Tassel Initiation is Synchronized to the Elongation Rates of Leaf Primordia in *Zea mays*

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ABSTRACT— Plants must coordinate the environmental conditions with internal cues in order to transition from vegetative to floral development at a time when reproductive success is likely. In this study, the association between primordial leaf growth and floral initiation in controlling that transition was examined. Maize plants grown under different photoperiod, light intensity and defoliation treatments were dissected at frequent intervals to measure the growth of exposed and unexposed leaves and determine the developmental stage of apical meristem.
A gradual decrease in relative elongation rates of successive leaf primordia and unexposed young leaves was observed as plants increased in size from seedling emergence until tassel initiation. Earlier-formed leaf primordia elongated at a higher relative rate than did younger, latter-formed leaf primordia. More importantly, this gradual decrease in early stage leaf growth and the resultant accumulation of unexposed leaves in the whorl were found to be associated with floral transition. Immediately prior to tassel initiation, the length of corresponding leaf primordia in plants of the same or different treatments was similar. Treatments including photoperiod extension and defoliation that delayed tassel initiation, and shading that reduced growth were also marked by a syncronized modification of leaf primordia elongation. Floral transition occurred when the slow-down in leaf primordia elongation and the resultant accumulation of leaf primordia reached a certain level. The elongation rate of leaf primordia could regulate floral transition in maize.
Keywords--- defoliation, flowering, growth, leaf elongation, maize, photoperiod, tassel initiation

1. INTRODUCTION

The time of year at which plants flower varies widely and is closely adapted to the environment in which they grow (Reeves and Coupland, 2000). In many species, the timing of flowering is primarily influenced by environmental factors including photoperiod, light quality and quantity, vernalisation (Dennis et al., 1996) and nutrient and water availability (King and Gocal, 1999, McIntyre, 1997). However, the sensitivity of plants to those environmental conditions depends on the size and developmental state of the responding plants. Plants of many species do not respond to photoperiods appropriate for flowering until they have reached a critical size (Sachs, 1999). Furthermore, plants can complete an entire life cycle in uniform environments without external cues that could specify the timing of developmental events. When sufficient developmental age is reached, transition to flowering can occur as a default under any photoperiod (Sachs, 1999). Some species are less sensitive to environmental variables and flower in response to internal cues such as plant size or number of vegetative nodes (Bernier et al., 1981, Levy and Dean, 1998). Plants use a combination of environmental conditions and internal cues to coordinate flowering time with the best-likelihood of reproductive success (Sachs, 1999, McSteen et al., 2000) but the way information is interpreted and integrated to optimize flowering time remains to be elucidated.

In a study on floral transition in sorghum, hundreds of plants were dissected to determine the developmental stage of apical meristems (Ockerby et al., 2000). It was noticed that there was always an accumulation of leaf primordia in the whorl before panicle initiation occurred and that the developmental stage of the meristem could be fairly predicted from the relative length of the last few leaf primordia. The coordinated decreasing in elongation rate of leaf primordia and the resultant accumulation of leaf primordia at the shoot apices were found to be associated with, and probably regulated the internal control of floral transition (Ockerby, 2001, Ockerby et al., 2014).

In the current study, leaf growth and floral transition was examined in maize, incorporating the effect of photoperiod, defoliation and shading treatments to modify plant growth and the timing of tassel initiation. The aim was to evaluate the association between primordial leaf growth and floral transition in maize in different environments. Mechanisms linking primordial leaf growth and developmental phase change are discussed.

2. MATERIALS AND METHODS

2.1 Study site

All work was done at Central Queensland University, Rockhampton, Australia (Lat. 23°19'S, Long. 150°31'E, Alt. 35m). Experiments 1 and 2 were conducted in a growth cabinet between February and May, 2002. The day/night temperature in the growth cabinet was 30/25 °C. Experiment 3 was conducted from July 22 to September 30, 2002 in a screen house. The temperature in the screen house during growth ranged between 10°C and 30°C.

2.2 Cultural details

Maize (*Zea mays* L. cv. DK689) seeds (12 per pot) were planted in 15-L black plastic pots each containing 13.5 kg sandy clay loam soil (field capacity: 0.25 g g^{-1}; permanent wilting point: 0.11 g g^{-1}). Water and fertilizer were supplied as needed. Five days after emergence, seedlings were thinned to eight plants per pot.

2.3 Experimental designs and treatments

Experiment 1- two photoperiod and two defoliation treatments were imposed to observe their effect on the time of tassel initiation and the morphological characteristics of the shoot apical meristem. There were eight pots per treatment. The growth cabinet was covered from 5 pm to 8 am, so plants under short-day (SD) treatment were maintained in short days of 9 h natural light at 30°C and 15 h darkness at 25°C. Plants of long-day (LD) treatment were maintained in 9 h natural light at 30°C, 6 h low light from incandescent lamps (from 5 am to 8 am and from 5 pm to 8 pm) at 25°C and 9 h darkness at 25°C. The photoperiod extension was imposed by suspending two 100 W incandescent bulbs 1 m above the pots providing about 10 µmol m^{-2} s^{-1} of PAR (photosynthetically-active radiation). Twelve days after sowing, half of the plants of the SD and LD treatments were defoliated. The defoliation was conducted by removal of all the shoot tissues 15 mm above the shoot apical meristem except for the first leaf and the sheath of the second leaf and when done the ligule of the 3rd or 4th leaf was exposed. The sheath of the second leaf was left to protect the shoot apical meristem. The first leaf was left intact to keep the plants alive but it was removed 5 days later when the partly removed leaves (the fifth and sixth leaves) appeared above the sheath of the second leaf. In this defoliation treatment, the 1st – 4th leaves were totally removed and the 5th and 6th leaves lost only the tips of their leaf blade. The four last-formed primordia were usually low enough to escape being damaged.

Experiment 2- two levels of photoperiod were imposed using the same method as Experiment 1 except that the 6-h low light from incandescent lamps was extended to 7 h (from 5 am to 8 am and from 5 pm to 9 pm). Thus plants of the short-day treatment were maintained under 9 h natural light at 30 °C and 15 h darkness at 25 °C, and plants of long-day treatment were maintained under 9 h natural light at 30 °C, 7 h low light from incandescent bulbs at 25 °C and 8 h darkness at 25 °C. Twelve days after sowing, half of the plants of the LD treatment were transferred to SD environment and half of the plants of the SD treatment were transferred to LD environment.

Experiment 3- one day after emergence plants were subjected to three levels of light intensity: viz. 100% 67% and 23% full sunlight (FS). Shading was imposed using one layer of semi-transparent plastic sheet for 67% FS and four layers for 23% FS. Eighteen days after sowing, half of the plants of the 67% FS treatment were subjected to defoliation using the same method used in Experiment 1. In this defoliation treatment, the 1st – 4th leaf blades were totally removed and the 5th and 6th leaf blades were partly damaged. The 6 last formed primordia were low enough to escape any leaf loss.

2.4 Measurements

When the shoot of a maize plant is vegetative, leaves are initiated from the shoot apical meristem located at the base of the "shoot" and entirely concealed by encircling leaves and sheaths of older leaves. What appears to be the stem is actually the collection of sheaths and young leaves rolled or folded one inside the other with the oldest sheath on the outside. Young leaves are extruded in succession through the centre of this pseudo-stem. Plants were dissected under a dissecting microscope at frequent intervals (10-12 plants per treatments on each occasion usually every three days) to count leaves and measure the length of exposed leaves and those unexpanded leaves and leaf primordia still in the whorl, and measure green leaf area and shoot dry weight.

2.5 Growth stage and shoot apical meristem description

The staging system used in this study divides plant development into vegetative and reproductive stages. The vegetative stage was defined by the time period during which leaves were initiated on the shoot apical meristem. The vegetative stage finished and tassel initiation was recorded when 70% of sampled plants had swelling at the base of the shoot apical meristem (stage 3 after Moncur, 1981). Both leaf primordia (LP) and immature leaves wholly concealed in the whorl were referred to as LP and numbered sequentially from the shoot apical meristem such that the youngest was deemed the 1st LP (Fig. 1).

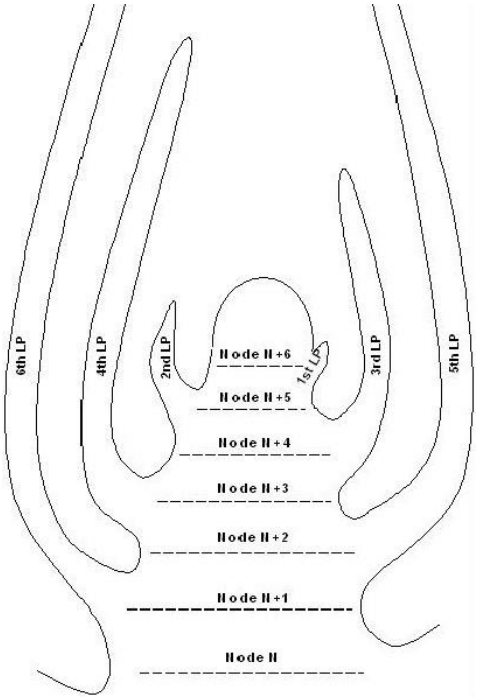

Fig 1. Diagram of the longitudinal section through a vegetative shoot apex of maize (*Zea mays*) showing its architecture and the numbering of leaf primordia and immature leaves wholly concealed in the whorl.

During the vegetative stage, the length of the 2nd LP varied within a stable range, and the lengths of older LP were observed to be closely coordinated with that of younger LP. Consequently the architecture of LP on the shoot apical meristem was described by plotting the length of older LP as a function of the length of the 2nd LP (Ockerby, 2001, Ockerby et al., 2014). In all cases, the data for the length of older leaf primordium on the y-axis are plotted on the log scale. Values on the right side of each graph show the slopes of the regression lines fitted to data without log-transformation such that:

$$L_n = aL_2 + b$$

where L_n is the length of the nth LP, L_2 is the length of 2nd LP, a is the slope and b is the intercept of the regression line.

Because LP initiate at a near-constant rate in intact plants, the elongation rate of an individual LP can be calculated by its length (L_n) and position (n, numbered from the meristem tip; Fig. 1.) at the time of measurement. By comparing the lengths of chronologically-identical LP, the difference between the average elongation rates over a time period from LP initiation to the time of measurement can be expressed. It is assumed that the range in length of the second LP can approximately represent the range in time from immediately after initiation of the youngest LP to immediately before initiation of the next new LP, so the lengths of older LP were plotted against the length of the second LP. In this way, the slopes of the regression lines can approximate the growth rates of LP during this period (a plastochron).

3. RESULTS

3.1 Tassel initiation was delayed by photoperiod extension and defoliation

In Experiment 1, long days achieved with low-light photoperiod extension (LD) delayed tassel initiation by about 4d and plants in LD produced 2 more leaves than plants in short day (SD) (Table 1). Defoliation delayed tassel initiation by 8d but defoliated and non-defoliated plants produced the same number of leaves. There was no interaction between photoperiod and defoliation treatments (Table 1). In Experiment 2, low light photoperiod extension delayed tassel initiation by about 4d, from 21d in the short-day control to 25d with photoperiod extension. The total leaf number of plants under photoperiod extension was also greater than that under short days (Table 1).

Table 1. The effect of photoperiod extension, defoliation and shading on total leaf number and the time from sowing to tassel initiation in maize (*Zea mays*). Treatments were: in Expt. 1 SD (9-h photoperiod) and LD (16-h photoperiod - extended with incandescent light) with and without defoliation (D); in Expt. 2 SD, LD, SD to LD and LD to SD plant exchanged at 12 days after sowing; and in Expt. 3 100%, 67% and 23% full sunlight (FS); and 67% FS with defoliation (FSD). Defoliation was done above the first leaf ligule at 12 days after sowing.

Treatment	Total leaf number	Time from Sowing to TI[2]
Experiment 1		
SD	15.5a[1]	20
SDD	15.7a	28
LD	17.5b	24
LDD	17.7b	32
Experiment 2		
SD	15.2a	21
SD to LD	17.8b	25
LD	17.8b	25
LD to SD	15.2a	21
Experiment 3		
FS	17.6b	30
67% FS	17.8b	30
23% FS	17.0a	29
67% FSD	17.7b	38

[1]Means followed by a different letter are significantly different ($P<0.05$).
[2]Tassel initiation (TI) was recorded when 70% of sampled plants within a treatment reached TI.

In experiment 3, plants in 23% full sunlight (FS) produced one less leaf and reached tassel initiation slightly earlier than plants in FS and 67% FS (Table 1). Defoliation delayed tassel initiation by 8d compared to the respective non-defoliation control in 67% FS, but defoliated plants did not produce extra leaves. The delay in tassel initiation was mainly associated with the cessation of leaf primordia (LP) initiation immediately after the defoliation was done. Because older leaves were removed and no extra leaves were produced, defoliated plants had less expanded leaves than control plants at the time of tassel emergence.

3.2 Tassel initiation was not dependent on shoot growth or green leaf area

In all experiments, the area of green leaf was dependent on shoot biomass (Fig. 2) although in 23%FS in experiment 3, plants produced consistently more green leaf area per unit of shoot biomass than less-shaded treatments (Fig. 2c). In experiments 1 and 3, shoot dry mass and green leaf area were reduced by defoliation per se, and despite that causing a delay in the timing of tassel initiation, those losses were not regained before tassel initiation. In contrast, delays in tassel initiation due to photoperiod extension in experiments 1 and 2 increased green leaf area and shoot biomass (data not shown) associated with having more leaves (Table 1).

3.3 Leaf initiation ceased with defoliation

Leaves of intact plants were initiated at a constant rate from seedling emergence until tassel initiation. In experiments 1 and 2, leaves were initiated at regular intervals of about 1.7d, and in the FS and 67% FS treatments in experiment 3 at about 2.2d (Fig. 3); the variation between experiments may have been caused by different ambient temperatures. Low-light extension of photoperiod had no effect on leaf initiation rate, but defoliation stopped leaf initiation immediately and it resumed 10 days later at its pre-defoliation rate (Fig. 3) when the length of partly-excised leaves had regrown to 10 cm. Reduced sunlight (23% FS) decreased leaf initiation rate only prior to the first sampling time (Fig. 3).

3.4 Leaf primordia elongation rate decreased over time and was slowest in the youngest primordium nearer the shoot apical meristem

In all experiments, LP elongated faster as they got older consistent with normal exponential growth in plants, thus at all sampling times older LP elongated at significantly faster rate than younger LP (Figs. 4 and 5). Concomitantly, as plants grew, the length of successive LP occupying a chronologically-similar position from the shoot apical meristem underwent a continuous decrease (the slope of the regression for LP decreased) and, as a result, more LP accumulated on the shoot apices as plants approached tassel initiation. The elongation rates of each LP were slower in those nearer the shoot apical meristem and slowest just prior to tassel initiation (Figs. 4 and 5).

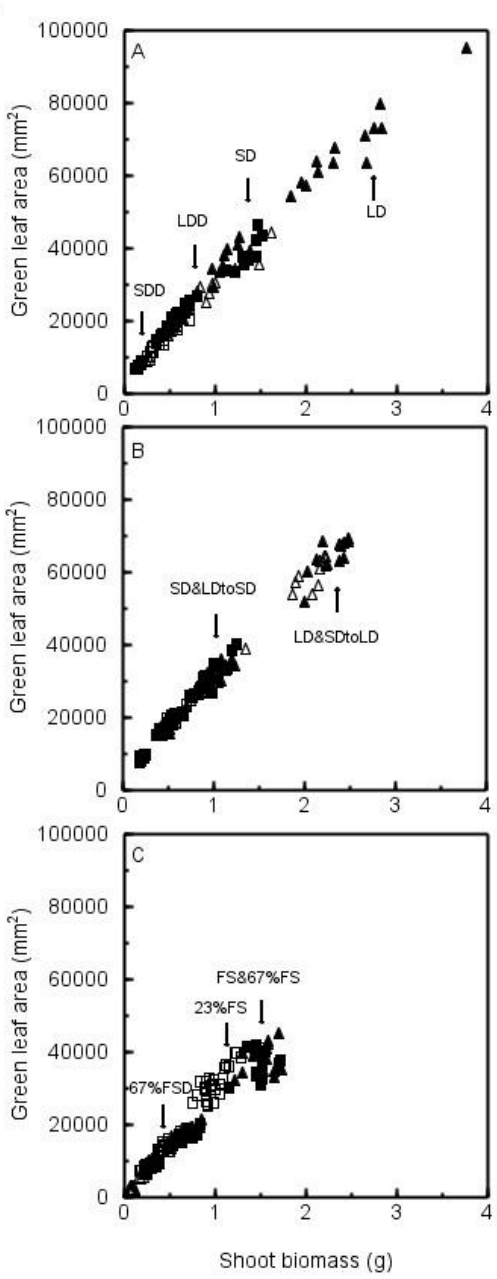

Fig 2. Green leaf area of maize (*Zea mays*) seedlings as affected by shoot biomass in (A) Experiment 1 as affected by photoperiod extension and defoliation: (■) short day (SD); (▲) long day (LD); (□) SD with defoliation (SDD); and (△) LD with defoliation (LDD), (B) Experiment 2 as affected by photoperiod: (■) SD; (▲) LD; (□) LD transfer to SD; and (△) SD transfer to LD, and (C) Experiment 3 as affected by shading and defoliation: (■) full sun (FS); (▲) 67% FS; (□) 23% FS; and (△) 67% FS with defoliation (67%FSD). The times of tassel initiation are indicated by arrows.

3.5 Leaf primordia elongation before tassel initiation was regulated by photoperiod

In experiments 1 and 2, at the times immediately before tassel initiation in SD and LD to SD plants, the plants in LD and SD to LD were vegetative and maintained faster rates of LP elongation (Figs. 4 and 5). Faster LP elongation of older LP resulted in one fewer whole leaf being recorded in the whorl. Just 4d later, the plants in LD and SD to LD progressed to tassel initiation and the elongation rate of LP slowed to resemble (in a chronological-sense and numbered from the shoot apical meristem) those on SD and LD to SD plants at tassel initiation. Plants in LD and SD to LD had slightly shorter LP and slower rates of LP elongation at tassel initiation than those of SD and LD to SD plants, but the differences were much less than the changes within treatments in the 4d before tassel initiation. Plants that had been transferred from SD to LD and vice-versa responded to the condition in which they were in at the time of tassel initiation.

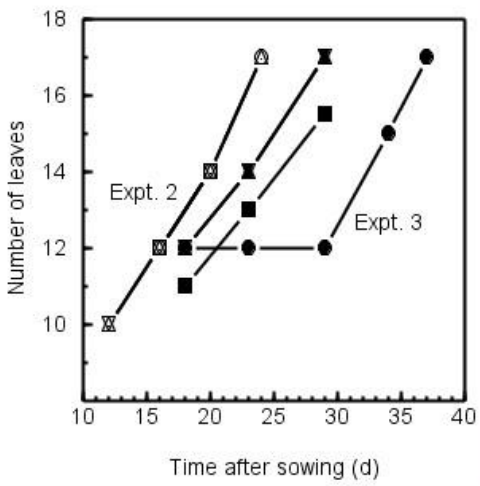

Fig 3. Leaf initiation rate of maize (*Zea mays*) seedlings in Experiment 2 as affected by photoperiod extension and defoliation: (▽) short day (SD); (△) long day (LD); (□) LD transfer to SD; and (O) SD transfer to LD, and in Experiment 3 as affected by shading and defoliation: (▲) full sun (FS); (▼) 67% FS; (■) 23% FS; and (●) 67% FS with defoliation.

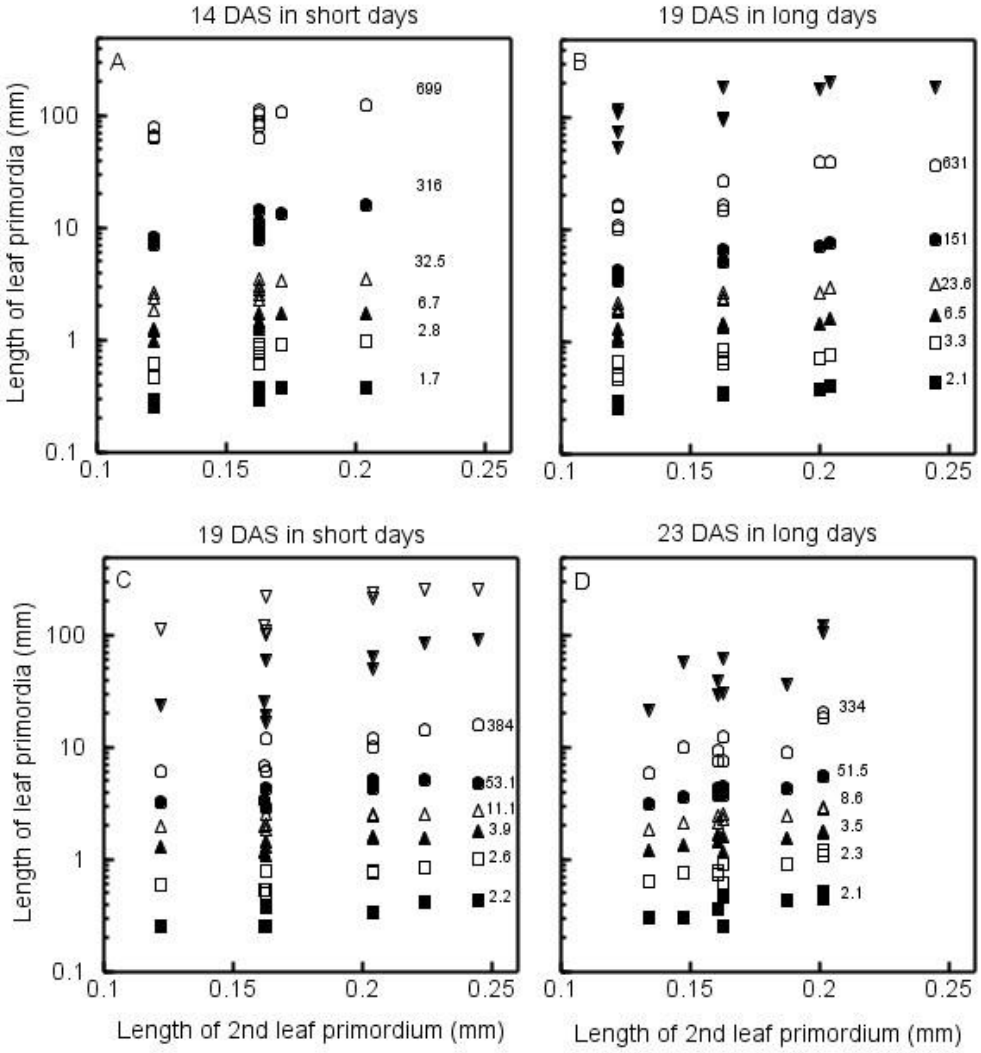

Fig 4. The lengths of leaf primordia (log scale) relative to the length of the second leaf primordium in vegetative maize (*Zea mays*) seedlings in experiment 1: short days at 14 (A) and 19 (B) days after sowing (DAS); and long days at 19 (C) and 23 (D) DAS. Linear slopes of lines fitted to the untransformed data are shown for leaf primordium (■) 3, (□) 4, (▲) 5, (△) 6, (●) 7, (O) 8, (▼) 9 and (▽) 10. Values of Y for each value on the X-axis represent the leaf lengths of a single plant. Tassel initiation was recorded in short days at 20 DAS and in long days at 24 DAS.

3.6 The shoot apical architecture at tassel initiation was not changed by shading or defoliation

In experiment 3, the elongation rates of the 3rd through to the 10th LP in plants immediately before tassel initiation were nearly identical across all treatments (Fig. 6). Thus the architecture of the shoot apical meristem at tassel initiation was the same despite reduced growth caused by shading and reduced growth (Fig. 2c) and delayed tassel initiation (Table 1) caused by defoliation.

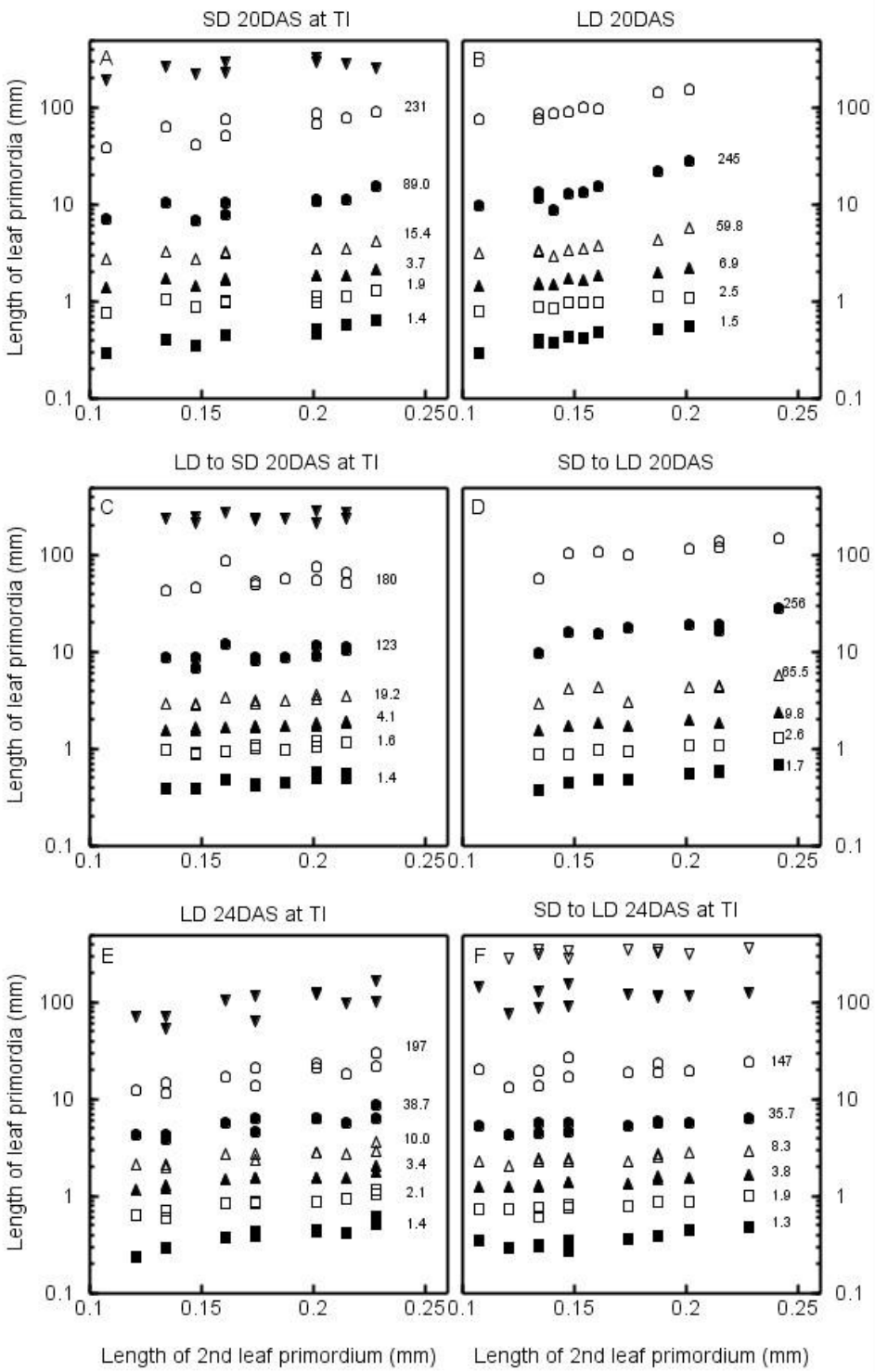

Fig 5. The lengths of leaf primordia (log scale) relative to the length of the second leaf primordium in vegetative maize (*Zea mays*) seedlings in experiment 2 at 20 days after sowing (DAS) in (A) short days (SD), (B) long days (LD), (C) LD transfer to SD and (D) SD transfer to LD; and at 24DAS in (E) LD and (F) SD transfer to LD. Linear slopes of lines fitted to the untransformed data are shown for leaf primordium (■) 3, (□) 4, (▲) 5, (△) 6, (●) 7, (**O**) 8, (▼) 9 and (▽) 10. Values of Y for each value on the X-axis represent the leaf lengths of a single plant. Tassel initiation (TI).

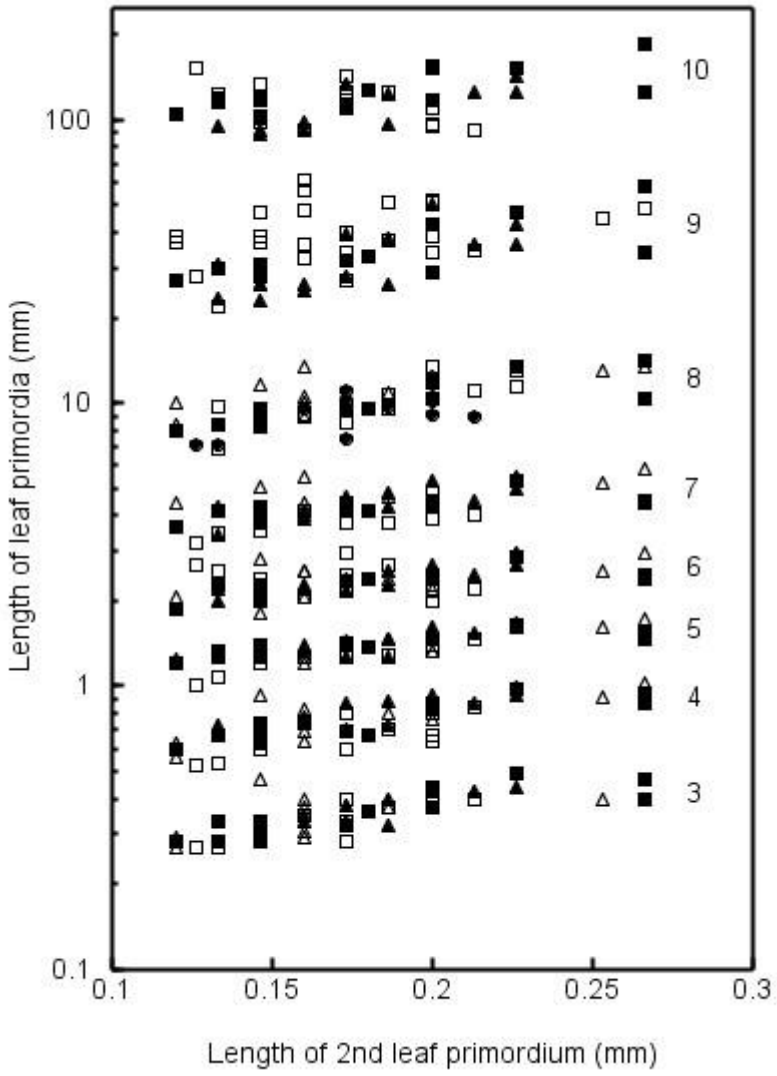

Fig 6. The lengths of leaf primordia (log scale) relative to the length of the second leaf primordium in vegetative maize (*Zea mays*) seedlings just prior to tassel initiation in Experiment 3; as affected by shading and defoliation: (■) FS (full sun); (▲) 67% FS; (□) 23% FS; and (△) 67% FSD (with defoliation). Data for all treatments are plotted as a grouping of equivalent leaf primordia (#3-10). Values of Y for each value on the X-axis represent the leaf lengths of a single plant.

4. DISCUSSION

In intact maize plants, leaves were initiated at a constant rate from seedling emergence until tassel initiation (Fig. 3) which is consistent with previous reports of canopy generation in grasses in a given environment (Evans & Barton, 1997; Hay & Ellis, 1998), Temperature is the major factor regulating leaf initiation rate but nutrient availability at non-extreme levels has little influence (Frank & Bauer, 1995).

Although the initiation of LP was constant in intact plants, as plants got older and bigger, the elongation rate of LP occupying the same position relative to the shoot apical meristem slowed (Figs. 4 and 5). This observation is consistent with a previous study in *Sorghum bicolor* (Ockerby, 2001; Ockerby et al., 2014). Decrease in the relative growth rates of the primordial leaves was also found in wheat plants as plant size increased from 8 leaves to 13 leaves (Kirby, 1990). The reduction in LP elongation rate and the consequent change in shoot apical architecture observed in this study occurred gradually from seedling emergence until tassel initiation indicating that an internal process (or factor) progressively modified LP growth.

The lengths and elongation rates of the LP on the shoot apical meristem in maize were found to be synchronized with the timing of tassel initiation. This observation is a new finding in maize but has been previously reported in *Sorghum bicolor* (Ockerby, 2001; Ockerby et al., 2014). The experimental method imposed natural and unnatural treatments that attempted to change the growth and the timing of tassel initiation in an attempt to disassociate LP elongation from the timing of tassel initiation. Despite using treatments with different sunlight intensity, defoliation and photoperiod, and

producing a range of shoot biomasses and different timings of tassel initiation, the data did not provide a single incidence where LP elongation and the timing of TI were not synchronised; nor was LP elongation ever slower than at the certain rates measured at tassel initiation.

The results give weight to the idea proposed by Ockerby et al., (2014) that the switch from vegetative to reproductive development in plants is somehow regulated by the accumulation of LP on the shoot apical meristem and the plant's failure to sustain rapid elongation of those primordia. Synchrony between the precise slowing in LP elongation and the timing of floral initiation implies that floral transition can be discretely regulated at the shoot apical meristem.

The locale of the shoot apical meristem could well be both the site of developmental 'time' measurement (via synchrony) and the activation of a flowering gene that differentiates reproductive anatomy. Within this hypothesis, the role of a specific floral messenger such as florigen (Chailakhyan 1937) is less certain. The putative floral-promoting gene in *Arabidopsis* is *FLOWERING LOCUS T (FT)* and its functional homolog in maize is ZCN8, however the latter's role was found to be more complex than simply regulating floral transition; it may also play a pleiotropic role in the generalized growth of vegetative and reproductive tissues (Danilevskaya et al., 2011). Mendez-Vigo *et al.*, (2010) linked natural variation for the rate of leaf production with flowering in *Arabidopsis thaliana* through distinct temporal and pleiotropic patterns of QTL effects. Their analyses showed that most of the genomic regions affecting flowering time or total leaf number also altered leaf production rate.

Young or newly-formed leaves may well be a potential site for 'floral stimulus' production (Colasanti et al., 1998; Irish & Jegla, 1997). In the current experiments, because of the slow-down in LP elongation, LP accumulated on the shoot apices as maize seedlings approached tassel initiation (Figs. 4 and 5). In *Nicotiana tabacum* the third and later leaves of plants raised in inductive condition were able to induce flowering but that ability was lost rapidly with age (Hopkinson and Ison, 1982). In transplanted seedlings those similar expanded leaves (despite their adequate size) were unable to influence the higher leaves that unfold and induce flowering; possibly that ability was lost with age or possibly the expansion of those leaves was so slow that there was insufficient young inductive leaf to promote floral induction. In maize, removing all but the one or two youngest leaf primordia on cultured excised meristems released the developmental control that limited the number of leaves the meristem subsequently produced. As the number of leaf primordia left attached increased, the proportion of meristems that were reset decreased and most plants produced no more leaves than untreated plants (Irish & Jegla, 1997; Irish & Nelson, 1988; Irish & Nelson, 1991). Maize plants that have mutation in the *indeterminate* gene (*id1*) did not undergo a normal transition to flowering but continued to produce leaves long after normal plants flowered (Colasanti et al., 1998). The *id1* mRNA was detected only in young leaves. In the current study, slowing in LP elongation was most significant for 5^{th}-8^{th} LP, consistent with the *id1*-mRNA localization pattern (Colasanti & Sundaresan, 2000).

The elongation rates of LP are sensitive to changes in the morphological environment of the leaf sheath tube. In perennial grasses, longitudinally-incision of the leaf sheath to allow light penetration (Wilson and Laidlow, 1985; Casey et al., 1999) and horizontally excision to reduce its height (Wilson and Laidlow 1985) reduced the length of emerging leaves. In both experiment, reduced leaf length was due to fewer and shorter laminar cells, a result associated with changes in the length of the leaf-blade elongation zone and (probably) the effect of the leaf tip having emerged from the sheath tube. Wilson and Laidlow (1985) also mentioned in passing that excision of the sheath tube appeared to delay leaf development, an effect that was associated with defoliation in the current study. Cell development and flowering may be connected; floral transition occurred normally in *Helipterum roseum* plants grown at 20°C and was accompanied by synchrony of steady-state cell-cycling; however at 25°C floral initiation was inhibited by the loss of steady-state cell-cycling (Sharman et al., 1990).

Leaf primordia are completely concealed in the sheaths of older leaves and are heterotrophic (Turgeon, 1989) and must import nutrients (carbohydrate, nitrogen and other mineral elements) from other parts of the plant to maintain their growth and activity. The observed slowing in LP elongation could be due to a nutrient deficit at the shoot apex. As plants grew in size, alternative sinks such as root or stem or just the increased anatomical distance to the root (Sachs 1999) may also have lessened the supply of nutrients to the shoot apices. Partially-exposed leaves that were rapidly expanding may have competed with the LP (Ockerby et al., 2014) and the shoot apical meristem for those nutrients and/or growth substances, required to maintain vigorous vegetative apical growth and proposed to be promoting or inhibiting floral transition (Bernier 1988). In sorghum (Ockerby et al., 2001) and in maize (this study) the removal of shoot tissues (especially parts of the immature expanding leaves) greatly delayed the onset of floral transition. In the purple-flowered *Impatiens balsamina*, when the leaves that unfolded during the photoperiodic induction treatment were removed, floral shoots reverted to vegetative growth (Tooke et al., 1998). If the same leaves were not removed, the plants did not revert after they were placed in non-inductive conditions.

The 'nutrient diversion' hypothesis postulates that floral induction is a means of modifying the source/sink relationships within the plant in such a way that the shoot apex receives a better supply of assimilates than under non-inductive conditions (Sachs, 1977). This hypothesis is supported by studies which showed that the concentration of carbohydrates increased dramatically in many parts of the plant during transition from vegetative to reproductive growth. This increase occurred early in the sequence of meristematic events related to the floral transition when no morphological changes were visible in the apices (Bodson & Bernier, 1985; Corbesier et al., 2002; Degli-Agosti & Greppin, 1998; Milyaeva et al., 1996; Van Nocker, 2001). The decrease in rate of LP elongation in sorghum (Ockerby et al., 2014) and

maize (the current study) as plants approached floral initiation suggests that lessening (rather than increasing) the supply of nutrients to shoot apices is central to control of transition to reproductive state.

Root-derived nutrients could also become limiting to apical growth. Nitrogen probably plays an important role as leaf extension rate is very responsive to nitrogen supply (Gastal et al., 1992; MacAdam et al., 1989; Thomas, 1983; Volenec & Nelson, 1984) and low internal N levels induce flowering (Corbesier et al., 2002; Ishioka et al., 1991; Raper et al., 1988; Rideout et al., 1992; Simmonds, 1982; Tanaka, 1986; Tanaka & Asagami, 1986; Yamasaki et al., 2000). Raper et al. (1988) proposed that floral transition is stimulated by an imbalance in the relative concentration of carbohydrate and nitrogen in the apical meristem rather than by the absolute concentration of either within the meristem. Nitrogen and carbohydrate metabolism are tightly linked in almost every biochemical pathway in the plant. According to Brouwer's hypothesis (Brouwer, 1962; Brouwer, 1983), and the transport-resistance models (Dewar, 1993; Thornley, 1998), the increase in carbohydrate levels could be either the cause or the result of the associated decrease in nitrogen levels.

The delay in tassel initiation by photoperiod extension was accompanied by the maintenance of faster rates of LP elongation for a longer duration (Figs. 4 and 5) such that photoperiod extension also modified the shoot apical meristem. Instead of stimulating the production of special 'flowering-time regulators', photoperiod extension appeared to decelerate the progress towards tassel initiation. Defoliation stopped leaf initiation entirely (Fig. 3) and LP elongation (Fig. 6) so both treatments affect LP growth and floral initiation via the reallocation of the plant's internal resources to initiate leaves or replace leaves removed. The vegetative development of the maize shoot can be divided into juvenile and adult phases based on the types of leaves produced at different times in shoot development. Changes in phase occur gradually, and leaves produced during the transition from juvenile to adult growth have a combination of juvenile and adult cell types and express a variety of other traits in a quantitatively intermediate fashion (Poethig, 1990). Orkwiszewski and Poethig (2000) found that phase identity of maize leaf is determined after leaf initiation, and they proposed that phase change is regulated by factors that originate outside the shoot apical meristem and act independently on leaf primordia and the shoot apical meristem. The slowing in LP elongation and the consequent change in shoot apical architecture observed in this study occurred gradually as plants increase in size from seedling emergence until tassel initiation. Factors affecting LP elongation could also exert control over leaf phase identity. Severely defoliated sorghum plants produced extra leaves compared with control plants but the final leaf length distribution of the plant that grew after defoliation was stopped was similar to control plants (Ockerby et al., 2001); leaf growth was a function of what was there and not what had been cut off.

It is our opinion that the developmental control of tassel initiation in maize is regulated through the growth of LP. The LP may just indicate or they may regulate the growth status of the shoot apical meristem. If so, then slowing in the elongation of LP to critical thresholds would engender conditions in the shoot apical meristem that triggers the transition from the vegetative to the floral phase. An alternate way of explanation maybe that the plant differentiates between its rate of producing new vegetative tissue and its ability to expand that tissue. New structures may be governed by cell division but growth is measured by cell expansion, and either may be physiologically-constrained by the resource supplied or the environmental controls that regulate growth. Consequently different cells in the shoot apical meristem are differentiated (Irish, 1998) and flowering happens.

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