

## **A SIMPLE METHOD FOR ESTIMATING THE END OF EFFECTIVE FLOWERING IN UPLAND COTTON (*GOSSYPIUM HIRSUTUM*)**

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### SUMMARY

In cotton (*Gossypium hirsutum*), it is hard to determine the exact date when reproductive growth ceases on the basis of field observations, as compared to more visible factors such as the onset of flowering or boll opening. It is, however, essential to characterize the growth cycle in order to determine what varieties are suitable for planting in different climatic and local cropping conditions. We estimated the end of the effective flowering period on the basis of the opening date of the last flower giving rise to a first-position boll on fruiting branches (LFP1), and propose a simple method for estimating this date. This study, conducted in 2002 and 2003 at Okpara, Benin, involved a comparison of six cotton varieties planted at two different dates (June and July). Plants were monitored to determine the dates when flowers opened at each position on fruiting branches. The LFP1 indicator made a clear distinction between varieties. This highly heritable trait, which was found to be closely correlated with other earliness criteria, could be used to characterize the length of the growth cycle in cotton varieties.

### INTRODUCTION

Cotton breeders would be better prepared to determine the cropping potential of any variety of *Gossypium hirsutum* if they had an accurate chronological picture of its growth cycle. This knowledge is especially important in rainfed cropping because the growth cycle of cropped varieties must be adapted so that the plants will thrive when planted at the recommended date under local conditions. Most breeders assess the earliness of yield of a variety according to the ratio of the weight of the first crop harvest (which is carried out when 50 % of the bolls on control plants are open) to the total harvest weight (Bilbro and Quisenberry, 1973; Ray and Richmond, 1966). This easy to calculate ratio can thus be used to compare the earliness of yield of several varieties. It is, however, not very accurate when the first harvest dates are delayed, and it is hard to make between-test comparisons because first harvest dates often differ between trials (Bourland *et al.*, 1991). A more reliable indicator is needed to characterize the length of the effective flowering period of cotton varieties.

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Cotton has a perennial growth habit, and the fruiting and vegetative growth phases overlap temporally, so the duration of these phases cannot be determined by simple observations. The two key points in the physiological development of cotton plants between seedling emergence and the completion of boll opening are the date of the beginning of reproductive growth and the date of completion of vegetative growth. Reproductive growth starts when the first flower bud is initiated, but this event is hard to observe. The date when the first flower appears is the most common indicator used to characterize the onset of the fruiting phase – this date can be easily and accurately pinpointed.

It is also complicated to determine accurately the date of completion of vegetative growth. This event comes at the end of a growth process when competition arises between fruiting and vegetative growth. Carbohydrates produced by photosynthesis are simultaneously used to sustain growth, the formation of new fruiting organs and maturation of already formed fruits. When reproductive growth starts, assimilates are preferentially allocated to fruiting organs (buds, flower and bolls) (Kerby *et al.*, 1990). Assimilates are shared between the fruiting and the vegetative organs (roots, stems and leaves) in such a way that plant growth slows down as the bolls develop. Flowering and growth cease once the plant is bearing its maximum boll load. This stage is called cutout (Mauney, 1986). The extent of cutout may differ between varieties – in some varieties, once the first bolls have reached maturity and if there are suitable temperature and humidity conditions, vegetative growth may begin again, followed by the formation of new fruiting nodes.

Bourland *et al.* (1992) estimated the cutout date by monitoring flowering progress along the main stem and noting changes in the number of nodes above the last flower in the first position on fruiting branches (NAWF); this is maximal at flowering onset and decreases thereafter as the bolls form and grow to maturity (Figure 1). They showed that physiological cutout occurred at  $\text{NAWF} = 5$ , at which stage approximately 95% of the bolls had formed. This indicator is commonly used by cotton crop managers to help them decide, for instance, on the best time to stop spraying insecticides or apply defoliant.

However, in Africa, under rainfed cropping conditions, we found that it was not always possible to monitor the date at which  $\text{NAWF} = 5$ , as cotton plants did not always attain it at the end of the growing period. We therefore proposed an alternative method for evaluating the date of completion of reproductive growth that involves estimating the opening date of the last flower giving rise to a first-position boll on fruiting branches (LFP1). We have also developed a method for estimating this indicator. Finally, we estimated the heritability of the LFP1 indicator to assess its use in cotton breeding programmes.

#### MATERIALS AND METHODS

The trials were conducted at the Centre de Recherches Agricoles Coton et Fibres at Okpara (Benin) in 2002 and 2003. Okpara ( $2^{\circ}41'E$ ,  $9^{\circ}18'N$ , 320 m asl) is located in

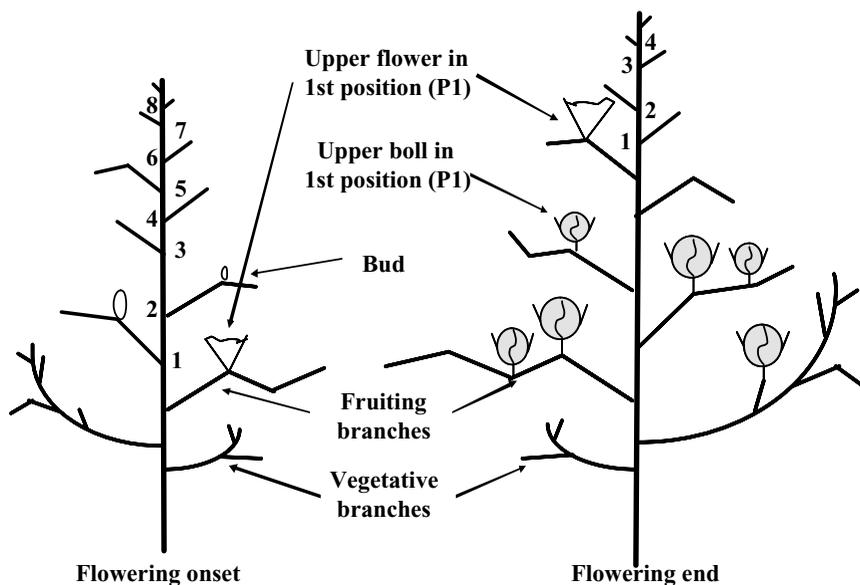


Figure 1. Flower and boll development in cotton. Left: at flowering onset, the upper open white flower in P1 is located on the first fruiting branch. At that time, the number of nodes above this upper white flower (NAWF) is equal to eight. Right: at the end of flowering, the upper open white flower in P1 is located on the fifth fruiting branch. At that time, NAWF is worth four. The upper formed boll on the plant in P1 is located on the third fruiting branch. The date at which it was only a flower corresponds to LFP1.

Table 1. Sowing dates, rainfall levels, and boll opening dates in the four trials conducted at Okpara, Benin, in 2002 and 2003.

Trial No	Year	Planting date	Effective rainfall <sup>†</sup>	First boll opening date <sup>‡</sup>
1	2002	June 26	826	108
2	2002	July 17	755	103
3	2003	June 20	858	113
4	2003	July 18	701	108

<sup>†</sup>Quantity of rainfall (mm) during the period from 10 days before planting to the first harvest.

<sup>‡</sup>Number of days between emergence and the first boll opening date per plant.

the middle of a cotton-growing area with abundant rainfall (around 1200 mm year<sup>-1</sup>). The tropical ferruginous soils that prevail are low in organic matter.

A split-plot experimental design with three replicates was used with two factors (Tables 1 and 2): two planting dates staggered by at least three weeks (June and July) and six varieties. Basic experimental plots (14.4 m<sup>2</sup>) were set up with three 6 m rows. Seedlings were thinned to one plant per hole. The stand density was 42 000 plants ha<sup>-1</sup>. We also implemented the crop management sequences generally recommended for cotton-growing areas in Benin. However, the plants were treated with pesticides to avoid shedding of fruiting organs induced by non-physiological factors. Plants in the middle row of each basic plot were monitored.

Table 2. Characteristics of the six cotton varieties.

Variety	Origin	Earliness	Habit
Mar 88-214	USA	Early	Short-compact
Oultan	Uzbekistan	Early	Slender-clustered
Chaco 520	Argentina	Medium	Compact
S 188	Nicaragua	Late	Slender-arborescent
Irma A1042	Cameroon	Late	Slender-arborescent
Stam 18A	Togo-Benin	Late	Slender-arborescent

Several phenological indicators were measured: node to first fruiting branch (NFFB), first flower opening date (FF), first opening boll date (FOB) and the production earliness ratio ( $H1/HT = \text{first harvest}/\text{total harvest}$ ). The dates of flower opening at all positions on fruiting branches were also monitored daily on five plants per plot, with two replicates per trial. These measurements enabled us to monitor the opening dates of the last effective flower (LEF), i.e. the flower that gave rise to a harvestable boll, as well as the branch number and the opening date of the LFP1 (Figure 1).

We considered that the opening date of the LEF gives a reliable estimate of the end of vegetative growth in cotton plants. In our study, this was used as a reference indicator to assess the quality of the other indicators.

Variance analyses were performed with the SAS<sup>®</sup> software package. Means were compared using the test of Tukey-Kramer (1956). The broad sense heritability coefficients for the opening date of the last flower giving rise to a LFP1 were estimated using a general linear model with random effects (Comstock and Robinson, 1952). The confidence interval of each heritability coefficient was calculated by the method described by Agresti and Coul (1998). Heritabilities were calculated for individual plants and for a set of five nearest neighbouring plants growing in the same plot.

## RESULTS

### *Estimation of the date of the LEF from the opening date of the LFP1*

As the vegetative phase ends when the last harvestable boll is initiated, we determined the opening date of the flower that gave rise to this last boll, i.e. LEF. More than 92 % of the plants (220 out of 238) produced this flower in the first position (P1) on a fruiting branch in each situation investigated (Table 3). The LEF date and the opening date of the LFP1 were closely correlated ( $R^2 = 0.99$ ). We thus propose LFP1 as a relevant criterion to estimate the end of the reproductive period.

### *The opening date of the LFP1*

*Genetic and heritability effects.* The variance analysis on the opening date of the LFP1 showed that there was a significant varietal effect ( $p < 0.01$ ), and that this factor was not correlated with the planting date (Table 4).

Stam 18 A and Irma A 1042 were later yielding varieties (LFP1 around 82 days post-emergence) and Mar 88-214 had the most determinate growth as it ceased fruiting after about 70 days. Stam 18 A, Irma A 1042 and S 188 stopped developing bolls 5–10

Table 3. Number of plants with the last harvestable boll at the first position (P1) on fruiting branches relative to the total number of monitored plants.

Variety	Planting date		Planting date		Total
	June 2002	July 2002	June 2003	July 2003	
Mar 88-214	8/9	9/10	8/10	9/10	34/39
Oultan	10/10	10/10	9/10	9/10	38/40
Chaco 520	8/9	10/10	10/10	10/10	38/39
S 188	10/10	9/10	9/10	8/10	36/40
Irma A 1042	10/10	10/10	8/10	9/10	37/40
Stam 18 A	10/10	10/10	8/10	9/10	37/40
Total	56/58	58/60	52/60	54/60	220/238

Table 4. Mean opening date of the last flower giving rise to a first-position boll on fruiting branches per variety and trial (in number of days post-emergence).

Variety	Planting date		Planting date		Mean
	June 2002	July 2002	June 2003	July 2003	
Mar 88-214	63.7	62.5	80.1	73.9	70.1
Oultan	69.8	68.7	83.6	78.9	75.3
Chaco 520	65.9	67.6	85.1	81.7	75.1
S 188	75.3	72.1	88.2	82.0	79.4
Irma A1042	80.5	72.7	89.5	85.2	82.0
Stam 18A	82.3	73.6	89.7	80.7	81.6
Mean	72.9	69.5	86.0	80.4	77.3
<i>s.e.</i>		3.8		2.5	3.5

Table 5. Broad sense heritability coefficients for the mean opening date of the last flower giving rise to a first-position boll on fruiting branches.

	Planting date		Planting date	
	June 2002	July 2002	June 2003	July 2003
$h^2$ plant level	0.48*	0.19	0.40*	0.35
$h^2$ plot level	0.67**	0.55	0.78**	0.53

\*, \*\* Heritability coefficients significant at the 5% and 1% levels, respectively.

days after Mar 88-214, Chaco 520 and Oultan, which are considered to be earlier varieties (Table 4). The cutout date estimates obtained via the LFP1 indicator clearly differentiated the varieties.

The heritability coefficients were quite high (Table 5) and close to values regularly obtained with indicators such as the first flower opening date or the first opening boll date (Godoy and Palomo, 1999; Lançon, 1994). The results did not vary between 2002 and 2003. The mean heritabilities per plant were higher in the early (June) planting schemes (2-year mean:  $h^2 = 0.44$ ) as compared to those of the July planting schemes (2-year mean:  $h^2 = 0.27$ ).

Table 6. Correlation coefficients between the mean opening date of the last flower giving rise to a first-position boll on fruiting branches and the earliness indicators: first harvest to total harvest ratio (H1/HT), first flower opening date (FF), first opening boll date (FOB), and node to first fruiting branch (NFFB).

Earliness indicators	Planting date		Planting date	
	June 2002	July 2002	June 2003	July 2003
H1/HT	-0.89*	-0.79	-0.98**	-0.81*
FF	0.91*	0.83*	0.82*	0.74
FOB	0.97**	0.86*	0.89*	0.80
NFFB	0.71	0.78	0.89*	0.45

\*, \*\* correlation coefficients significant at the 5% and 1% levels, respectively. Each correlation coefficient was calculated with six pairs of observations (six varieties).

*Correlations between LFP1 and other phenological indicators.* For all varieties, the opening date for LFP1 was closely correlated with the other earliness indicators such as H1/HT, FF, FOB and NFFB (Table 6). Like the LFP1 heritability findings, these correlations were higher in the early June planting schemes than in the July schemes.

#### *Simple indirect estimation of LFP1*

It is not easy to monitor LFP1 directly. To determine this date accurately, the dates of appearance of all flower buds at the end of the growth cycle have to be noted and then correlated with the number of bolls actually harvested at these positions. However, this would be a time- and labour-intensive operation. We thus propose a simple method that involves monitoring flowers only at the first positions on a few fruiting branches.

Cotton flowers open simultaneously along the main stem and along the fruiting branches at a rate that varies between genotypes and according to climatic conditions. Mauney (1986) reported that at 30 °C the vertical flowering interval (i.e. the time between two flowering events at the same position on two consecutive fruiting branches) ranges from 2.2 to 4.0 days depending on the variety and cycle stage. However, this interval could be considered as constant at peak flowering, provided water availability and temperature were not limiting factors (Croizat, 1995). We thus hypothesized that flowering progress at P1 along the main stem could be modelled by a linear equation:

$$y = ax + b \quad (1)$$

where  $y$  is the opening date of a flower at position 1, and  $x$  is the number of the corresponding fruiting branch (Figure 2). Parameters  $a$  and  $b$  in the equation correspond respectively to the number of days between two successive flowers in P1 and to the approximate date of flowering onset. They were estimated on the basis of evenly spaced observations (Table 7). Note that the flowering rates were slightly slower in early varieties planted in June.

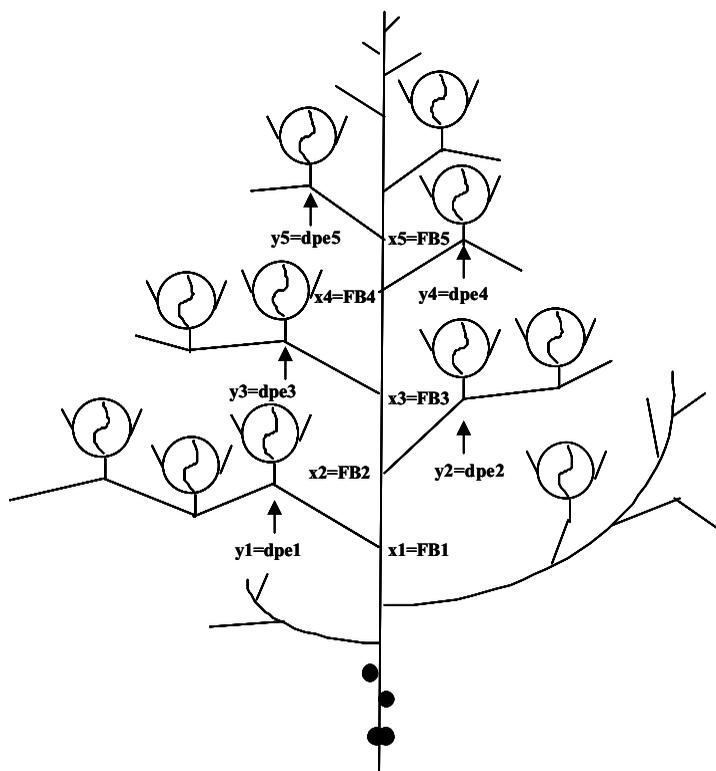


Figure 2. Diagram showing regression variables x and y, y being expressed in days post-emergence (dpe) and x in fruiting branches number (FB).

Table 7. Regression equation parameters per variety and planting date for the number of days post-emergence according to the number of fruiting node with an open flower in P1.

Variety	Planting date						Planting date					
	June 2002			July 2002			June 2003			July 2003		
	a	b	R <sup>2</sup>	a	b	R <sup>2</sup>	a	b	R <sup>2</sup>	a	b	R <sup>2</sup>
Mar 88-214	4.35	45.6	0.99	4.00	40.7	0.94	3.85	47.5	0.99	3.70	50.7	0.99
Oultan	4.17	45.0	0.99	4.17	36.4	0.88	4.00	42.5	0.98	3.57	51.1	0.98
Chaco 520	4.35	45.7	0.95	4.17	40.4	0.90	4.17	44.3	0.98	4.00	48.9	0.98
S 188	4.35	45.4	0.89	4.17	42.5	0.94	3.85	50.7	0.99	3.70	56.4	0.98
Irma A 1042	4.17	48.8	0.98	4.17	42.1	0.97	4.35	45.7	0.98	3.85	54.6	0.98
Stam 18 A	4.55	45.3	0.92	4.17	41.8	0.96	4.17	46.8	0.98	3.85	51.1	0.98

a: linear regression slope (days per fruiting node); b: ordinate at the origin (days post emergence); R<sup>2</sup>: determination coefficient, each R<sup>2</sup> was calculated with 12 pairs of observations.

Based on the node number of the last fruiting branch bearing a boll at the first position, we used this regression to determine the date at which the corresponding flower appeared, thus providing an estimation of LFPI. Values estimated by this method were compared to the actual dates of flowering at the highest first positions

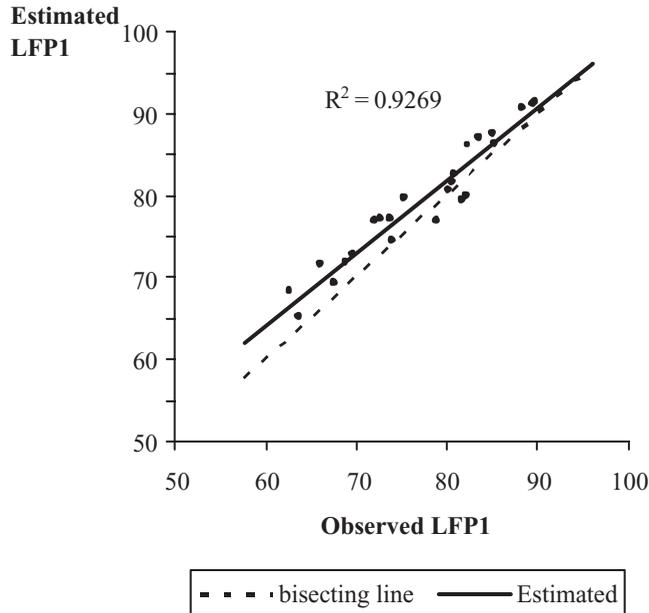


Figure 3. Opening date of the last flower giving rise to a first-position boll on fruiting branches (LFP1 in days post-emergence)—a comparison of estimated and observed values.  $R^2$  was calculated with 24 pairs of observations (2 years  $\times$  2 planting dates  $\times$  6 varieties).

that gave rise to a harvestable boll (Figure 3). The actual LFP1 values and those estimated via equation (1) were very closely correlated ( $R^2 = 0.93^{**}$ ), indicating that this estimation method was satisfactory. The estimated values were very close to the bisecting line, especially when the growth cycles got longer and closer to the genetic potential. In contrast, with shorter growth cycles (varieties planted in July, or when rainfall was insufficient), the calculated values were slightly overestimated, thus reducing the gap between the early and late varieties, but this deviation was never high or significant ( $\chi^2 = 0.99$ ,  $d.f. = 22$ ).

#### DISCUSSION AND CONCLUSION

The opening date of the last flower giving rise to a first-position boll on fruiting branches (LFP1) is a good indicator of the opening date of the last effective flower (LEF), leading to a harvestable boll. Flowering dynamics along the main stem can be readily monitored, so this criterion is easy to estimate. Contrary to earliness indicators such as the production earliness ratio ( $H1/HT = \text{first harvest}/\text{total harvest}$ ), or the first opening boll date (FOB) that were discussed in Bourland *et al.* (1991), the LFP1 measure is relatively independent of the conditions prevailing at the very end of the growth period, as bolls are already formed at that time. Actual LEF could be different from LFP1 if uneven pest attacks, for example topping due to *Earias* sp., were particularly affecting the number of bolls produced next to the main stem in P1, in comparison with the bolls produced in the other positions.

LFP1 was closely correlated with – but not really equivalent to – all of the following earliness indicators: the H1/HT ratio, FOB, the node to first fruiting branch (NFFB) and the first flower opening date (FF). This indicator can be used as a complement to the flowering date to assess the effective flowering duration – a period when the plant maintains its potential for producing flowers that could be transformed into harvestable bolls.

LFP1 heritability was found to be high at both the plant and whole-plot scale, especially under early planting conditions. In cotton breeding programmes, when cycle length and earliness are crucial traits, LFP1 could be used in varietal screening tests to predict the genetic potential of varieties according to local cropping conditions. Our results suggest that it might be a better strategy to estimate LFP1 in early planting trials in order to clearly highlight the genetic differences. LFP1 would, however, be unsuitable for programmes involving large-scale breeding of individual plants because the labour costs would be excessively high.

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