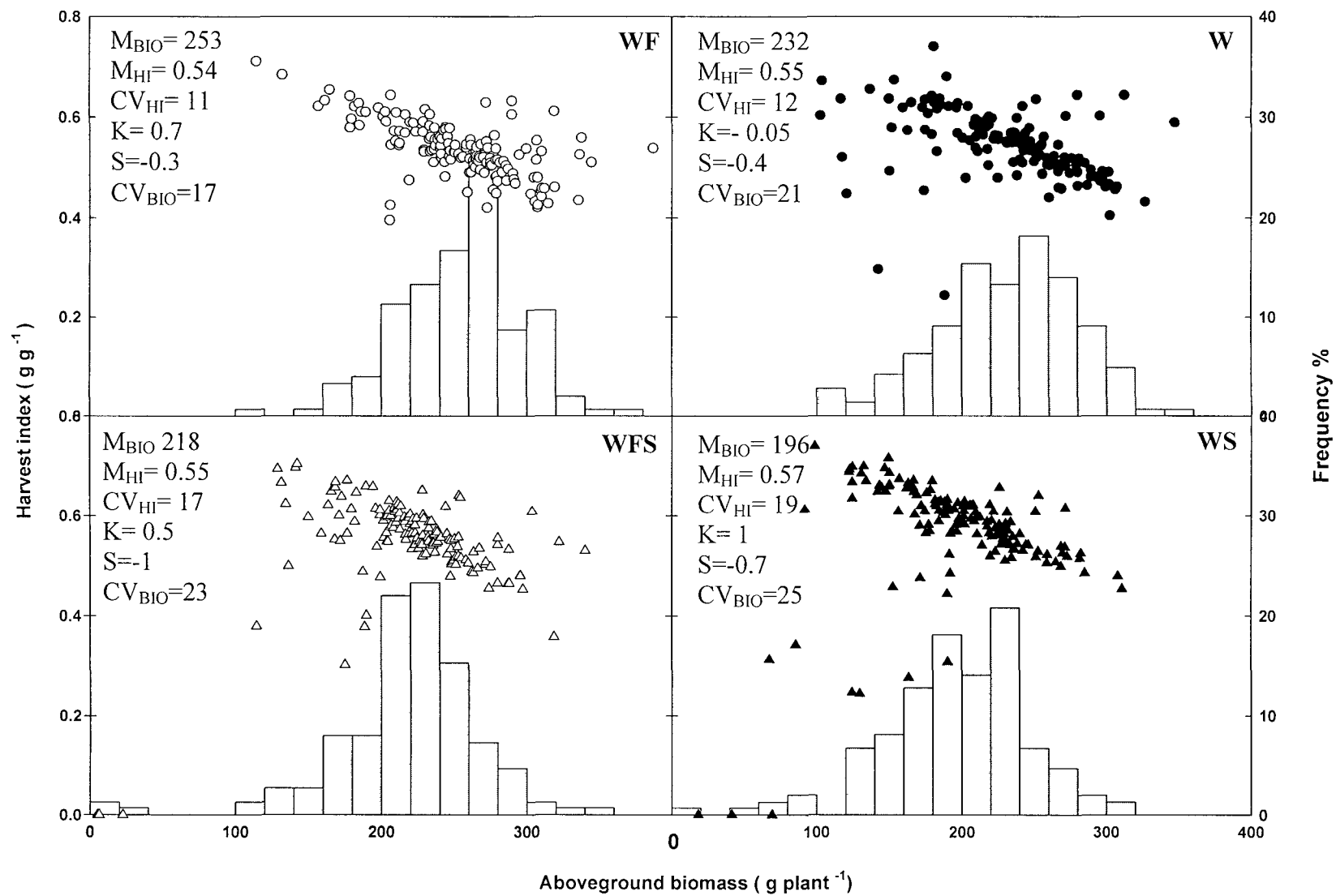
Figure 8. Aboveground biomass at maturity and harvest index of plants that developed under (WF) weed-free conditions, (W) weedy conditions, (WFS) weed-free + stress conditions and (WS) weedy + stress conditions in 2009. The Kolmogorov-Smirnov two-sample test also indicated that the frequency distribution of the individual plants differ among weedy, weed-free + stress and weedy + stress treatments ($P < 0.001$) compared to weed-free. M_{BIO} = mean for aboveground biomass. M_{HI} = mean for harvest index. The coefficient of variation (CV), skewness (S), kurtosis (K) of the frequency distribution are also presented.
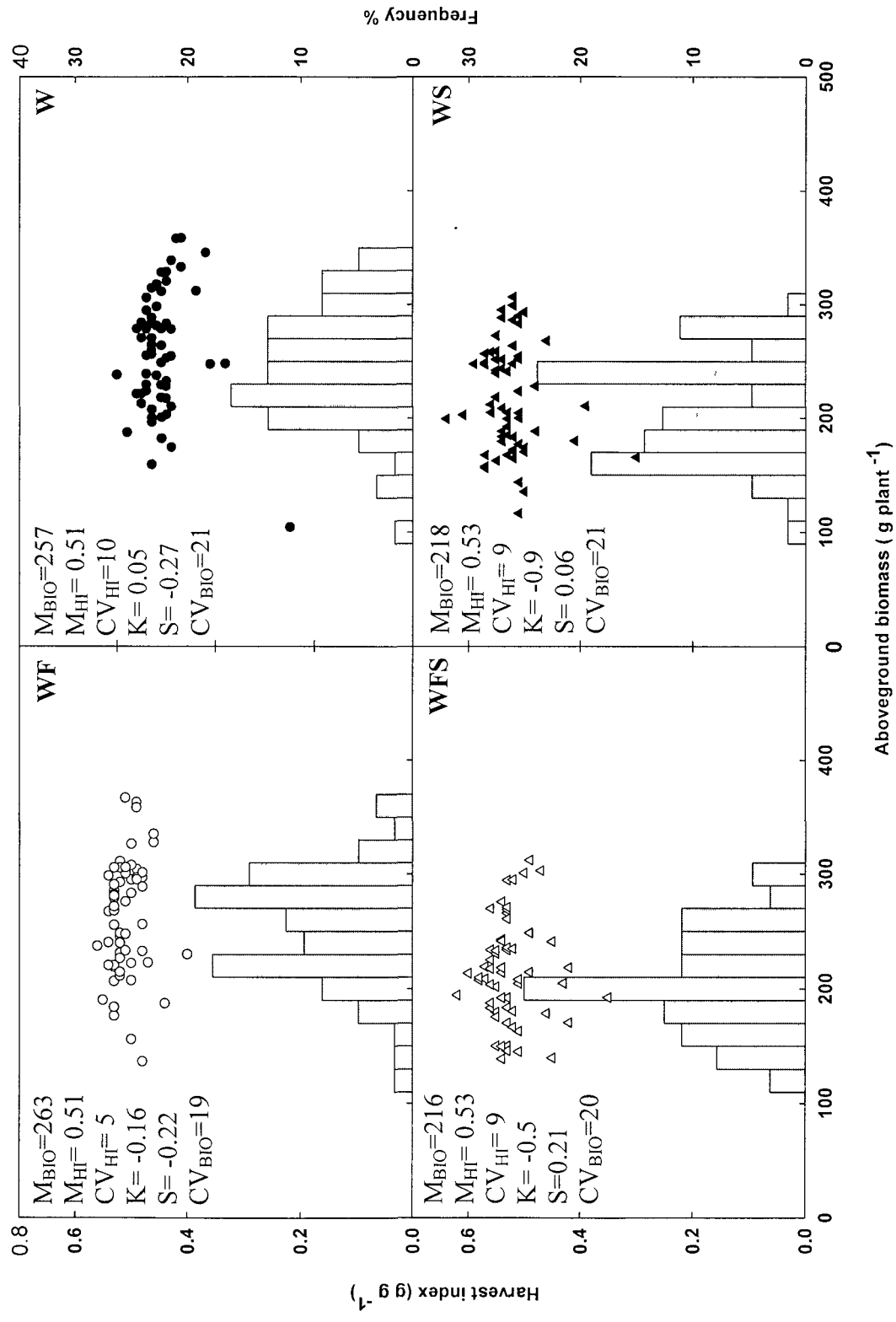
Figure 9. Aboveground biomass at maturity and harvest index of plants that developed under (WF) weed-free conditions, (W) weedy conditions, (WFS) weed-free + stress conditions and (WS) weedy + stress conditions in 2010. The Kolmogorov-Smirnov two-sample test also indicated that the frequency distribution of the individual plants did not differ among non stress treatment ($P > 0.05$), however it differed for stress treatments weed-free + stress and weedy + stress treatments population compared to on stress treatments ($P < 0.0001$). M_{Bio} = mean for aboveground biomass. M_{HI} = mean for harvest index. The coefficient of variation (CV), skewness (S) and kurtosis (K) of the frequency distribution are also presented.
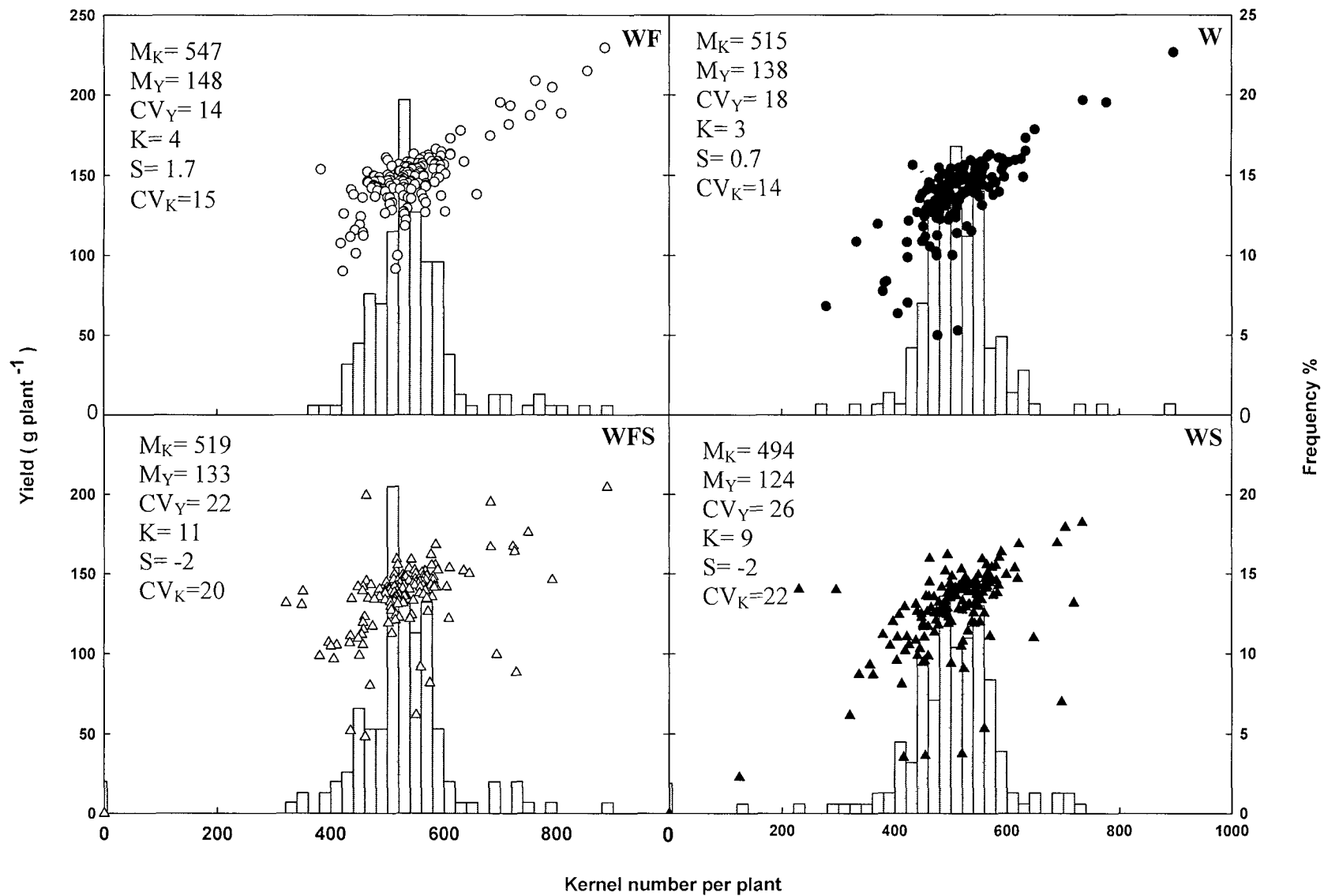
Figure 10. Kernel number at maturity and kernel weight of plants that developed under (WF) weed-free conditions, (W) weedy conditions, (WFS) weed-free + stress conditions and (WS) weedy + stress conditions in 2009. The Kolmogorov-Smirnov two-sample test also indicated that the frequency distribution of the individual plants differed among weedy and weedy + stress treatments ($P < 0.0001$, and $P < 0.0002$) compared to weed-free and weed-free + stress, respectively. M_K = mean for kernel number. M_Y = mean for yield. The coefficient of variation (CV), skewness (S) and kurtosis (K) of the frequency distribution are also presented.

Figure showing four scatter/histogram panels (WF, W, WFS, WS) of Yield (g plant⁻¹) versus Kernel number per plant.

WF
$M_K= 525$
$M_Y= 135$
$CV_Y= 18$
$K= 2.1$
$S=-1.24$
$CV_K= 13$

W
$M_K=530$
$M_Y= 130$
$CV_Y= 24$
$K= -0.5$
$S= -0.4$
$CV_K= 17$

WFS
$M_K= 492$
$M_Y=113$
$CV_Y= 21$
$K= 0.98$
$S= -0.6$
$CV_K= 14$

WS
$M_K= 488$
$M_Y= 114$
$CV_Y= 23$
$K= 0.03$
$S= -0.7$
$CV_K= 16$

Yield (g plant^{-1})

Frequency %

Kernel number per plant

45

Figure 11. Kernel number at maturity and kernel weight of plants that developed under (WF) weed-free conditions, (W) weedy conditions, (WFS) weed-free + stress conditions and (WS) weedy + stress conditions in 2009. The Kolmogorov-Smirnov two-sample test also indicated that the frequency distribution of the individual plants did not differ among non stress treatments ($P > 0.05$), however it differed among stress treatments weed-free + stress and weedy + stress treatments ($P < 0.003$ and $P < 0.001$, respectively) , compared to their respective no stress treatments. M_K = mean for kernel number. M_Y = mean for yield. The coefficient of variation (CV), skewness(S) and kurtosis (K) of the frequency distribution are also presented.

Discussion

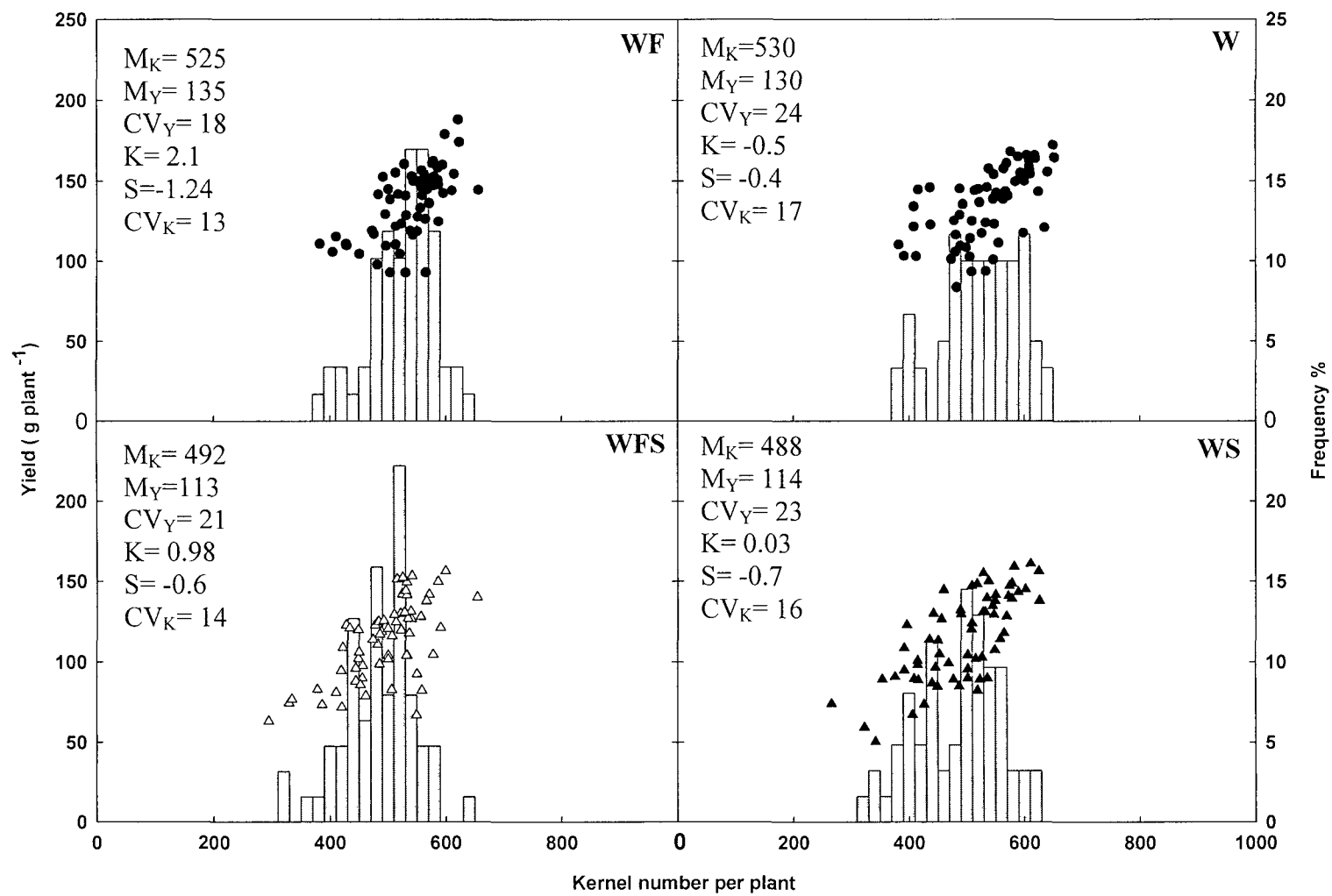Our field methods were designed to establish two different light quality environments (high R:FR and low R:FR), while minimizing the potential confounding effects of direct competition for water, nutrients and light. Maize seedlings responded to the presence of neighbouring weed competitors soon after emergence by triggering a shade avoidance response. This response consisted primarily of an increase in plant height, a reduction in leaf tip number and in the root: shoot ratio. These morphological changes illustrated the level of plasticity within maize and demonstrated the complexity of the relationship between plant development and light quality environments (Ballaré *et al.* 1990; Smith 1982). These responses are typical as shade avoidance is considered to be an adaptive response enabling a plant to anticipate and avoid competition for light (Ballaré *et al.* 1990; Ballaré *et al.* 1987; Schmitt and Wulff 1993).

An increase in plant height and a reduction in rate of leaf appearance caused by low R:FR light has been reported previously in several studies (Gramig and Stoltenberg 2009; Green-Tracewicz *et al.* 2011; Kasperbauer *et al.* 1994; Liu *et al.* 2009; Maddonni *et al.* 2002; Markham and Stoltenberg 2009; Page *et al.* 2009; Rajcan *et al.* 2004). For example, Liu *et al.* (2009) documented an increase in plant height in maize from the reflected low R:FR light in the presence of *Amaranthus retroflexus* L. (redroot pigweed) between 6 to15 leaf tip stage. In addition, Page *et al.* (2009) also reported a reduction in rate of leaf appearance between 9 to 15 days after planting compared to the weed-free control.

Low R:FR reflected from the aboveground leaf and stem tissues of neighbouring weeds reduced maize seedling root: shoot ratio and root biomass. Page *et al.* (2009)

reported a reduction in root biomass 12 days after emergence (DAE) and a lower root to shoot ratio three DAE. Similarly maize seedlings developed a greater shoot biomass relative to the root biomass when exposed to low R:FR from the 4 to the 9 leaf tip stage of maize (Rajcan *et al*. 2004). In addition, root analysis revealed that aboveground weeds reduced root surface area, root volume and root diameter. Recently, Afifi and Swanton (2011) reported that low R:FR light delayed the emergence of the root radical, reduced crown root length, diameter, leaf surface area at 4 leaf tip stage. In the current experiment, when maize seedlings were harvested following the implementation of the drought stress, seedlings grown under both WFS and WS experienced a reduction in plant height, stem diameter, stem biomass, leaf biomass, total plant biomass, leaf surface area and leaf tip number appearance. Hsiao (1973) suggested that stem and leaf elongation and root growth were the most sensitive parameters to mild drought stress. NeSmith and Ritchie (1992) reported that adding drought stress for a limited period of time such as between the 8 or 9 leaf tip number (i.e., approximately 18 to 21 days after emergence) reduced leaf surface area and delayed rate of leaf appearance. Moreover, in other studies (Bruce *et al*. 2002; Earl and Davis 2003; Wolfe *et al*. 1988) drought stress caused a reduction in leaf surface area as result of temporary leaf rolling and leaf wilting or accelerated leaf senescence. In addition, a reduction in C fixation per leaf as a result of stomatal closure was reported to reduce photosynthesis (Michelena and Boyer 1982). Jones *et al*. (1986) suggested that a reduction in leaf area and corresponding increase in root biomass caused by drought stress alone was a adaptive strategy to enhance water and nutrient uptake.

The interaction of light quality × drought stress varied between years. The interaction of these two variables was detected only in 2010. This was similar to Page *et al.* (2011), who found that the interaction of light quality and drought stress applied at silking was variable between years. For example, in the first year of their experiment the relative reduction in grain yield was 1%, 24% and 37%, and similarly the reduction in KNP was 1%, 24% and 35% in the W, WFS and WS treatments respectively. In the second year of the same experiment no interaction was observed. This lack of consistency may be attributed to the timing of the stress relative to the growth stage and the magnitude of the stress.

Drought stress delayed silking in 2009 and low R:FR light delayed silking in 2010. The delay in silking in 2009 caused by drought stress also reduced rate of leaf appearance and leaf surface area development. These changes would have contributed to a reduction in the rate of photosynthesis (Edmeades *et al.* 2000) . This effect of drought was not evident in 2010. The observed delay in silking was attributed only to the effect of low R:FR. Page *et al.* (2011) reported a delay in silking date of 1.6 days caused by drought stress compared to the control treatment in 2008; however, in 2009 this relationship was not evident.

Drought stress increased HI measured at maturity in both years of the study. HI for drought stress plants ranged from 0 to 0.74 compared to a range of 0.4 to 0.71 in the WF control. A similar increase was reported by Cerrudo *et al.* (2010). In this research HI increased with increasing delaying weed control from the 3 to the 10 leaf stage of maize. This delay in W control resulted in an increase in HI of 4.5% to 12%, respectively compared to the WF control. The ability of HI to remain stable or to increase under stress

conditions may be attributed to partitioning of plant dry mater to reproductive structures at the critical time when kernel number is determined (Andrade et al. 1999). This would suggest that maize plants when stressed may suffer a higher reduction in plant dry weight than grain yield.

Maize kernel number per plant was influenced in an additive manner by low R:FR plus drought stress in 2009. In 2010, only drought stress contributed to the reduction in kernel number. Previous research has shown that low R:FR reflected from the green stem and other tissues of neighbouring weeds can reduce kernel number. Page *et al.* (2010) reported that maize plants set fewer kernels per plant and partitioned less biomass to the developing ear in response to neighbouring weeds. In 2010, this response was not evident and may be attributed to ideal growth conditions that occurred in this year. In 2010, the average temperatures for the months of July and August were 21° and 19° C, respectively, compared to 16° C and 17° C in 2009 (see Appendix I). Temperature may play a modifying role in the expression of the physiological responses triggered by shade avoidance; however, no research has been reported to date on this interaction.

The aboveground biomass and grain yield of maize were reduced both by low R:FR and drought stress in 2009. Similar to kernel number, only drought stress affected aboveground biomass and grain yield in 2010. Page *et al.* (2011) reported that in the absence of non limiting resources, the presence of neighbouring weeds from seedling emergence until the 10th leaf tip stage followed by drought stress two weeks before silking, reduced aboveground biomass in both years. Over the two years of their experiment, the reduction in biomass ranged from 2 to 4%, 15 to 22 % and 16 to 25 %, in WF control, WFS and WS, respectively compared with the weed-free control. In similar

fashion, maize grain yield was reduced in both years in the present work; this reduction ranged from 1%, 18 to 24% and 35 to 37 % in the W control, WFS and WS treatments, respectively. The extent of yield loss in maize caused by weeds is determined by the time of weed emergence relative to the crop (John *et al.* 1985; Kropff and Spitters 1991) . Weeds that emerge with the crop are most competitive. For example, Bosnic and Swanton (1997) reported that, in maize, yield loss from early competition with barnyard grass was 35% compared with 6% yield loss from weeds that emerged after the 4leaf tip. This observation suggests that yield loss caused by weeds that emerge with the crop is possibly explained by understanding the role of low R:FR and the sensitivity of differing maize hybrids to this signal.

The hypotheses for this study stated that a reduction in root biomass caused by low R:FR will reduce the ability of maize to recover from subsequent drought stress. This hypothesis was predicated upon two premises. The first premise was based upon the observation that low R:FR reflected from neighbouring weeds can reduce root biomass and the R: S. Such changes would suggest that under field conditions, failure to control weeds that emerge with the crop would potentially alter the ability of maize to tolerate subsequent abiotic stresses. The second premise is based on work published by Weinig and Delph (2001). In this paper the authors suggest that once plasticity has been expressed in response to shade avoidance, the opportunity costs associated with this response will limit a plant's ability to express a second adaptive response to subsequent stress.

This is the first study to explore the response of maize seedlings to the interaction of low R:FR and subsequent drought stress. Although this interaction was not evident in either year of the study, the results of this study confirm the potential for this interaction and the need for further research.

Chapter 3: General Discussion

Research contributions

Understanding the physiological processes that respond to biotic or abiotic stresses is an important step toward future improvements in grain yield. In this study, the interaction of shade avoidance and subsequent drought stress was explored in terms of morphological changes and yield components. The hypothesis for this study stated that early shade avoidance and drought stress will interact to reduce fitness in maize seedlings.

Maize seedlings grown under conditions of non limiting resources underwent morphological changes triggered by the R:FR ratio reflected from the leaf and stem tissue of the neighbouring weeds. The presence of aboveground weeds reduced root surface area, root volume and root diameter. These changes in root morphology influenced the ability of maize to withstand subsequent drought stress. As a result of this study the following key observations were made:

- Variation in the response of maize to neighbouring weeds and subsequent drought stress occurred between years.

- Maize growth stage and the severity of the drought influenced the interaction of shade avoidance and drought stress

- Maize silking dates was effected by drought stress in 2009 and by low R:FR in 2010.

- Drought stress increased HI and reduced aboveground biomass and grain yield.

- Drought stress reduced KNP.

The increase in HI caused by drought stress was a direct result of how HI was calculated. HI is defined as (HI (%) = (grain weight / above ground plant weight) × 100). Harvest index was expected to be reduced, however, the reduction in aboveground biomass was larger than the reduction in grain yield, hence HI increased.

Research limitations

The measurement of drought stress used in this study may limit the ability to interpret the outcome of this experiment. In this study drought stress was measured primarily through visual observation. Once >95% leaf rolling occurred the drought stress was terminated 24 hours later. Determination of the severity of the drought stress using only visual characterization may limit repeatability of this variable. Within the published literature, various methods have been used including thermocouple psychrometry (Bassetti and Westgate 1993), and individual pail weights (Farid 2011). Such diversity in methods maybe attributed to level of accuracy required and the associated cost.

In addition, the response to drought stress will be influenced by hybrid selection and maize seedling density. New drought trait technology, for example, will influence the degree to which yield will be lost as result of drought stress. If drought stress increases plant to plant variability then as maize seedling density increases, intraspecific competition will play a greater role in determining the extent of the interaction of the R:FR ratio and drought stress. Page *et al.*, (2010) reported that the onset of intraspecific competition differed with seedling density. At 16 plants m^{-2} intraspecific competition was evident at the 12 leaf tip compared to the 14 leaf tip when maize was grown at 8 plants m^{-2}.

Future research

The results presented in this thesis provide an important insight into the potential for the interaction of shade avoidance and subsequent drought stress. Morphological changes which occur in roots caused by neighbouring weeds may be critical to understanding the cumulative processes that determine grain yield in maize. Specifically, future research should investigate the role of short- term repeated stress or continuous long-term stress on the yield components of maize. In addition, the potential interaction of drought stress by low R:FR by nitrogen deficiency should be explored.

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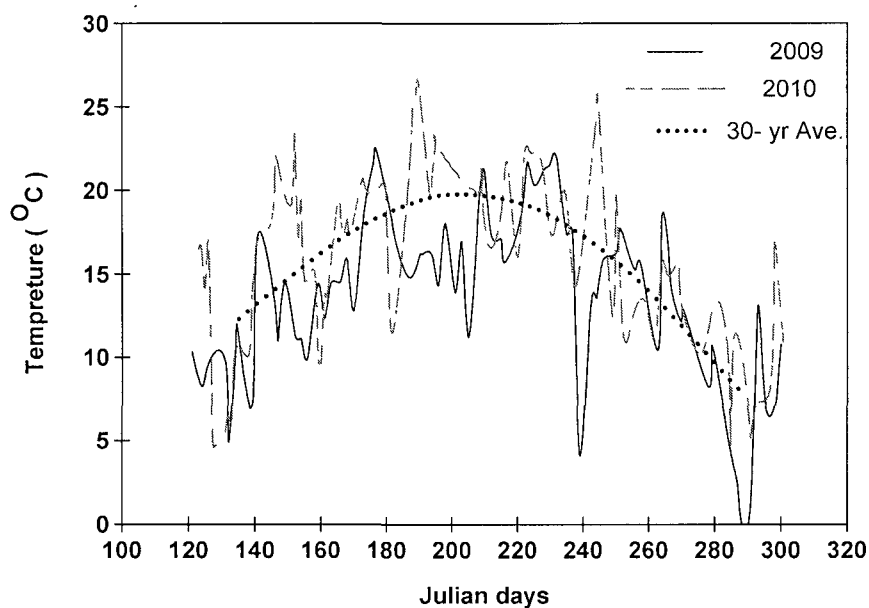
Figure 12. Daily mean air temperature in 2009 and 2010 at the Arkell Research Station.

Thirty year averages are based on meteorological data collected from 1980- 2010 at the

Environmental Canada weather station located at the Guelph Arboretum.