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Effect of Pulp Cell Number and Assimilate Availability on Dry Matter Accumulation Rate in a Banana Fruit [*Musa* sp. AAA group 'Grande Naine' (Cavendish subgroup)]

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Fruit position on the bunch (inflorescence) is an important part of variability in banana fruit weight at harvest, as fruits at the bottom of the bunch (distal fruits) are approx. 40 % smaller than those at the top (proximal fruits). In this study, the respective roles of cell number and cell filling rate in the development of pulp dry weight are estimated. To this end, the source/sink ratio in the plant was altered at different stages of fruit development. Leaf shading (reducing resource availability), bunch bagging (increasing sink activity by increasing fruit temperature), and bunch trimming (decreasing sink size by fruit pruning), applied once cell division had finished, showed that the pulp filling rate depends on resource availability. Bunch bagging and bunch trimming were also carried out before the end of cell division to estimate the role of pulp cell number in the development of pulp dry weight. A sampling method was calibrated to evaluate pulp cell number from the digestion of a fixed portion of the pulp in a solution of chromic and nitric acids. A relationship was found between pulp cell number and fruit length at the end of cell division. It was observed that pulp cell number is a determining factor in pulp dry weight variability within a bunch. On the other hand, the cell filling rate was identical for all fruits in the bunch and was influenced by the source/sink ratio. A Michaelis-Menten relationship was invoked to relate the cell filling rate in a bunch to the source/sink ratio during bunch filling.

Key words: Banana fruit, *Musa* sp., fruit growth, cell number, cell filling rate, source/sink ratio, temperature.

INTRODUCTION

Fruit weight and size (length and diameter) are important commercial criteria for export bananas, as they influence the selling price on the European market. In the French West Indies, harvest occurs when a reference fruit in the bunch (inflorescence) reaches a diameter of 34 mm, corresponding to a fruit of suitable size and maturity for export. An important source of variation in fruit weight and size is fruit position within the bunch: fruits are arranged in clusters called hands that are inserted helicoidally around a central axis called the stalk. Hands at the top of the bunch are the first to be initiated on the meristem (Alexandrowicz, 1955; Ganry, 1980) and bear fruits (proximal fruits) that are 40 % bigger and heavier than those at the bottom of the bunch (distal fruits) (Robinson, 1996). This negative gradient is detrimental for banana growers because the entire bunch is harvested at the same time and, so as to qualify for export from the French West Indies to continental France, a fruit must be at least 30 mm in diameter and 17 cm long. To reduce this gradient and improve the yield of premium fruit, it is important to study weight and size determination in relation to fruit position within the bunch.

According to recent studies (Jullien *et al.*, 2001), the negative gradient in fruit weight and size is related to a difference in developmental stage between proximal fruits (initiated first) and distal fruits that leads to a difference in pulp cell number between fruits. Due to competition for assimilate, pulp cell number is reduced in the younger fruits. We may thus hypothesize that pulp cell number is a determining factor for fruit growth rate, as has been shown for other species e.g. grain legumes (Munier-Jolain and Ney, 1998), wheat (Brocklehurst, 1977) and melon (Higashi *et al.*, 1999). However, the growth rate of banana fruit also seems to vary with the source/sink ratio during fruit growth. Removal of hands (thereby decreasing the sink size: Meyer, 1975; Daniells *et al.*, 1994; Johns, 1996), leaf shading (reducing resource availability: Israeli *et al.*, 1995) or bunch bagging (increasing sink demand by elevating the air temperature: Turner and Rippon, 1973; Ganry, 1975; Johns and Scott, 1989*a*, *b*; Daniells *et al.*, 1992; Jannoyer and Chillet, 1998; Turner, 1998) modify fruit growth rates and, consequently, the commercial harvest date.

Effects of source/sink modifications on banana fruit growth rate differ between studies and may be related to differences in fruit age at the time a treatment is applied. Indeed, treatment may have a different effect on cell number and the rate and duration of cell filling depending on the developmental stage at which it is applied. For pea, soybean and lupin, modification of the source/sink ratio after the end of cell division affects the duration of grain filling but not the

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rate (Munier-Jolain *et al.*, 1998). The grain filling rate depends only on the cell number at the beginning of filling (Munier-Jolain and Ney, 1998). For apricot, Jackson and Coombe (1966) have shown that differences in fruit volume within a tree were related to differences in cell number, but that between trees the contribution of cell volume was important. In peach, the leaf/fruit ratio at the branch level modifies the fruit filling rate (Ben Mimoun, 1997): when the leaf/fruit ratio was decreased, the filling rate, filling duration and final weight also decreased. These results suggest that both cell filling rate and cell filling duration may be modified by the source/sink ratio.

For banana fruit, pulp developmental stages have been dated in degree-days (dd) cumulated since flower emergence (Jullien *et al.*, 2001). These authors have shown that cell division starts about 70 dd after flower emergence and stops about 350 dd after flower emergence in proximal hands. Results also showed that cell division starts and stops about 50–70 dd later in distal hands for a bunch of eight hands. This developmental lag is maintained during the whole fruit growth period. Rapid starch accumulation begins in the pulp after the end of cell division (Jullien, 2000). Cell filling duration is fixed by the commercial harvest date; harvesting occurs before maximum pulp dry weight has been achieved (Barnell, 1940; Jullien, 2000). In this study, we modified the source/sink ratio at different stages of fruit development [between inflorescence emergence (anthesis) and commercial harvest] to evaluate the respective roles of pulp cell number, cell filling rate and cell filling duration in the determination of banana fruit weight.

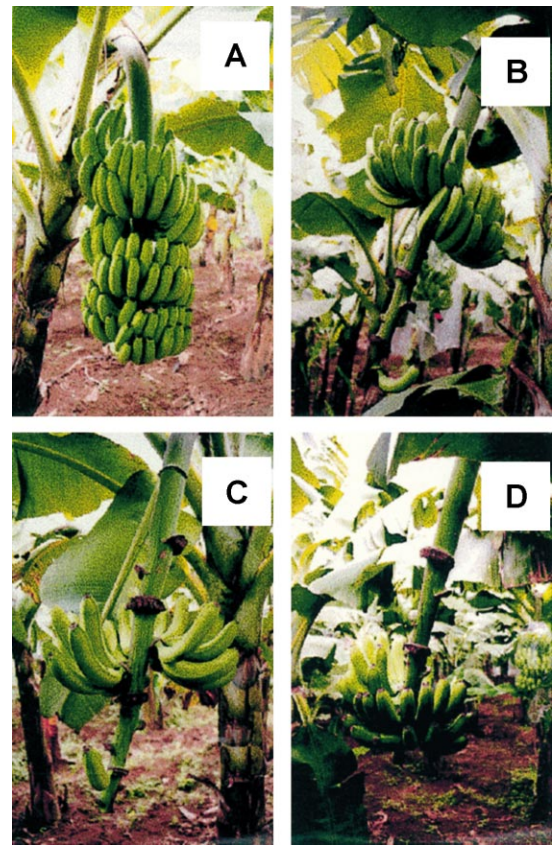

FIG. 1. Pruning treatments (expts 1 and 3). A, Control (H8CA); B, H2UA and H2UB; C, H2MA and H2MB; D, H2LA and H2LB.

MATERIALS AND METHODS

Planting material and growing conditions

Field experiments were conducted in Guadeloupe (French West Indies: 16°N, 62°W), with *Musa* sp. (AAA Group Cavendish Subgroup) 'Grande Naine'. Plants raised from tissue culture were planted on 15 Jun. 1997 (expt 1), 10 Nov. 1998 (expt 2) and 3 Jul. 1999 (expt 3). A first group of plants at the same developmental stage (inflorescence emergence) was selected in February 1998, June 1999 and March 2000, respectively, in expts 1–3. Of these plants, only those with seven or eight hands and an equivalent number of fruits per hand were retained after the bracts covering the distal hand had lifted. Hands were numbered from top to bottom of the bunch (hand 1: proximal hand, top of the bunch; hand 8: distal hand, bottom of the bunch).

Treatment application was chosen according to developmental stages, as determined by Jullien *et al.*, (2001): cell division ceased around 350 dd from inflorescence emergence in proximal fruits and around 420 dd from inflorescence emergence in distal fruits.

Increasing the source/sink ratio during fruit filling by pruning hands (expts 1 and 3)

Hand pruning was carried out on two different dates in expts 1 and 3: at 350 dd (about 5 weeks) after inflorescence emergence, i.e. when approx. 80 % of cell division had occurred in the bunch (Jullien *et al.*, 2001) in expt 1 (excision A) and between 0 and 180 dd, i.e. during the cell division phase in expt 3 (excision B). Each experiment consisted of three different pruning situations (Fig. 1), i.e. leaving (1) the two upper hands [hands 1 and 2 in excision A (H2UA) and excision B (H2UB)]; (2) the two middle hands [hands 4 and 5 in excision A (H2MA) and excision B (H2MB)]; and (3) the two lower hands [hands 7 and 8 in excision A (H2LA) and hands 6 and 7 in excision B (H2LB)] on the bunch. Pruning treatments were applied to five replicates per treatment in excision A and to two (H2UB, H2LB) or three (H2MB) replicates per treatment in excision B. Treatments were compared with a control that consisted of 15 bunches with eight hands in expt 1 (control 1: H8CA) and of 30 bunches with seven hands in expt 3 (control 3: H7CB). Thus, there were 30 plants in expt 1 and 35 plants in expt 3. The experimental design was completely randomized. Bunches were sampled at the beginning and in the middle of the filling period for destructive measurements in H8CA (five bunches at 6 and 10 weeks after flower emergence) and in H7CB (ten bunches at 6 and 9 weeks after flower emergence). The remaining bunches were harvested at the same time for all treatments within an experiment, when the mean diameter of the reference fruits on hand 4 had reached 34 mm (commercial harvest) in any treatment (expt 1, 14 weeks after flower emergence) or in the control (expt 3, 14 weeks after flower emergence).

TABLE 1. *List of treatments and respective abbreviations*

Experiment	Treatment	Abbreviation	Number of plants sampled		
			Beginning of filling	Middle of filling	Commercial harvest
Expt 1	Excision A hands 1&2	H2UA	0	0	5
	Excision A hands 4&5	H2MA	0	0	5
	Excision A hands 7&8	H2LA	0	0	5
	Control 1	H8CA	5	5	5
Expt 3	Excision B hands 1&2	H2UB	0	0	2
	Excision B hands 4&5	H2MB	0	0	3
	Excision B hands 6&7	H2LB	0	0	2
	Control 3	H7CB	10	10	10
Expt 2	Shading	Shading	5	5	5
	Bagging	Bagging	5	5	5
	Early bagging	Early Bagging	0	0	5
	Control 2	Control 2	5	5	5

Number of plants sampled for destructive measurements during pulp filling.

Decreasing the source/sink ratio by leaf shading and bunch bagging (expt 2)

Experiment 2 included three treatments and a control. The first treatment (shading) was initiated 6 weeks after inflorescence emergence (400 dd), after the end of cell division, and consisted of shading the plant with a pyramidal umbrella placed 5 m above it (at inflorescence emergence banana plants were approx. 4 m tall). This treatment reduced irradiance by 60 %. The expected effect was a reduction in the amount of assimilates available for fruit growth due to lower photosynthetic activity. The second treatment consisted of bunch bagging (bagging) after the end of cell division: a blue polythene bag (18 μm thick) was placed around the bunch 6 weeks after inflorescence emergence (400 dd). The expected effect was an increase in fruit demand for assimilates as a result of increased air temperature in the bag (Turner, 1998). It was assumed that fruit temperature was also increased. The bag was sealed to the bunch stalk above the first hand and left open at the bottom. The third treatment also consisted of bunch bagging but was applied earlier (early bagging): the bag was placed on the bunch 1 week after inflorescence emergence, i.e. at the beginning of cell division in the fruit. This treatment was designed to study the effect of temperature elevation on cell division. Treatments included 15 (shading and bagging) or five (early bagging) bunches. Treatments were compared with a control (control 2) that included 15 bunches, each with eight hands; expt 2 thus included 35 plants. Each individual plant was a replication of one treatment. To avoid interaction between treatments (especially with the umbrella of the shading treatment), plants chosen for the experiment were always separated by at least one guard plant. Treatments were randomly allocated to bunches. Of these bunches, five were sampled in shading, bagging and control 2 treatments at the beginning of, and in the middle of, the filling period (6 and 11 weeks after flower emergence) for destructive measurements. As in expt 1, the remaining bunches were sampled at the same time, i.e. when control 2 had reached commercial harvest (15 weeks after flower emergence).

Treatments, their respective abbreviations, and the number of individual plant replications for each sampling are summarized in Table 1.

Fruit measurements

Within the bunch, each hand has two rows of fruits: one inner and one outer whorl. All measurements were made on the fruit located in the middle of the outer whorl of the hand (reference fruit). Fruit diameter and length were measured weekly on the reference fruit of hands 1, 4 and 7 in controls (H8CA, control 2 and H7CB), and plants in the bagging, shading and early bagging treatments; of hands 1 and 2, 4 and 5, and 7 and 8 respectively for H2UA, H2MA, H2LA, and on hands 1 and 2, 4 and 5, and 6 and 7 respectively for H2UB, H2MB and H2LB.

Fresh and dry weights of the pulp were recorded for the reference fruit of the hands cited above. The destructive measurements (made at the beginning and middle of filling and at harvest) allowed us to calculate a relationship between the product of the square of fruit diameter and its length (G^2L, 10^{-6} m^3) and pulp dry weight (P, g) during the fruit filling period:

$$P = 0{\cdot}1026\ G^2L - 1{\cdot}619\ (r^2 = 0{\cdot}88,\ n = 317)$$

Pulp dry weight could then be estimated weekly using non-destructive measurements of diameter and length in all treatments.

Estimating pulp cell number

Pulp cell number (PCN) was estimated following digestion in a 750 ml 20 % chromic acid: 250 ml 20 % nitric acid solution according to the protocol of Brown and Rickless (1949). We first developed a sampling method to estimate pulp cell number by digestion of a fixed portion of the pulp.

For this purpose, 19 fruits were sampled after the end of cell division (500 dd from inflorescence emergence) on three different plots independently from those used in expts 1–3. Samples of pulp were taken from the fruit apex (sample 1), from the middle of the fruit (sample 2) and from near the fruit peduncle (sample 3). The rest of the pulp was mixed and constituted six other samples (samples 4–9). Mean cell density (cell number per unit of pulp weight) was constant between samples. All sampling methods gave a good estimation of PCN, calculated from the digestion of the whole pulp. The closest correlation was found using the mean pulp cell number calculated from samples 4–9 ($y = 0{\cdot}96x + 4 \times 10^6$, where y is the cell number calculated by digesting the whole banana pulp and x is the pulp cell number calculated by the sampling method; $r^2 = 0{\cdot}94$, $n = 19$). Statistical analysis showed that the relationship was not significantly different from $y = x$ ($P < 0{\cdot}01$). This sampling method was thus used in further PCN estimations.

We then developed a simple and non-destructive estimator of PCN that would satisfy two conditions: (1) it should be a result of the cell division phase (measured at or near the end of cell division); and (2) it should be measured early enough to be independent of growing conditions during bunch filling. According to Jullien (2000), at the end of the cell division phase, i.e. 350 dd after emergence, a fruit has reached 80 % of its final length but only 10 % of its final pulp dry weight. Length at this stage thus seems to meet both criteria described above. Consequently, we tried to link PCN counted at 500 dd to fruit length measured at 350 dd from inflorescence emergence (L350). Therefore, PCN was counted in six fruits from expt 2 (early bagging treatment) and in 14 fruits from expt 3. In expt 3, fruits were sampled on eight banana plants that differed from those used for the treatment. Fruit lengths were measured at 350 dd from inflorescence emergence and PCN was counted at 500 dd according to the sampling method described above, to be sure that PCN did not increase further.

Estimation of the source/sink ratio during bunch filling

The source/sink ratio was calculated as the ratio between the quantity of assimilates available for pulp filling and the sink size estimated by the total cell number of the bunch. Following Jullien *et al.* (2001), we assumed that pulp filling starts at 350 dd from inflorescence emergence and ends at harvest. The amount of resource available during the pulp filling period (S_o) was calculated using the following equation (Monteith, 1977):

$$S_o = R_{IE}\ R_{UE}\ S_{PAR} / [d\ (S_H - 350)]$$

where S_o is dry matter produced (in g per plant per dd), R_{IE} is the radiation interception efficiency of the canopy, R_{UE} is the radiation use efficiency, S_{PAR} is the photosynthetically active radiation (PAR) cumulated during the bunch filling period and expressed in MJ m^{-2}, d is the planting density in plants m^{-2} and S_H is the cumulated degree-days from inflorescence emergence to harvest. According to Turner (1990), the mean R_{UE} of the banana plant is 1·5 g MJ^{-1}. The light interception coefficient was formulated by Bonhomme and Ganry (1976) as follows: $R_{IE} = 1\text{-}e^{-0{\cdot}7L}$ where 0·7 is the extinction coefficient calculated for the canopy of Cavendish subgroup banana plants and L is the leaf area index. The leaf area index was calculated from leaf area measurements using the following equation (Champion, 1963): $L_A = 0{\cdot}83L_l\ L_w \times 10^{-4}$ where L_l (m) and L_w (m) are leaf length and width respectively. Leaf area was measured on 20 banana plants for each experiment.

The sink size was calculated as the total number of cells at the beginning of pulp filling:

$$S_i = \sum_{i=1}^{N_H} N_{Fi} N_{PCi}$$

where S_I is the sink size expressed in number of cells, N_H is the number of hands, N_{Fi} is the number of fruits in hand i and N_{PCi} is the pulp cell number of the reference fruit of hand i. The source/sink ratio was calculated by dividing S_o by S_i and is expressed in grams of dry matter per cell and per degree-day during the pulp filling period.

Meteorological data

Daily thermal time was calculated using 14 °C as the base temperature (Ganry and Meyer, 1975). Air temperature was measured outside and under the bags in the bagging treatments at three different heights [top (hand 1), middle (hand 4) and bottom (hand 7) of the bunch] using copper-constantan thermocouples. Fruit age was expressed in thermal time cumulated from inflorescence emergence and expressed in degree-days using these temperatures.

Statistical analysis

To compare means, Newman-Keul's tests at the 0·05 probability level were performed using STATITCF software (STAT-ITCF5·0, 1991). Non-linear adjustments were performed using the non-linear SAS procedure (SAS Institute, 1987).

RESULTS

Estimation of pulp cell number

A close correlation was found between fruit length measured 350 dd after flower emergence (L350) and PCN (Fig. 2). This result confirms that L350 is a good estimator of fruit PCN at the beginning of bunch filling. As a consequence, L350 was used in all treatments to estimate PCN.

Effect of source/sink ratio on fruit growth

Pulp dry weight (PDW) at harvest was significantly increased in all hands in the pruning treatments compared with controls (Table 2). Shading significantly decreased PDW for all hands, but bagging had no effect on PDW compared with control 2. The early bagging treatment

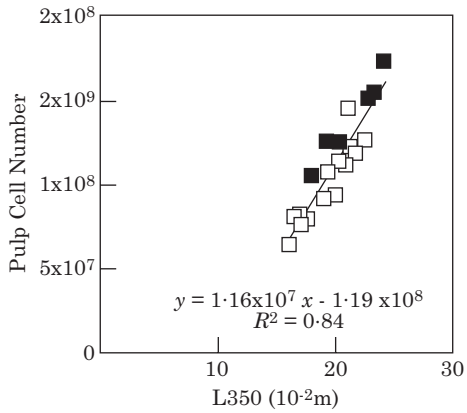

FIG. 2. Relationship between pulp cell number per fruit counted at 500 dd and fruit length (10^{-2} m) measured at 350 dd (L350). ■, Expt 2; □, expt 3.

significantly improved PDW in hands 1 and 4 but had no significant effect on PDW in hand 7.

PCN was significantly increased by early bagging in hands 1 and 4 but not in hand 7. Statistical tests could not be applied to results from the excision B treatment because of the small number of replicates. PCN was significantly higher in hand 1 than in hand 7 in all treatments. A positive relationship was found between PDW and counted or estimated PCN for each treatment. This relationship explained 50–90 % of the variation in PDW between hands of the same bunch (r^2 of the relationships between 0·5 and 0·9).

As the harvest date was the same for all treatments within an experiment, the filling duration was identical for all treatments. For treatments applied after 350 dd, i.e. after the end of cell division, variations in PDW at harvest could be interpreted as variation in the cell filling rate. Pruning

TABLE 2. *Effect of different treatments on pulp dry weight (PDW), pulp cell number (PCN) and mean air temperature under the leaf canopy*

	Hand	PDW (g per fruit)	PCN (Number per fruit)	PDW = f(PCN)	Mean air temperature during pulp filling (°C)
H2UA	1	31·08* A	*1·22 × 10^8 A*	$2{\cdot}44 \times 10^{-7} \times NC - 2{\cdot}59$ $r^2 = 0{\cdot}70$	
H2MA	4	31·12* A	*1·12 × 10^8 A*	$1{\cdot}30 \times 10^{-7} \times NC + 15{\cdot}9$ $r^2 = 0{\cdot}52$	
H2LA	7	26·71* B	*8·55 × 10^7 B*	$2{\cdot}83 \times 10^{-7} \times NC + 1{\cdot}13$ $r^2 = 0{\cdot}82$	25·4
H8C	1	24·01 C	*1·19 × 10^8 A*		
	4	21·84 C	*1·15 × 10^8 A*	$1{\cdot}48 \times 10^{-7} \times NC + 4{\cdot}87$ $r^2 = 0{\cdot}56$	
	7	17·57 D	*9·06 × 10^7 B*		
lsd ($P < 0{\cdot}05$)					
Treatment effect		2·70	$9{\cdot}20 \times 10^{-1}$		
Hand effect		1·32	$1{\cdot}11 \times 10$		
Shading	1	20·25*	*1·34 × 10^8*		
	4	18·77*	*1·26 × 10^8*	$1{\cdot}55 \times 10^{-7} \times NC + 1{\cdot}01$ $r^2 = 0{\cdot}61$	25·9
	7	14·28*	*1·04 × 10^8*		
Bagging	1	29·33	*1·34 × 10^8*		27·6
	4	26·93	*1·29 × 10^8*	$2{\cdot}45 \times 10^{-7} \times NC + 5{\cdot}82$ $r^2 = 0{\cdot}62$	27·2
	7	20·94	*1·08 × 10^8*		26·6
Early bagging	1	34·46*	**1·49 × 10^8***		27·6
	4	30·78*	*1·36 × 10^8**	$3 \times 10^{-7} \times NC - 9{\cdot}97$ $r^2 = 0{\cdot}90$	27·2
	7	22·34	**1·07 × 10^8**		26·6
Control 2	1	29·31	**1·32 × 10^8**		
	4	27·48	**1·30 × 10^8**	$2{\cdot}17 \times 10^{-7} \times NC - 0{\cdot}33$ $r^2 = 0{\cdot}63$	26·06
	7	22·29	**1·07 × 10^8**		
lsd ($P < 0{\cdot}05$)					
Treatment effect		1·93	$7{\cdot}50 \times 10^6$		
Hand effect		1·67	$6{\cdot}00 \times 10^6$		
H2UB	1	40·55	*1·19 × 10^8*		
H2MB	4	37·4	*9·63 × 10^7*		
H2LB	7	30·75	*8·34 × 10^7*		24·10
Control 3	1	27·11 A	**1·08 × 10^8 A**		
	4	25·33 A	**9·90 × 10^7 A**	$2{\cdot}28 \times 10^{-7} \times NC + 2{\cdot}5$ $r^2 = 0{\cdot}85$	
	7	19·35 A	**7·49 × 10^7 B**		
lsd ($P < 0{\cdot}05$)					
Hand effect		2·27	$8{\cdot}60 \times 10 + 06$		

Numbers in italics indicate estimated PCN while numbers in bold type indicate counted PCN. * indicates values significantly different from the control ($P < 0{\cdot}05$). Letters indicate uniform groups defined in the analysis of variance ($P < 0{\cdot}05$) by the Newman Keul's test. lsd, least significant difference ($P < 0{\cdot}05$).

TABLE 3. *Slopes of lines of* $X(t) = C_{w(t)} - C_{W(BFYC)}$

Treatment	Slope (pg per cell per dd)		lsd (pg per cell per dd)
H8CA	288	D	
H2UA hand 1	375	C	
H2MA hand 4	401	BC	
H2MA hand 5	455	AB	
H2LA hand 7	480	A	
			0·54
Shading	160	C	
Control2	244	B	
Bagging	235	B	
Early bagging	281	A	
			0·27
H7CB	290	B	
Excision B	384	A	
			0·35

When fruit position on the bunch had no significant effect on the slope, a mean slope was calculated for hands 1 and 7; otherwise different slopes were calculated for each hand (H2MA, hands 4 and 5).

lsd, Least significant difference between treatments; dd, degree-days; C_w cell dry weight (g); BFYC, beginning of filling of the youngest cells.

(excision A) increased PDW while shading decreased it. This indicated that the source/sink ratio during bunch filling affected the cell filling rate.

Variation in cell filling rate in relation to fruit position in the bunch and source/sink ratio

According to Jullien *et al.* (2001), cell division in the bunch ceases at around 420 dd after inflorescence emergence. In the following, we therefore assumed that the youngest cells in the bunch accumulate dry matter from 500 dd. This stage was called the 'beginning of filling of the youngest cells' (BFYC). Dry matter accumulation in the cell was represented by a new variable calculated as follows:

$$X(t) = C_W(t) - C_{W(BFYC)}$$

where $C_W(t)$ is the cell dry weight at time t and $C_{w(BFYC)}$ is the cell dry weight at BFYC. Cell dry weight was calculated by dividing PDW by PCN, where PCN was calculated from L350. $X(t)$ was plotted against time expressed in degree-days accumulated since BFYC for all treatments (Fig. 3). Meteorological measurements showed that air temperature was identical for hands 1 and 7 in all treatments except bagging and early bagging. In the case of bagging, there was a negative gradient in temperature between the top and the bottom of the bag (Table 1): air temperature under the bag was raised for hand 1 but not for hand 7, compared with air temperature measured under the canopy. Thus for bagging and early bagging treatments, temperature was corrected using the air temperature measured inside the bag. Shading did not modify the air temperature.

Slopes of the curves of $X(t) = C_w(t) - C_{w(BFYC)}$ represent cell filling rate (CFR) in g per cell per dd (Fig. 3). To compare slopes, curves were fitted to straight lines whose slope was calculated for each treatment. Statistical analysis of the slopes shows that there was no significant difference in CFR between hands of the same bunch except between hands 4 and 5 in H2MA. Thus a mean slope was calculated for hands 1 and 7 in H8CA, control 2, H7CB, shading, bagging and early bagging, for hands 1 and 2 in H2UA, and for hands 1 and 7 in H2LA. Two different slopes were calculated for hands 4 and 5 in H2MA. A mean slope was calculated for H2UB, H2MB and H2LB (Table 2). For treatments applied after 350 dd, results show that: (1) shading significantly decreased CFR; (2) bagging did not modify CFR significantly; and (3) excision A increased CFR. CFR was significantly higher in H2UA compared with H8CA, in H2MA compared with H2UA, and in H2LA compared with H2MA. Treatments applied before 350 dd (excision B and early bagging) significantly increased CFR.

CFR was then plotted against the mean source/sink ratio during bunch filling (cf. Materials and Methods; Fig. 4). A Michaelis-Menten equation was fitted to the data: $C_{FR} = [(C_{FRmax} \times S_o/S_i)/(K + S_o/S_i)] + c$, where $C_{FRmax} + c$ is the maximal value of C_{FR}, S_o/S_i is the source/sink ratio during the bunch filling period, and K and c are constants. K represents the inverse of the cell affinity for assimilates (Marcelis, 1996). The fitted function is: $C_{FRmax} = 5{\cdot}35 \times 10^{-10}$ g per cell per dd, $K = 4{\cdot}55 \times 10^{-10}$ and $c = 0{\cdot}52 \times 10^{-10}$. Fruits from pruning treatments (excisions A and B) have the highest source/sink ratio and CFR. Fruits from the shading treatment have the lowest source/sink ratio and CFR. Agricultural conditions of fruit growth (H8CA, control 2 and bagging) correspond to intermediate source/sink ratios. In these cases, the CFR was lower than its maximal calculated value ($C_{FRmax} + c$).

DISCUSSION

The results enable us to estimate the respective roles of pulp cell number and cell filling rate in determining banana pulp dry weight. The methods employed assured the independence of these two variables. Pulp cell number was estimated from fruit length at 350 dd, i.e. at the end of cell division and the beginning of bunch filling. Variation in estimated pulp cell number is thus independent of growing conditions during bunch filling, which only affected the cell filling rate. Relationships between pulp cell number, cell filling rate and pulp dry weight were analysed for different source/sink ratios.

Variation in pulp cell number

A close linear relationship was found between pulp cell number and fruit length at 350 dd. This estimator was thus used to characterize the variation in pulp cell number in the bunch and between different growing conditions. Pulp cell number is higher in fruits from hand 1 (proximal fruits) than in fruits from hand 7 (distal fruits). This is in accordance with histological results reported by Jullien *et al.* (2001). These authors suggested that the difference in

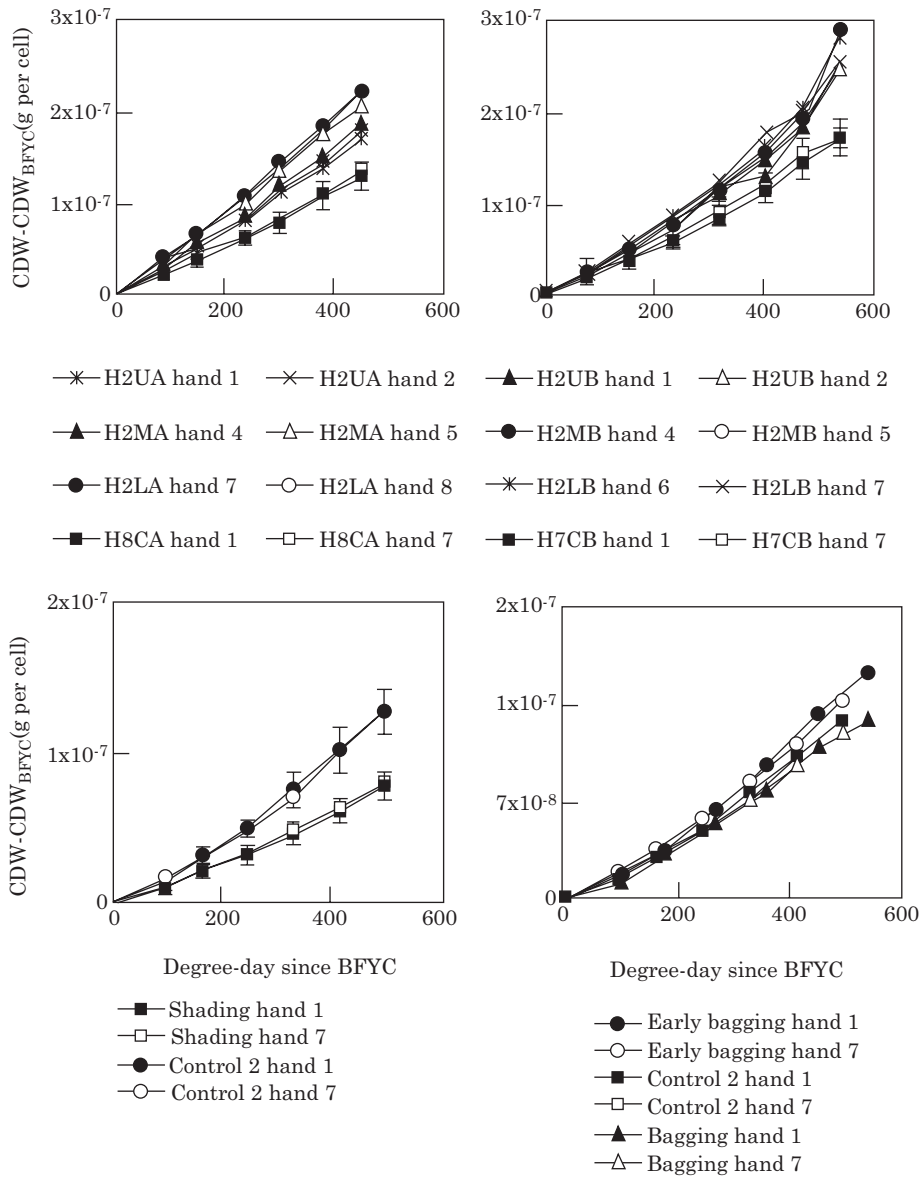

FIG. 3. Course of the variable $X(t)=C_{W(t)}-C_{W(BFYC)}$ against time expressed in degree-days cumulated from the beginning of filling of the youngest cells (BFYC). $C_{W(t)}$, Cell dry weight at time t; $C_{W(BFYC)}$, cell dry weight at BFYC; dd, degree-days.

cell number between hands is due to a difference in resource availability during the cell division phase related to the developmental lag between fruit (fruits on proximal hands are in an advanced stage compared with fruits on distal hands): when cell division starts in fruits on hand 1, competition for assimilates is low as only hand 1 fruits are undergoing cell division. When cell division starts in fruits on hand 7, all the fruits on the bunch are in the cell division phase, hence competition is increased and cell division may be limited by assimilate availability. Our results are in accordance with this hypothesis: pruning during cell division (excision B) seemed to have a positive effect on pulp cell number, but these results need to be confirmed because of the small number of replicates in our experiments. However, our results are in accordance with results obtained for pea (Munier-Jolain and Ney, 1998), wheat (Brocklehurst, 1977; Gleadow *et al.*, 1982) and apple (Coffinet *et al.* 1995) that show that resource availability during the cell division phase influences final cell number.

The second cause of variation in pulp cell number is temperature. The rise in air temperature around hands 1 and 4 following bunch bagging during the cell division phase had a positive effect on pulp cell number. Results obtained for other species are variable. For wheat (Wardlaw, 1970) and maize (Jones *et al.*, 1985), a temperature rise increased the cell division rate but decreased the length of the cell division phase, so that the final cell number was not affected. At extreme temperatures, cell number may be reduced (Jones *et al.*, 1985). For melon, Higashi *et al.* (1999) have shown that variations in the mean seasonal temperature during the cell

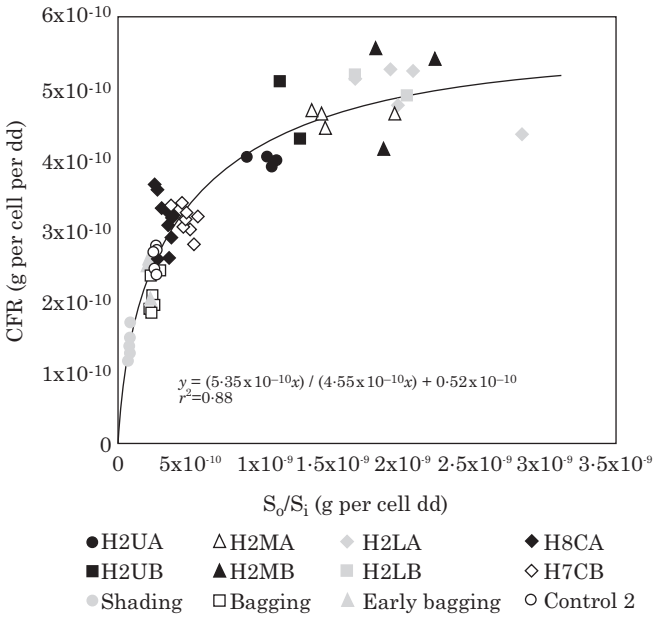

FIG. 4. Relationship between cell filling rate (CFR) and source/sink ratio during bunch filling period (S_o/S_i). The line represents the Michaelis-Menten equation fitted to the data. dd, Degree-days.

division phase are positively correlated with final cell number. The response of biological processes and, in particular, enzymatic activity, to temperature depends on the cardinal temperatures, i.e. the minimum, optimum and maximum (Lüttge *et al.*, 1996; Weikai and Hunt, 1999). Cardinal temperatures vary between species (Weikai and Hunt, 1999). Thus, the effect of temperature on the cell division rate will depend on the species and the temperature range studied. Our results showed that under the experimental conditions employed, temperatures (26–28 °C) were below the optimum for cell division.

Variation in cell filling rate

Cell filling rate was calculated using estimated pulp cell number calculated from fruit length at 350 dd (L350). Hand pruning at 350 dd (excision A) may have modified pulp cell number, especially in the youngest fruits (hand 7). Indeed, according to Jullien *et al.* (2001), cell division occurs until approx. 420 dd in distal hands (hand 7). Thus, the cell filling rate might have been overestimated in excision A treatments. However, results obtained for excisions A and B (hand pruning at the beginning of cell division) agree: cell filling rates and source/sink ratios obtained for both experiments are the same. This suggests that pulp cell number and cell filling rate were correctly estimated in the excision A treatment.

This study has shown that from the time every cell is in the filling phase (from BFYC), the cell filling rate may be regarded as constant during the pulp filling period. This result is similar to that observed for pea and soybean, where the rate of grain filling is fixed at the beginning of the filling period by the grain cell number (Munier-Jolain and Ney, 1998). In our experiments, this result could be explained by the fact that source/sink ratios were kept constant during the whole filling period. It would be interesting to study the effect of short-term alterations of the source/sink ratio on fruit growth rate. Such studies have already been carried out for peach (Grossman and Dejong, 1995*a*, *b*); these authors showed that variation in the fruit growth rate during growth was the result of seasonal patterns of resource dynamics.

We have also shown that the cell filling rate was identical for fruits of upper, middle and lower hands in a bunch. This means that cell filling rate is not a determining factor in fruit weight variability within the bunch, and may indicate that there is no priority in assimilate allocation. This could be confirmed by an histological study of the stalk anatomy to determine the number of vessels present at each hand and their course in the bunch stalk. Skutch (1937) stained vessels along the stalk but did not find any difference in vessel density.

There is a relationship between cell filling rate and the mean source/sink ratio over the whole pulp filling period. This relationship has two components: for source/sink ratios between 0 and $1{\cdot}14 \times 10^{-9}$ g per cell per dd, cell filling rate increases with the source/sink ratio. In this situation, cell filling is limited by the source. Hand pruning increases the cell filling rate while leaf shading decreases it. For source/sink ratios higher than $1{\cdot}14 \times 10^{-9}$ g per cell per dd, cell filling rate tends to a maximal value. In this situation, fruit growth is limited by the number of cells to fill (sink size). For species such as peach (Grossman and Dejong, 1995*a*, *b*) and apple (Denne, 1960; Westwood *et al.*, 1967), the quantity of assimilate available during fruit filling (after the end of cell division) influences fruit growth rate, i.e. the cell filling rate. These results correspond to those obtained for banana plants with source/sink ratios between 0 and $1{\cdot}14 \times 10^{-9}$ g per cell per dd. Conversely, for legumes such as pea, lupin and soybean, Munier-Jolain *et al.* (1998) have shown that alterations to the source/sink ratio made after the end of cell division do not modify the grain filling rate, i.e. the cell filling rate. Only the duration of grain filling may be affected by resource availability during grain filling. In the same way, Jones and Simmons (1983) showed that for cereals such as maize, pruning carried out after the end of cell division does not affect the grain filling rate. The authors explain these results by the existence of other factors limiting growth or by the fact that the genetic potential was already reached before pruning. These situations correspond to our results obtained for banana plants with source/sink ratios above $1{\cdot}14 \times 10^{-9}$ g per cell per dd. Actually, over this part of the curve, resources are not limiting fruit filling: cell filling rate is almost at its maximal value and modification of the source/sink ratio would have little effect on cell filling rate.

A Michaelis-Menten equation was fitted to the data. The fitted curve did not pass through zero at the origin. This may signify that carbon remobilization from vegetative parts of the plant contributes to bunch filling. This suggestion is corroborated by the results of Eckstein *et al.* (1995) who showed that a substantial amount of carbon is remobilized from vegetative parts of plants during fruit growth. The

maximum cell filling rate calculated by statistical fitting was $5{\cdot}87 \times 10^{-10}$ g per cell per dd ($K + c$). Another explanation may be that fruits that have a proper photosynthetic activity contribute to pulp filling. Indeed, for peach, the contribution of fruit photosynthesis to fruit growth was estimated to be about 10 % (Pavel and Dejong, 1993).

Respective roles of pulp cell number and cell filling rate in pulp dry weight determination

Pulp cell number at the end of cell division determines the potential fruit filling rate. The maximum fruit filling rate is the product of pulp cell number and maximum cell filling rate. Our study confirms the importance of cell number in final fruit weight determination for banana plants. This has already been shown for other species such as maize (Jones *et al*., 1985), wheat (Feucht and Höfner, 1985), pea and soybean (Munier-Jolain and Ney, 1998), apple (Denne, 1960) and melon (Higashi *et al*., 1999).

In agricultural situations (bunches with eight hands in H8CA, control 2 and H7CB), cell filling rate was below its maximal value. Fruit filling was thus source-limited. In these conditions, an increased fruit demand in assimilates could not be satisfied. Bagging had no significant effect on cell filling rate (bagging *vs*. control 2). This result agrees with those obtained for cucumber by Marcelis (1993, 1996); indeed, this author noticed that temperature did not have a positive effect on fruit growth rate when fruit growth was source-limited. When the bunch was bagged at the beginning of the cell division phase (early bagging), pulp cell number was increased and the source/sink ratio was decreased. But the results showed that, contrary to results obtained in the bagging treatment, cell filling rate was increased. We can hypothesize that the early augmentation of sink demand, via the increase in pulp cell number, increases the quantity of dry matter allocated to the bunch. This may be due to an increase in leaf photosynthetic activity or to an increase in the percentage of total dry matter allocated to the bunch. Indeed, the influence of sink demand on leaf photosynthesis has already been demonstrated for apple (Gucci *et al*., 1994) and peach (Ben Mimoun *et al*., 1996). Feedback mechanisms of this nature have been widely described at a cellular level (Leegood, 1996). For cucumber, Marcelis (1993) has shown that the fruit load may modify the percentage of total assimilates allocated to fruits.

Fruit growth limitation by the source may also explain why increasing plant density has a negative impact on fruit and bunch weight at harvest (Daniells *et al*., 1985; Robinson, 1996). Indeed, in this case, an increase in sink size (number of bunches per hectare) and an increase in source size (leaf area index) can only explain a decrease in fruit weight up to a certain point. Further work is needed to confirm this hypothesis.

We conclude that: (1) cell number estimated at the beginning of pulp filling is a good indicator of fruit sink strength within the bunch; and (2) cell filling rate is influenced by the source/sink ratio during bunch filling. We have evidence that in agricultural conditions and with the Grande Naine cultivar, cell filling rate was less than maximal and that pulp filling was source-limited. Thus, this variety could be improved by decreasing the number of fruits initiated or by increasing resources available for fruit growth (photosynthetic production, percentage of assimilates allocated to the bunch, remobilization of reserves). These conclusions differ from those of Daniells *et al*. (1994) who considered that the banana cultivar 'Williams' (similar to 'Grande Naine') is generally sink-limited. However, the results are difficult to compare for two reasons. First, Daniells *et al*. (1994) considered the total bunch weight and not the fruit weight. In this case, bunch trimming reduced final bunch weight because the increase in fruit weight did not compensate for the decrease in fruit number. Secondly, in the study by Daniells *et al*. (1994), bunches of different treatments (leaf removal, bunch trimming) were not harvested at the same time, but only when the reference fruit had reached a fixed diameter. Thus, fruit growth duration differed: bunches from the leaf removal treatment had the same final weight as the control, but they were harvested later. In fact, fruit growth rate was actually reduced.

Our results also indicate that techniques to optimize cell division rate would have an important effect on final fruit weight. This was particularly noticeable in our early bagging treatment and is in accordance with results obtained by Daniells *et al*. (1992). In our experiment, early bagging was applied 1 week after flower emergence, i.e. at the beginning of cell division in the pulp, while bagging was applied about 1 week after the end of cell division. In agricultural conditions in the French West Indies, bunch bagging is carried out about 3 to 4 weeks after flower emergence, i.e. 2 weeks before the end of cell division. According to our results and those of Daniells *et al*. (1992), earlier bagging would allow an increase of fruit weight and size on the upper hands. It would be worthwhile investigating whether a similar effect could be obtained on lower hands by modifying the size of the bag (for instance using a longer bag). Early bagging thus appears to be a cultural technique that warrants further study.

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