Leaf Growth Controls the Timing of Panicle Initiation in *Sorghum bicolor*

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ABSTRACT—*Flowering is initiated in grasses when the developmental program at the shoot apical meristem switches from producing vegetative to reproductive structures. The switch occurs via endogenous controls and in response to cues from the environment. Day-length is a major factor controlling flowering time and it is commonly believed to generate a signal in the green leaves which is transferred to the shoot apical meristem. Whether the signal is the flowering hormonal complex 'florigen', an electrical signal, or a multi-factorial process involving phytohormones and sugars remains inadequately backed by research. Many genes are said to control the onset of flowering in plants but the physiological mechanisms that switch those genes on-and-off remain hidden. Recent evidence shows that small signaling molecules may be implicated in but again not necessarily causal to the flowering process.*

In the monocotyledon Sorghum bicolor the elongation rates of leaf primordia and of unexposed leaves (those wholly within the whorl) slowed during the vegetative developmental phase during a defined period prior to the initiation of panicle structures. The response was the same in treatments where the timing of panicle initiation was varied by season, agronomy, photoperiod, cultivar or defoliation. Moreover, in all treatments, panicle initiation was coincident to the attainment of both a common architecture of the shoot apex and comparable slowing of the elongation rates of leaf primordia and unexposed leaves. During vegetative development the total length of unexposed leaves was strongly dependent on the area of green leaf. We hypothesize that growth-related processes that slowed the elongation rate of leaf primordia and unexposed leaves might also have triggered panicle initiation. In a hereditary sense, this effect would ensure that the plant had sufficient leaf area and vegetative biomass to grow seed, in the same trend that those similar ancestors had adequate plant architecture and thus reproduced and survived.

Keywords: defoliation, flowering, leaf elongation, panicle initiation

1. INTRODUCTION

Numerous factors affect the 'ripeness' of a plant to flower (Hopkinson and Ison, 1982) and the onset of flowering itself (Bernier, 1988). Genetic studies confirm the existence of metabolic pathways of gene action that effect autonomous plant development or respond to photoperiod, vernalisation or gibberellin (Simpson *et al.*, 1999), or possibly, carbon metabolism (Levy and Dean, 1998). In many species leaf-derived signals are said to regulate floral induction (Pouteau *et al.*, 1997) and the determination of floral structures (Tooke and Battey, 2000), but the nature of the 'florigen' signal (Zeevaart, 1976) and how it is transported to the shoot apical meristem remains unclear (Evans, 1993; Machackova and Krekule, 2001). Recent studies suggest that specific flowering genes (e.g. *CONSTANS*) working in accord with the small signaling molecule FT may be involved in floral initiation (Jaeger *et al*., 2006). Alternately, on a physiological level, floral initiation is considered to be a function of the cumulative distance of the shoot apical meristem from roots, such that the number of intervening nodes is a reliable determinate of when a plant will flower (Sachs, 1999).

It has been shown that leaf primordia and unexposed leaves are involved in the floral signal. In experiments with *Zea mays* (Irish and Jegla, 1997; Irish and Karlen, 1998) or *Sorghum bicolor* (Ockerby, 2001; Ockerby *et al.*, 2001), the period from treatment to floral initiation was longer when fewer leaf primordia or less unexposed leaf were left attached to the shoot apical meristem in excised meristems or defoliated plants, respectively. With fewer attached primordia or less unexposed leaf, leaf development was reset to an earlier or younger developmental time.

The study by Ockerby *et al.* (2001) also showed that a plant which was severely defoliated then allowed to re-grow produced chronologically older leaves that had essentially the same architecture as the original leaves on an intact plant. Implicitly, it follows that the plant had no 'memory' of the defoliation *per se*, and that leaf development and growth were strictly internally-regulated.

While dissecting the shoot apical meristem of sorghum we noticed that each leaf primordium was a predictable fraction of the length of the next older primordium. In this paper, we report on the elongation rates of those leaf primordia and their relationship to green leaf area and the timing of panicle initiation which was altered in plants by natural and artificial factors: season, agronomy, photoperiod, cultivar or defoliation.

2. MATERIALS AND METHODS

The method of the four experiments and treatments was to establish populations of sorghum plants in which both the characteristics of leaf growth and the timing of panicle initiation were varied either by natural or artificial means. Our purpose was to identify relationships between leaf development and the timing of panicle initiation.

2.1 Study site

Experiments were conducted during 1999 and 2000 at Mareeba or nearby at Walkamin Research Station (17°08'S, 145°26'E, altitude 591 m) in north Queensland, Australia. The soil was a Euchrozem, a deep red, pedal, uniform clay soil.

2.2 Cultural details

In Mareeba, plants were grown in a glasshouse. Four sorghum [*Sorghum bicolor* (L.) Moench] plants were grown in each 4.5-L black plastic pot and received complete nutrients and daily watering. At Walkamin, sorghum was grown in the field at a plant-to-plant spacing of 0.2 m in small plots of cultivated soil and supplied with complete nutrients and watered twice-weekly. Plants were kept unaffected by insects or weeds.

2.3 Experimental designs and treatments

Experiment 1- the purpose of this experiment was to describe the developmental pattern of leaf primordia grown under natural conditions. Sorghum (cv. Boomer) was sown in the field on 31st May, 2000. The experiment used three blocks of entire plants and no treatments were applied.

Experiment 2- five sorghum cultivars were sown in the glasshouse on 24th September, 1999. The experiment used a split-plot design with two replications and two randomly-assigned, whole-plot treatments. The whole plots were either natural (~12.9 h) or extended (15 h) photoperiods. Within each whole-plot, the treatment structure was five levels of cultivar: 38-day Milo, Buster, Boomer, M35-1 or QL24 arranged randomly. Photoperiod was extended with fluorescent tubes and incandescent bulbs suspended 1 m above the plant canopy and provided 12-20 µmol photons m^{-2} s^{-1} PAR from before dusk to 8 pm and from 5 am until after dawn. Natural photoperiod was 12 h 30 min at the start of the experiment and 13 h 20 min at the time when the latest cultivar QL24 initiated a panicle. In this experiment, we expected that panicle initiation (in most cultivars) would be delayed by extended photoperiod however no effect of photoperiod on panicle initiation was evident. Hence data for photoperiod treatments were combined.

Experiment 3- sorghum (cv. Boomer) was sown in the field on 10th May, 1999. The experiment was a split-plot design with two main-plot treatments and two replications. The main plots were either natural (c. 11.5 h) or extended (14 h) photoperiod. Photoperiod was extended with incandescent bulbs suspended 1.2 m above the ground providing 2-12 µmol photons m^{-2} s^{-1} PAR at canopy level. Lamps were turned on before dusk and off at 7 pm and on at 5 am and off after dawn. Photoperiod extension did not delay panicle initiation and the photoperiod treatments' results were combined. Within each main plot, the treatment structure was a random design. Sub-plot treatments were nine levels of defoliation (using scissors) of which four levels are reported here: no defoliation (control); defoliation of all exposed leaf blade if the ligule was visible; defoliation of all leaf blades and sheaths at just above the second leaf ligule; and defoliation of all leaf blades and sheathes just above the first ligule (Fig. 1). Defoliation treatments with 3 to 4-day intervals commenced when the second leaf ligule was visible and ceased at panicle initiation in control plants. There was minimal (<10 mm) stem elongation before panicle initiation, so defoliation did not remove the shoot apex.

Experiment 4- sorghum (cv. Boomer) was sown in the field on 29th October, 1999. The experiment used a randomized-block design with three replications. The treatments were four levels of defoliation: control (no defoliation);

defoliation of exposed leaf blade if the ligule was visible; defoliation of leaf blades and sheaths at just above the first ligule; and defoliation of unexposed and expanding leaves and sheaths - using a scalpel blade to incise through the leaf sheaths opposite the first ligule then pulling the excised, partially exposed fourth leaf and all younger leaves upwards from the whorl (Fig. 1). Defoliation was done four times over twelve days commencing when the second leaf ligule was first visible and ceasing at panicle initiation in control plants.

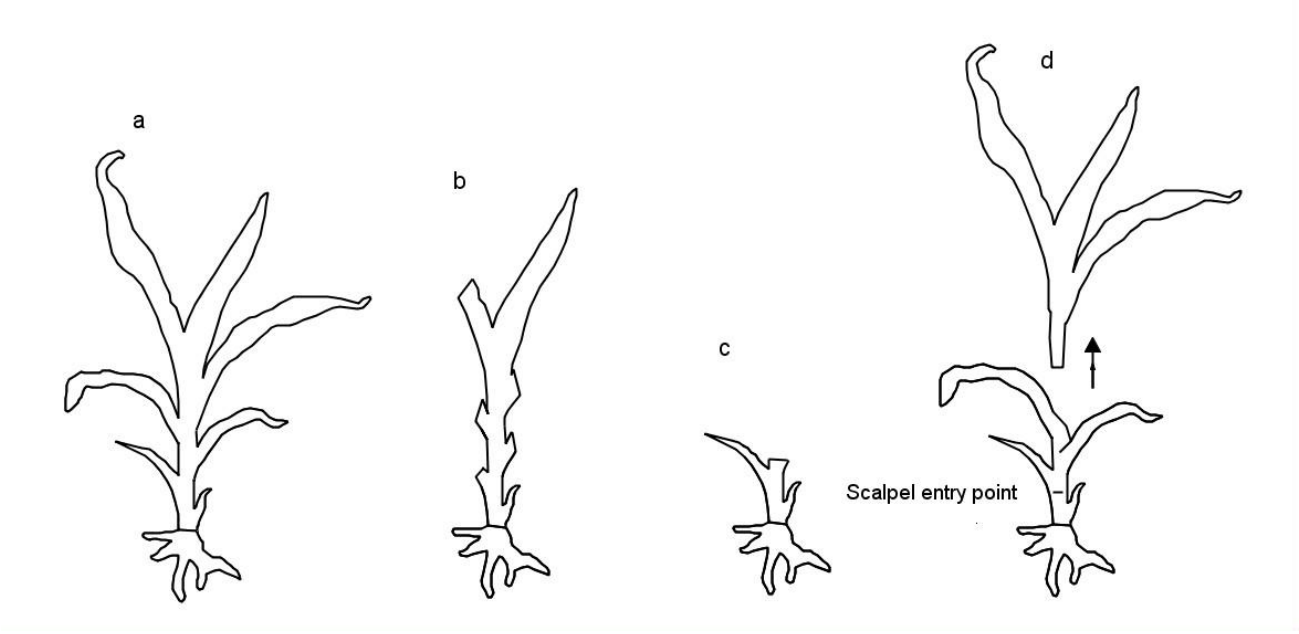

Fig 1. Defoliation treatments in experiments 3 and 4: (a) control – no defoliation, (b) removing the fully-exposed leaf blade, (c) removing all leaf and sheath above the second leaf ligule, and (d) removing the partially exposed fourth leaf and all younger leaves from the whorl at the height of the first ligule.

2.4 Measurements

Plants were sampled twice-weekly until spikelets were visible on the inflorescence in each treatment during dissection. In the laboratory, exposed green leaf area was measured (Paton Electronic Planimeter). Leaves that were partly or wholly unexposed and leaf primordia were removed with a scalpel under a dissecting microscope, and the unexposed leaf blade length was measured. Panicle initiation was recorded when the primary branch meristem was observed as a swelling at the base of the shoot apex according to Moncur (1981). In the analyses we used only those plants that showed no evidence of having initiated the primary branch of the panicle. Although working with destructive sampling made it difficult to quantify, it appeared that generally one leaf was initiated on the shoot apical meristem but did not develop; instead it appeared to coalesce into the panicle structure. Those few plants which exhibited a discontinuity between the lengths of the youngest and next older leaf primordium and those plants in which expansion of the apical dome suggested that panicle initiation had started were excluded from the analyses.

2.5 Statistical methods

The linear relationship between unexposed leaf length and the length of the second youngest leaf primordium on the shoot apex was investigated. Separate lines were fitted for each primordium or unexposed leaf (numbered from the youngest). The variance around lines changed with leaf age, so mixed models estimated by the Restricted Maximum Likelihood procedure (REML; Patterson and Thompson, 1971) were fitted. These models were specified in such a way that the error structure (R-structure in REML terminology) had separate error terms for each age of leaf. This allowed for the variance heterogeneity. The slopes of the lines (fixed effects) were tested using Wald tests. The models were fitted using the SAMM function (Butler *et al.*, 1999) in SPLUS.

The non-linear smooth trend in the relationship of the total or combined length of unexposed leaves on green leaf area was investigated using a smoothing spline. This spline was fitted using mixed models and REML estimation (Verbyla *et al.*, 1997). The splines represent a straight line with smooth departures away from the straight line. Inferences were made about the fitted lines by testing the smooth departures away from the straight line using a log likelihood statistic for random effects. Inferences were made about the underlying straight line using Wald statistics.

3. RESULTS

3.1 Autonomous elongation of unexposed leaves

During vegetative development, the leaf primordia and unexposed leaves of untreated *Sorghum bicolor* (cv. Boomer) plants grown in the field (Expt. 1) elongated at a logarithmic, size-dependent rate (Fig. 2). As each leaf aged, it elongated faster (P<0.001). The elongation rate of the third youngest primordium was mainly constant across time, changing (P<0.001) only between 28 and 39 days after sowing (DAS). Thus, it is deduced that the apical dome and youngest primordia also maintained stable sizes, and that elongation of the youngest primordium was dependent on the initiation of next leaf primordium to develop. In contrast to the constant relative elongation of new leaf primordia across time, the relative elongation rates of equivalently-positioned unexposed leaves decreased (P<0.001) across time, attaining their slowest rate at 70 DAS, immediately before panicle initiation (Fig. 2).

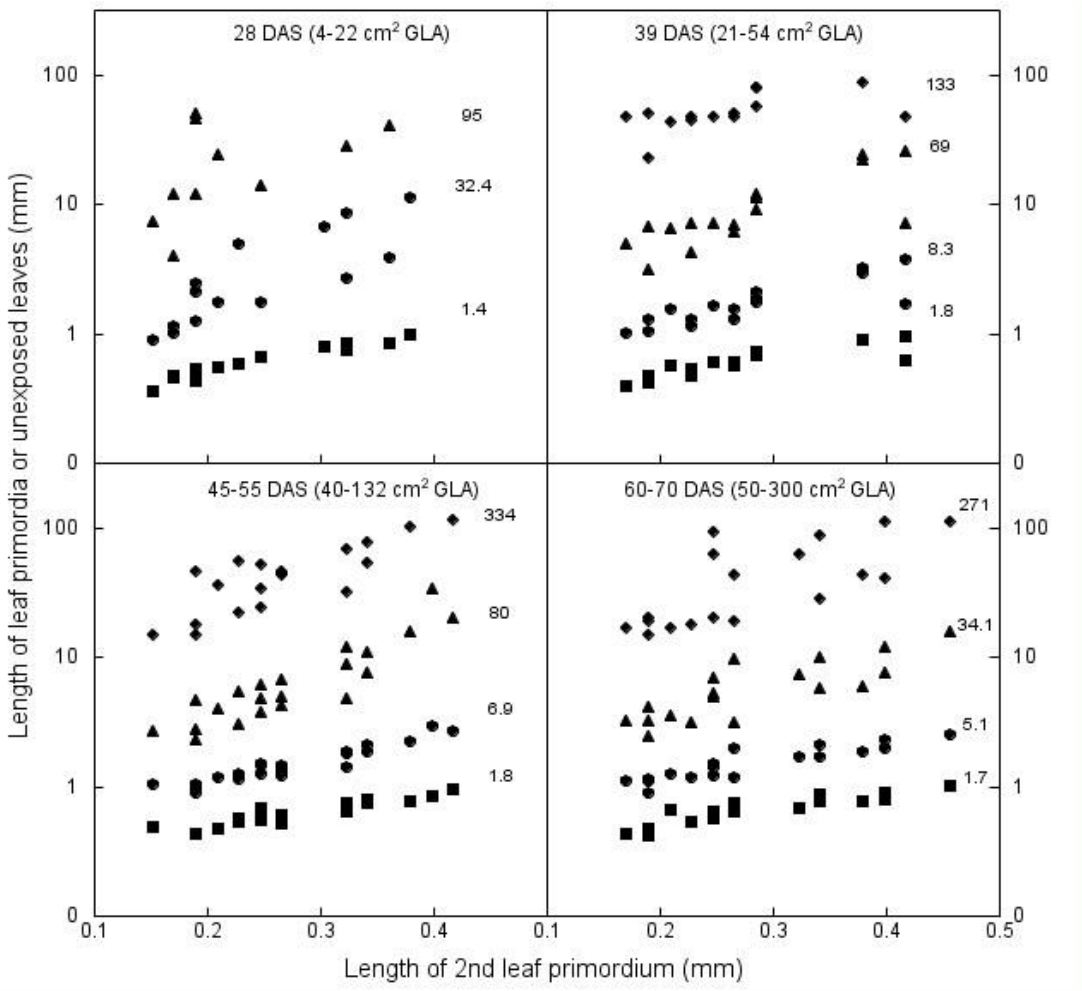

Fig 2. Lengths of leaf primordia and unexposed leaves (log scale) relative to the length of the second leaf primordium in vegetative grain sorghum plants (*Sorghum bicolor* cv. Boomer). Measurements were made in Expt. 1 at four times (days after sowing - DAS) with a range of green leaf area (GLA) as indicated. Linear slopes of lines fitted to the untransformed data are shown for leaf primordia (■) 3 and (●) 4; and unexposed leaves (▲) 5 and (♦) 6. Values of Y for each value on the X-axis represent the leaf lengths of a single plant.

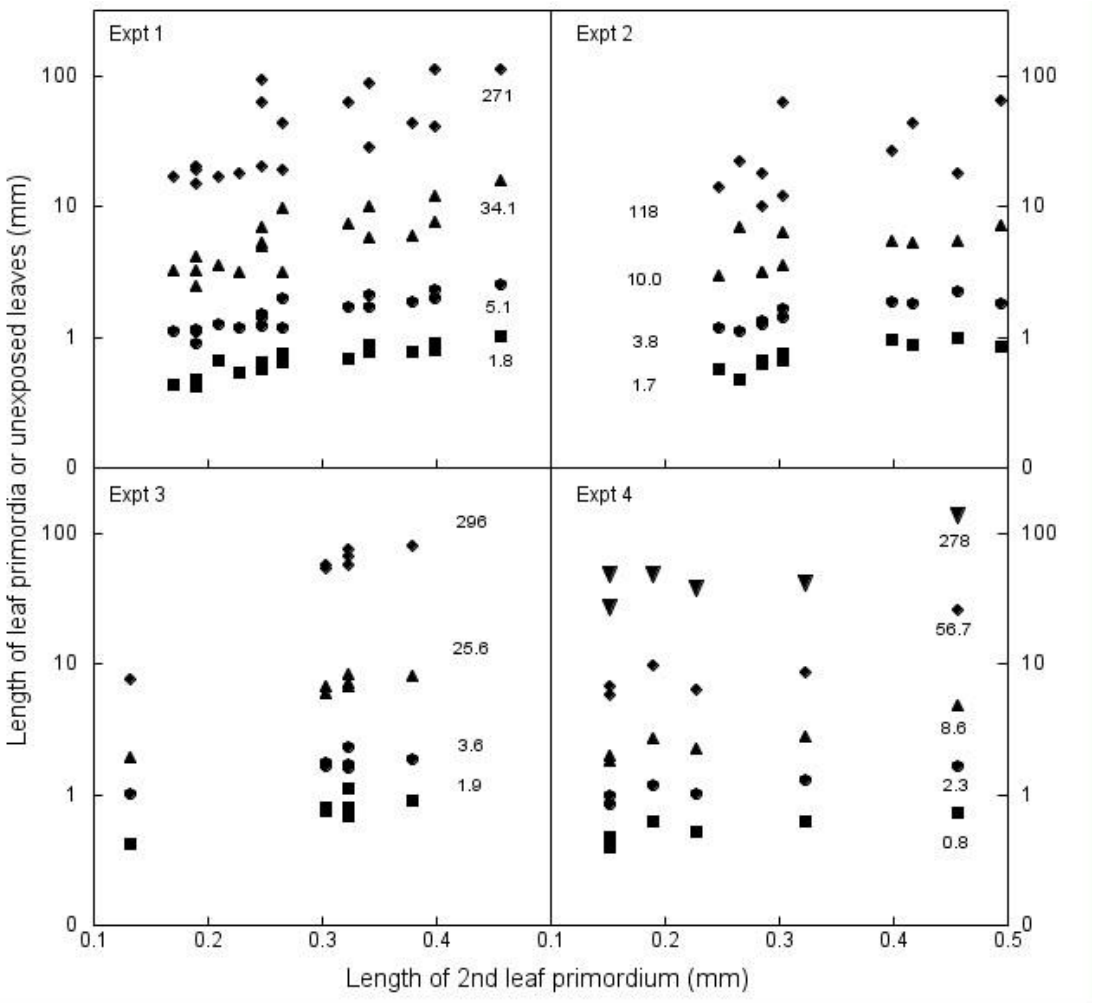

Fig 3. Lengths of leaf primordia and unexposed leaves (log scale) relative to the length of the second leaf primordium in untreated grain sorghum plants (*Sorghum bicolor* cv. Boomer) immediately prior to panicle initiation in experiments 1-4. Linear slopes of lines fitted to the untransformed data are shown for leaf primordia (■) 3 and (●) 4; and unexposed leaves (▲) 5, (♦) 6 and (▼) 7. Values of Y for each value on the X-axis represent the leaf lengths of a single plant.

3.2 The leaf primordia architecture just prior to panicle initiation was similar

The architecture of leaf primordia and unexposed leaves at the shoot apical meristem in cv. Boomer plants sampled immediately before panicle initiation in all four experiments was similar (Fig. 3). The phenomenon was marked by in-union slowing of the elongation rate of each adjacent primordia and unexposed leaves.

3.3 Unexposed-leaf elongation was dependent on green leaf area within a cultivar

The total length of unexposed leaf and green leaf area were strongly correlated (Fig. 4). As green leaf area increased, the increase in unexposed leaf length was less dependent on green leaf area. In all four experiments this relationship was similar; the linear component did not vary in slope ($P>0.05$) although the smooth variation in each line differed ($P<0.001$) between experiments. This latter variation indicates differences in the absolute rate of new unexposed leaf development per unit of green leaf area. Even so, relationships were sufficiently strong and consistent across different experimental conditions to conclude that unexposed leaf development was closely related to, and most likely regulated, by green leaf area expansion. As plants approached panicle initiation the elongation of unexposed leaves became less dependent on green leaf area indicating the onset of a limiting factor on unexposed leaf elongation.

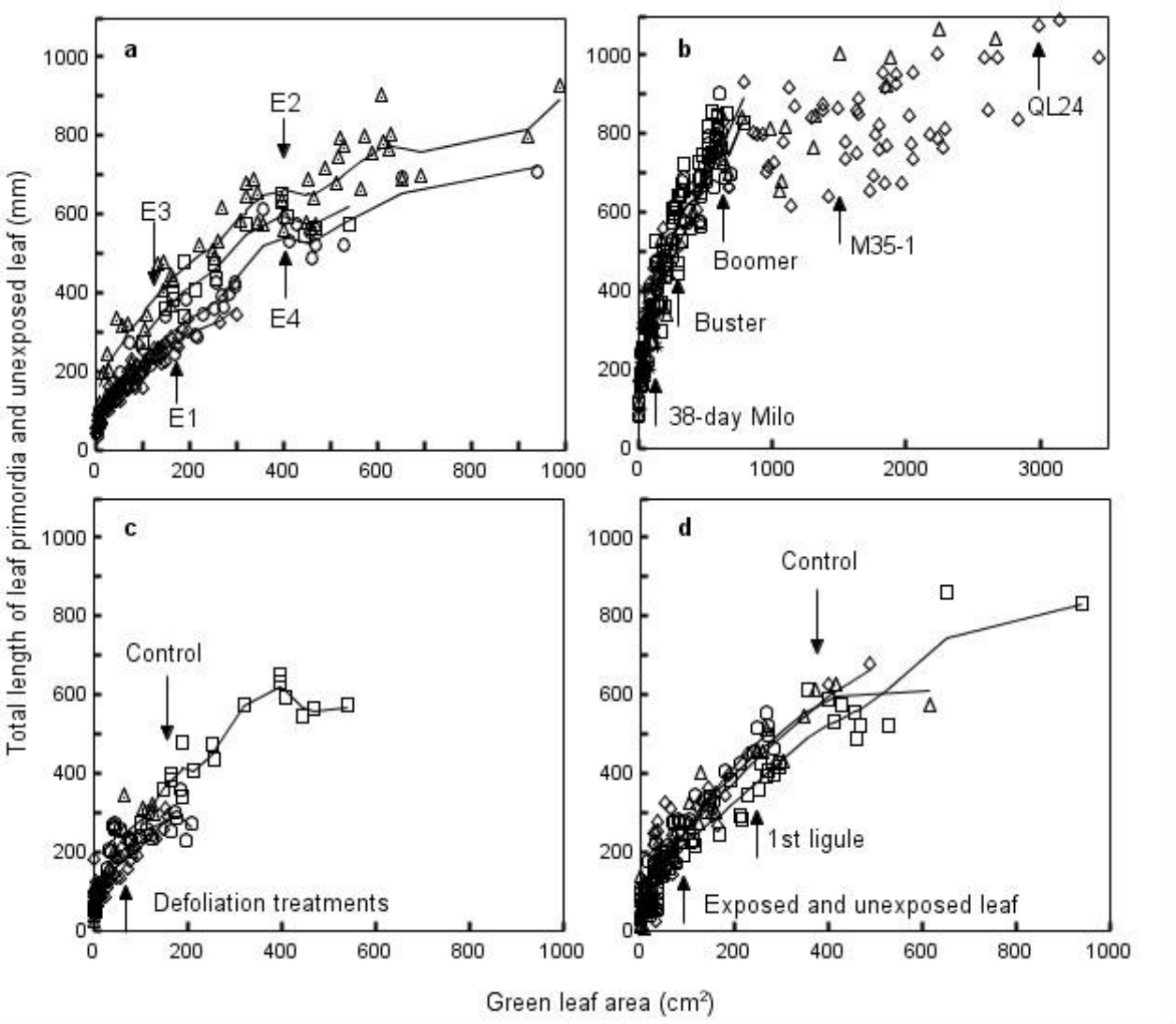

Fig 4. The lengths of leaf primordia and unexposed leaf combined as a function of green leaf area in *Sorghum bicolor*. Arrows mark the green leaf area at the time of panicle initiation in each treatment: (a) control plants of cv. Boomer in four experiments: (◊) E1 - field, winter; (Δ) E2 - glasshouse; (□) E3 - field, winter; (○) E4 - field, spring; (b) cultivars in E2: (*) 38-day Milo, (□) Buster, (○) Boomer, (Δ) M35-1 and (◊) QL24; (c) defoliation treatments in E3: (□) control, (○) exposed leaf, (◊) second ligule and (Δ) first ligule; and (d) defoliation treatments in E4: (□) control, (○) exposed leaf, (Δ) first ligule and (◊) unexposed leaf.

3.4 Cultivars had similar apex development correlated with green leaf area

In Expt. 2 which compared sorghum cultivars, panicle initiation occurred at 20 DAS in 38-day Milo, at 29 DAS in Buster and Boomer, at 45 DAS in M35-l and at 69 DAS in QL24. Leaf number at panicle initiation also varied, increasing from four fully-exposed leaves and 10 total leaves in 38-day Milo through to *c*. 14 fully-exposed and 25 total leaves in QL24.

Despite those natural differences between cultivars, the elongation of leaf primordia and unexposed leaves showed consistent patterns among cultivars, both during early vegetative development and just before panicle initiation. Successively older leaf primordia or unexposed leaves either elongated faster or were longer (if the slopes were not different the intercept was larger; Table 1). Also, the relative elongation rates of equivalently-positioned, unexposed leaves decreased between sampling times.

As panicle initiation was delayed across cultivars, there was an in-union slowing of leaf elongation, thus QL24 the cultivar to last initiate a panicle had the most and shortest leaves, and the slowest elongation rates of equivalently-positioned leaf primordium (Table 1). Changes in leaf elongation rate were most evident in the larger unexposed leaves and least so in the third youngest leaf primordia (Table 1). As previously noted for cultivar Boomer (Fig. 2), the elongation rate and size of the younger leaf primordia across time also did not vary so much within a cultivar (Table 1).

Table 1. Variation in elongation rates (slopes; mm/mm) and sizes (intercepts; mm) of successively older leaf primordia and unexposed leaves (relative to the second leaf primordium) of five cultivars of *Sorghum bicolor* grown in a glasshouse in experiment 2.

Cultivar	38-day Milo		Buster		Boomer		M35-1		QL24	
(n=5-21)	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
					Early vegetative phase					
Days after sowing	12		12-17		12-20		12-17		31-42	
Leaf 3	1.68	0.2	2.37	0.0005	2.2[a]	0.089	2.91	-0.075	1.5	0.16
Leaf 4	13.22	-0.75	16.97	-2.11		2.26	50.97	-8.985	3.56	0.112
Leaf 5	265.66	8.94	483.73	-74.94		28.36	228.43	-11.99	14.08	-1.44
Leaf 6						128.69			76.53	-12.96
Leaf 7									474.14	-94.95
Leaf 8									316.18	122.24
P value	P<0.001		P<0.001		n.s.	P<0.001	P<0.001		P<0.001	
					Just before panicle initiation					
Days after sowing	17-20		28-29		24-29		26-45		52-69	
Leaf 3	2.12	0.078	2.95	-0.093	1.69	0.16	1.48	0.23	1.86	0.08
Leaf 4		1.038	6.8	0.176	3.75	0.28		1.53	2.58	0.36
Leaf 5		14.638	38.1	-5.073	10.0	1.5		5.38	4.82	0.41
Leaf 6			503.31	-95.967	117.5	-9.98		20.0	27.82	-2.37
Leaf 7					460.1	-		97.23	113.95	-14.7
Leaf 8						21.83		270.44	701.41	-114.11
Leaf 9									944.93	-41.94
P value	n.s.	P<0.001	P<0.001		P<0.011		n.s.	P<0.001	P<0.001	
					Change between sampling times					
Regression parameter	Slope		Slope		Intercept[b]		Slope		Slope	
Leaf 3	n.s.		n.s.		-0.105 (*P*<0.005)		-1.48 (*P*<0.008)		n.s.	
Leaf 4	-7.33 (*P*<0.051)		-10.17 (*P*<0.051)		-1.56 (*P*<0.006)		-47.18 (*P*<0.003)		n.s.	
Leaf 5	-		-445.67 (*P*<0.016)		-24.45 (*P*<0.018)		n.s.		-8.27 (*P*<0.003)	
Leaf 6			-		-105.32 (*P*<0.001)				-49.71 (*P*<0.009)	
Leaf 7									-360.71 (*P*<0.001)	

[a]Slope assigned to all leaves not significantly different (*P*<0.05).
[b]Slope not significantly different between sampling times, n.s. not significant (*P*=0.05).

At panicle initiation, green leaf area was greater in later-developing cultivars (Fig. 4b). When green leaf area was <800 cm^2, the underlying slope of total unexposed leaf length on green leaf area was not significantly different (*P*>0.05) between cultivars, and the smooth curve was common (*P*<0.001); 38-day Milo was excluded from this analysis because it had a very small range for green leaf area. In both M35-1 and QL24 the slope of relationship changed when green leaf area exceeded 800 cm^2, and in both cultivars this change coincided with the onset of rapid stem elongation: from less than 50 mm increasing to 350 mm at panicle initiation. Stem elongation was minimal in the other cultivars before panicle initiation.

3.5 Leaf initiation and panicle initiation were delayed by some defoliation treatments

In Expt. 3, leaf initiation proceeded at a near linear rate in control plants (Fig. **5**a). Defoliation of exposed leaf blades slowed leaf initiation but leaf initiation was delayed markedly when all leaf above the second leaf ligule was removed, and essentially ceased when all the leaf and leaf sheaths above the first ligule were removed.

Panicle initiation was not delayed by the defoliation of exposed leaf blades, but was delayed when all leaf above the second leaf ligule was removed, and further delayed when all leaf above the first ligule was removed (Fig. 5a).

3.6 Defoliation reduced leaf area development but it did not correlate with the onset of panicle initiation

In Expt. 3, at the time of panicle initiation in the control and when defoliation was ceased, green leaf area was reduced in all defoliation treatments as consequence of the defoliation *per se* (Fig. 5b). Subsequently, in response to defoliation of exposed leaves and defoliation at the second ligule, those plants at panicle initiation had much less green leaf area than control plants at panicle initiation. In contrast, in response to defoliation above the first ligule, those plants at panicle initiation had regenerated the same green leaf area as control plants at panicle initiation.

The length of unexposed leaves at panicle initiation was affected by defoliation in a similar way to green leaf area (Fig. 5c.), although the response to defoliation of only the exposed leaves was clearly a plant growth response as unexposed leaves were not removed.

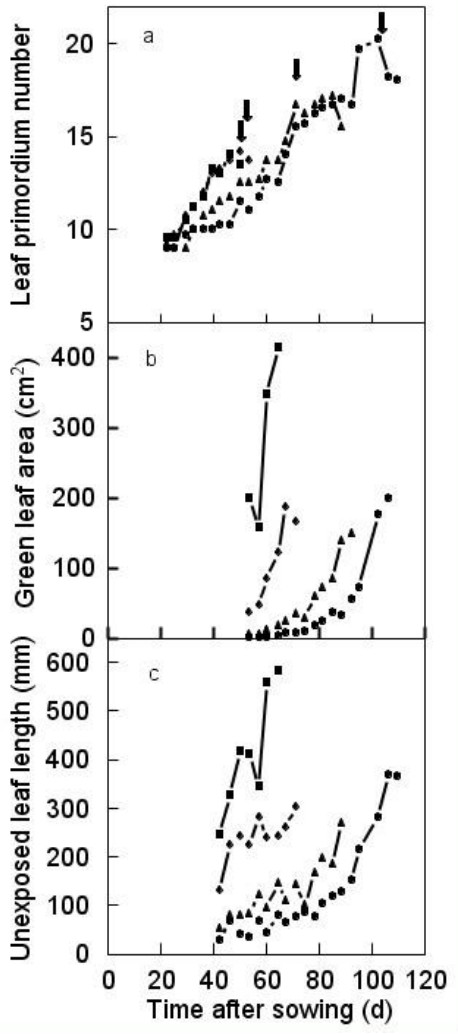

Fig 5. Defoliation treatment effects on (a) leaf primordium initiation, (b) green leaf area and (c) the length of unexposed leaves in vegetative grain sorghum plants (*Sorghum bicolor* cv. Boomer). Measurements were made in experiment 3 for (■) control plants, and in defoliation treatment removing: (◆) the exposed leaf blade; (▲) all leaf and sheath above the second leaf ligule; and (●) all leaf and sheath above the first leaf ligule; twice-weekly from the time the second leaf ligule was visible until panicle initiation in the control. The time of panicle initiation in treatments is indicated by arrows in (a).

3.7 Defoliation reset leaf development but didn't change the pattern of leaf elongation

In Expt. 3, twice-weekly defoliation above the second or first leaf ligules caused long delays in panicle initiation from 25 to 51 d, respectively. In contrast, delay was absent if only the exposed leaf blade was removed. Despite those different timings of panicle initiation, induced artificially, the relative elongation rates of equivalently-positioned leaf primordium and unexposed leaves just before panicle initiation were essentially the same across treatments (Table 2).

In Expt. 3, defoliation treatments reduced the amount of green leaf area at panicle initiation compared with the control plants (Fig. 4c), although the underlying linear slopes for total unexposed leaf length on green leaf area were not significantly different ($P>0.05$). Removing only the exposed leaf blade increased the intercept value; which is mostly a defoliation effect *per se*, because exposed leaf was removed until panicle initiation of the control. The smooth curve was not common for all defoliation treatments ($P<0.01$), reflecting the different range of green leaf area across treatments (Fig. 4c).

Table 2. Variation in elongation rates (slopes; mm/mm) and lengths (intercepts; mm) of successively older leaf primordia and unexposed leaves (relative to the second leaf primordium) of *Sorghum bicolor* (cv. Boomer) subjected to defoliation. Treatments were: control with no defoliation, defoliation of exposed leaf, defoliation above the level of the first or second leaf ligule or defoliation of unexposed and expanding leaf at height of the first leaf ligule.

Defoliation treatment	Leaf 3		Leaf 4		Leaf 5		Leaf 6	
Expt. 3	*Immediately before panicle initiation*							
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Control	1.38[a]	0.37	4.03	0.51	26.6	-1.49	298.1	-32.2
Exposed leaf		0.30	4.80	0.33	41.2	-3.96	154.4	38.4
First leaf ligule		0.33	9.91	-0.39	46.1	0.44	98.6	10.1
Second leaf ligule		0.24	2.94	0.56	13.1	0.17	122.2	9.0
P value (n>5)	n.s.	0.001	0.016	-	0.001	-	0.001	-
Expt. 4	*Early vegetative development or end of defoliation treatments*							
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Control	1.64	0.19[A]	3.98	0.19	9.93	0.20	23.72	6.38
Exposed leaf					23.41	-2.11	168.06	1.63
First leaf ligule					157.97	-32.57	n.a.	n.a.
Unexposed leaf					28.91	-2.10	319.99	-36.34
P value (n>5)	n.s.	n.s	n.s.	n.s.	0.002	-	0.025	-
Expt. 4	*Immediately before panicle initiation*							
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Control	0.83	0.35	2.31	0.59	8.6	0.52	18.05	14.6
Exposed leaf	2.33	-0.04	9.77	-0.85		9.82	n.a.	n.a.
First leaf ligule	1.15	0.30	2.64	0.71		6.46	214.22	4.19
Unexposed leaf	1.31	0.29	3.17	0.52		2.79	337.38	-50.39
P value (n>5)	0.001	-	0.001	-	n.s.	0.017	0.012	-

[a] Slope or intercept assigned to all leaves not significantly different ($P<0.05$). n.s. not significant ($P=0.05$). n.a. leaf tip exposed.

In Expt. 4, twice-weekly defoliation of all exposed leaf blades resulted in an 11-d delay in panicle initiation ($P<0.05$) compared with control plants; which is a substantially different result than the no delay associated with this treatment in Expt.3. Defoliation above the first ligule delayed panicle initiation by 25 d and defoliation of unexposed leaves excised from within the whorl delayed panicle initiation by seven days ($P<0.05$).

In Table 2, the leaf elongation rates of control plants before defoliation was started was compared with those of defoliated plants at the end of the defoliation period (three days before panicle initiation in control plants). The elongation rates of the fifth and sixth unexposed leaves were faster in defoliated than control plants. Natural vegetative development was associated with a gradual slowing of leaf elongation (Fig. 2) but defoliation resets leaf elongation to a faster rate. Reductions in the rates of leaf elongation were observed in control and defoliated plants between their respective vegetative sample and the sample just before panicle initiation; the largest reductions were in the fifth unexposed leaf (Table 2). There were small differences in the elongation rates of the third and fourth leaves between control plants and those with only the exposed leaves defoliated.

The smooth curve of total unexposed leaf length on green leaf area was common to all defoliation treatments ($P<0.001$), however, the underlying slope of the line was less ($P=0.003$) for the control compared to defoliated plants (Fig. 4d). Thus, defoliated plants developed unexposed leaves more efficiently per unit of green leaf area. The green leaf area at panicle initiation was much greater in the control and first ligule defoliation treatments than in the exposed leaf or unexposed leaf defoliation treatments.

4. DISCUSSION

Growth played an important role in leaf initiation at the shoot apical meristem. With defoliation that left a little green leaf on the plant, leaf initiation was not affected. In contrast, with severe defoliation that removed nearly the whole of the green leaf on a recurring basis, leaf initiation stopped, and when that defoliation ceased, leaf initiation restarted at its natural linear rate. Severe defoliation did not cause any discontinuity between the elongation rate relationships of successive leaf primordium and, although leaf initiation stopped, the architecture of the shoot apical meristem was unchanged. The fact that the shoot apical meristem was responsive to the removal and then new growth of green leaves validates a functional and dependent growth relationship between green leaf and the shoot apical meristem.

During vegetative development, the two youngest leaf primordia had constant elongation rates and relative sizes. Leading up to panicle initiation, each new leaf primordium replaced the former in such a way that the length of the second leaf primordium remained within a precise range, over time and for cultivars and defoliation treatments. There was similar coordination in the relative elongation rate of the third compared with the second leaf primordium. The recurring architecture of the shoot apical meristem infers homoeostasis between the 'perfect' shoot apical meristem and widely-different phenotype expressed by plants in different treatments.

Of importance to the timing of panicle initiation was that the elongation rates of earlier-formed primordia, relative to the second-youngest primordium, were continually slowing during vegetative development; and precisely slower when the time of panicle initiation was near. This result is analogous to that of Kirby (1990) who found in wheat that non-emerged leaves accumulated on the shoot apex because the rate of leaf appearance was half that of the rate of leaf initiation. The attainment of a similar architecture and slow elongation rates of primordia on the shoot apical meristem across cultivars, sowing time and defoliation treatments may trigger floral initiation or (at least) signal the physiological condition necessary to achieve a hereditarily-successful flowering time. The elongation rates of leaf primordia may be a functional component of a switch where the 'ripeness-to-flower' status moves progressively towards or away from the 'slow' homeostasis that is necessary to trigger panicle initiation. Foliar expansion can impact on floral initiation. Hopkinson and Ison (1982) noted that despite their adequate size the slow expansion of leaves of seedbed tobacco plants failed to promote floral initiation, and Lauri (1992) found that floral zones in cherry were characterized by a predominance of foliar components over stem components on the brachyblast branches.

Various hypotheses about the floral induction (introduced earlier in the paper) link observational and measurable environmental criteria with the timing of floral initiation or the onset of flowering but they do not describe a causal mechanism or truly functional process. Evans (1993) elaborated about the need not only to describe how the floral signal is produced and transported, but also how it acts when it reaches the shoot apical meristem. The experiments and analyses in this paper gave an insight to a causal mechanism because there was a measurable conditioning of the shoot apical meristem before panicle initiation and it was repeated in all treatments. Also, although green leaf area increased in all treatments leading up to panicle initiation, plants accumulated unexposed leaf at slower rates per unit of leaf area. Reduced growth of the unexposed leaves is evidence of competition from an alternate sink, possibly each subsequent expanding leaf being, as it was, increasingly larger (Kaitaniemi *et al.*, 1999; Ockerby *et al.*, 2001).

No measurement of leaf elongation rate was slower than those obtained at the time of panicle initiation, so the common slow growth and precise architecture of the unexposed leaves and shoot apical meristem might have been the switch-point at which panicle initiation occurred. Support for this hypothesis may be found in the work of Sunderland (1961) who reported a progressive decline in the rates of cell multiplication and expansion in each successive primordium generated in the apical dome of winter rye and lupin, and that of Williams and Williams (1968) who showed that the relative growth rates of the youngest leaves and the inflorescence of wheat were similar. Further work may show that the definitive switch could be as simple as (speculative) a sink-induced limitation on the supply of energy to the shoot apical meristem determining whether it will differentiate a vegetative or reproductive structure.

The time delay to panicle initiation after defoliation ceased varied when different leaves were removed. When unexposed leaf was removed there was a short delay to panicle initiation; when exposed leaf (green leaf area) was removed it was a longer delay; and when the plant was defoliated just above the 1st ligule the delay was longest. When more leaf was removed by defoliation it took longer to re-establish the balance between green leaf and the architecture and the slow function of the shoot apical meristem necessary to switch to panicle initiation. This finding is analogous to those of Irish and Karlen (1998) who showed that plants regrown from smaller excised meristems of maize regrew to be developmentally younger plants.

One troubling aspect of this work was the failure of sorghum to respond to photoperiod extension with incandescent light. Tropical sorghum is classified as a quantitative short-day plant (Quinby and Karper, 1945) and photoperiod extension longer than 13h should have delayed panicle initiation (Hammer et al., 1989). Even so, the natural photoperiod at panicle initiation was not the same across all the experiments as some were done in winter and some in summer. It may still have been the case that plant growth was responsive to endogenous signals and cues from the environment (Muchow and Carberry, 1990) but its effect was mediated by the greater biomass of non-emerged leaves which reduced the supply of growth resources to each primordial leaf structure (Williams, 1975). The possibility exists that natural day-length variations affected the timing of floral initiation but did not perturb the analyses or interpretation of results, that leaf growth controlled the timing of panicle initiation.

A plant that has seeded has also successfully balanced its growth between vegetative and reproductive architectures. The data and analyses in this work raised the possibility that plants initiate flowering by a sink-induced regulation of growth at the shoot apical meristem. Growth was determined by the capacity of green leaves and reckoned against the increasing size of developing leaves still inside the whorl. A mechanism that 'recognized' when the plant had allocated only enough resources to leaf area would be a successful evolutionary process.

5. REFERENCES

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