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MORPHOMETRY AND SURFACE GROWTH DYNAMICS OF THE SUNFLOWER. *(Helianthus annuus* L.) RECEPTACLE. ITS IMPORTANCE IN THE DETERMINATION OF YIELD

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RESUMEN

MORFOMETRIA Y DINAMICA DEL CRECIMIENTO EN SUPERFICIE DEL RECEPTACULO DE GIRASOL *(Helianthus annuus* L.). SU IMPORTANCIA EN LA DETERMINACION DEL RENDIMIENTO

Este trabajo describe un análisis cuantitativo de la morfología del capítulo del girasol *(Helianthus annuus* L.) en etapas tempranas de su desarrollo. Se utilizaron plantas de girasol crecidas en condiciones controladas de fotoperíodo, intensidad lumínica, humedad y temperatura. Se determinó la evolución de la superficie y del volumen del receptáculo, como así también el número total de células del tejido meristemático superficial y del meristema sub-superficial del mismo, desde el inicio del desarrollo reproductivo (18-20 días desde la siembra) hasta la aparición de los primordios florales (32-34 días desde la siembra). Los resultados obtenidos son utilizados para proponer un modelo de la generación de primordios florales en el capítulo.

Palabras clave: Crecimiento, girasol, *Helianthus annuus*, morfogénesis.

SUMMARY

This paper describes a quantitative analysis of the morphology of the capitulum of sunflower *(Helianthus annuus* L.) during early stages of its growth. Plants of sunflower were grown under a controlled photoperiod, light intensity, air humidity and temperature. The dynamics of surface and volume expansion of the receptacle was evaluated. The number of cells in the surface layer and sub-surface meristem from the begining of the reproductive state (18-20 days from sowing) until the appeareance of floret primordia (32-34 days from sowing) was also determined. The results are used to elaborate a model of floret primordia generation in the capitulum.

Abbreviations: BA: N^6-benzyladenine; DFS: Days from sowing; FS: Floral stage; LD: Long day photoperiod; SD: Short day photoperiod; SL: Surface layer; SSM: Subsurface meristem.

Key words: Growth, *Helianthus annuus*, morphogenesis, sunflower.

INTRODUCTION

Mathematical studies simulating plant shape, morphology, growth and development have been made in order to understand the physical

changes in plant structures as they develop or mature (Gifford and Kurth, 1963; Erickson, 1966; Gordon, 1966; Niklas and Chaloner, 1976; Niklas, 1977, 1979; Moens and Moens, 1981) and to interpret the underlying events that are taking place at the whole organ level (Mauseth and Niklas, 1979; Niklas and Mauseth, 1980; Mauseth, 1984; Goodall and Green, 1986).

The young capitulum of sunflower is an organ with radial symmetry. The surface of the receptacle changes from a characteristic dome-shape in the transitional stage to become a nearly flat disc-shaped surface developing a raised toroidal rim before the floret initials start to be seen at the receptacle rim (Marc and Palmer, 1981). During its early development it is not possible to distinguish zonation patterns, so there is a clear separation between the surface layer (SL) and the rest of the sub-surface meristem (SSM), with no other distinct zonation.

Because the number of floret primordia to be developed in the capitulum is closely related to the dimensions attained by the receptacle (Hernández and Palmer, 1988) it is important to have a precise mathematical description of the changes occuring during its early growth. This paper describes a quantitative evaluation made in order to know more in detail the dynamics of growth of the receptacle before the floret primordia become differentiated. The information obtained can be used for modelling the inflorescence development.

MATERIALS AND METHODS

Plan cultivation

Plants of sunflower hybrid, cultivar Hysun-30, were grown under an 11 hr short day (SD) or 18 hr long day (LD) photoperiod (control), 28 °C air temperature, relative humidity between 60-70 % and 550 μmol m^{-2} s^{-1} of photon flux density at the canopy height. Inflorescence development was followed during the experimental period by sampling and scoring as described by Marc and Palmer (1981) starting at floral stage (FS) 2 and finishing at FS 5 when the first incipient peripheral ring of floret initials becomes visible at the receptacle rim.

Aplication of a cytokinin

Preliminary evidence showed that cytokinins are able to induce a significant expansion of the receptacle surface giving the possibility of more floret primordia to be set up (Palmer and Hernández, 1988). One batch of plants growing under LD was then treated by daily applications of an ethanolic solution of the cytokinin N^6-benzyladenine (BA) (100 mg BA L^{-1} in ethanol 20 % (v/v)), to the whole upper shoot by foliar spray, starting in FS 3 (21 DFS) and finishing in FS 5 (30-32 DFS). Each application gave a total amount applied of 0.3 mg BA $plant^{-1}$. In BA-treated plants (LD + BA treatment) capitula were sampled at the same time as the control plants. Another batch of SD, LD and LD + BA-treated plants was allowed to grow until maturity for identifi-

cation and counting of the number and arrangement of floral parstichies.

Histological methods

Sampled capitula were dissected out and the surface diameter of each receptacle was measured. The capitula were then fixed in 8% glutaraldehyde in phosphate buffer (pH 6.8) at 2 °C for 3 days, dehydrated in an ethanol series and embedded in acrilic resin LR White (The London Resin Co., England). The blocks were sectioned at 1-2 μm using glass knives and the sections stained for 2 min with toluidine blue 0 (Sigma Chemicals, USA), 0.05 % in acetate buffer (pH 5.4), mounted in immersion oil and photographed. Only the median section of embedded capitula were used. Four capitula were sectioned for each floral stage and treatment.

Volume and surface area calculation

The outline for each median section taken from enlarged photographic prints, was traced on 1 mm division translucent graph paper. The outlines of the limits between the surface layer (SL) and the sub-surface meristem (SSM) were also traced. The base of the SSM was defined by drawing a straight line between the intersection of the receptacle surface and the axil of the last formed leaf or involucral bract. The position of the top median point of symmetry of the section and the intersection between the surface edges and the axil of the last formed rim leaf or involucral bract was then used to transfer the outlined graph to a pair of coordinate axes. Assuming that the receptacle outlines were the result of a mathematical function plotted against the Cartesian coordinates, a set of coordinate points (X_i, Y_i) for each outline was then obtained (Figure 1). The function of the shape for each set of coordinate points, for each outline and floral stage, was computer-obtained (Fig. 1).

A computer program was written for calculating both the surface area and volume of solids of revolution and for creating a three-dimensional (3-D) perspective of the receptacle, using the polynomials obtained for the section profiles integrating them by the method of slicing solids (Thomas and Finney, 1984) and rotating them about the Y axis (Mauseth and Niklas, 1979). The list of sub-routines in BASIC developed for the surface area and volume calculation is available on request.

From enlargements of the prints of longitudinal sections (Figure 2a), the height and lenght of the cells of the SL and SSM were measured in diferent positions. The same specimens used to obtain transverse sections were also re-oriented in the block and sectioned in the transverse plane, tangential to the surface and photographed (Figure 2b). Even though cells generally have a polyhedral shape, for reason of convenience the cells were assumed to be cylindrical so that the cell volume could be calculated as *Cell volume* = $\pi \times (\text{Cell width}/2)^2 \times \text{Cell length}$.

More than 60 cells were measured from each print and its mean volume calculated. Using the derived volume and the estimated mean cell volume

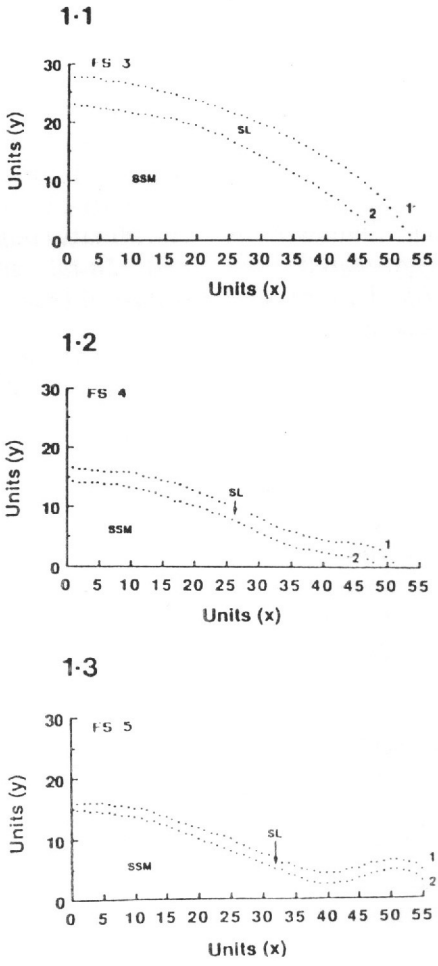

FIG. 1.–*Plotting of the mean surface outlines in FS 3, 4 and 5 obtained from the prints of the transverse sections of the capitulum. 1-1: FS 3; 1-2: FS 4; 1-3: FS 5. The values in coordinate axes are given in arbitrary units to increase the speed of calculation. Each unit has a corresponding scale value in relation to the scale of enlargement of each print. SL: surface layer; SSM: sub-surface meristem.*

Polynomials for Fig. 1-1:

$Y_1 = 26.89 - 0.17X + 0.007X^2 - 0.0086X^3 + 2.32.10^{-5}X^4 - 2.23.10^{-7}X^5 \quad r^2 = 1$

$Y_2 = 22.28 - 0.41X + 0.053X^2 - 0.0037X^3 + 9.07.10^{-5}X^4 - 7.95.10^{-7}X^5 \quad r^2 = 1$

X, Y units $= 5.84$ *μm.*

Polynomials for Fig. 1-2:

$Y_1 = 15.70 - 0.30X + 0.047X^2 - 0.0037X^3 + 9.40.10^{-5}X^4 - 7.87.10^{-7}X^5 \quad r^2 = 1$

$Y_2 = 13.28 - 0.12X + 0.011X^2 - 0.0014X^3 + 3.94.10^{-5}X^4 - 3.17.10^{-7}X^5 \quad r^2 = 1$

X, Y units $= 12.50$ *μm.*

Polynomials for Fig. 1-3:

$Y_1 = 15.84 - 0.35X + 0.046X^2 - 0.0035X^3 + 8.77.10^{-5}X^4 - 6.96.10^{-7}X^5 \quad r^2 = 1$

$Y_2 = 14.31 - 0.41X + 0.051X^2 - 0.0037X^3 + 9.16.10^{-5}X^4 - 7.23.10^{-7}X^5 \quad r^2 = 1$

X, Y units $= 17.90$ *μm.*

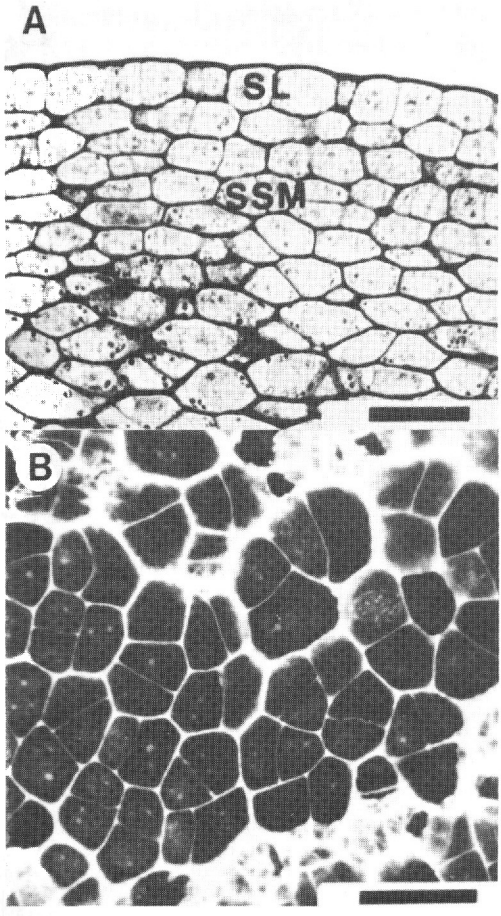

FIG. 2.—*Enlargement of a longitudinal radial section (A) and a transverse section (B) of the capitulum in FS 3, to show the shape of the cells in the surface layer (SL) of the receptacle and the sub-surface meristem (SSM).*
Bar = 30 μm.

obtained from the prints, the number of cells was calculated for the succesive floral stages, for the SL and the SSM. A minimum of 4 median longitudinal and transverse sections was examined for each developmental stage and the mean value for all the parameters previously mentioned calculated. The values obtained for the number of cells in each zone of the receptacle (SL and SSM), were used to estimate the rate of increase in cell number per mm^3 per day for the intervals between the different sampling times.

RESULTS AND DISCUSSION

Quantitative description of the receptacle growth with time

The changes procuced in the surface area of the receptacle during flowering, up to FS 5 are shown Figure 3. The results obtained of estima-

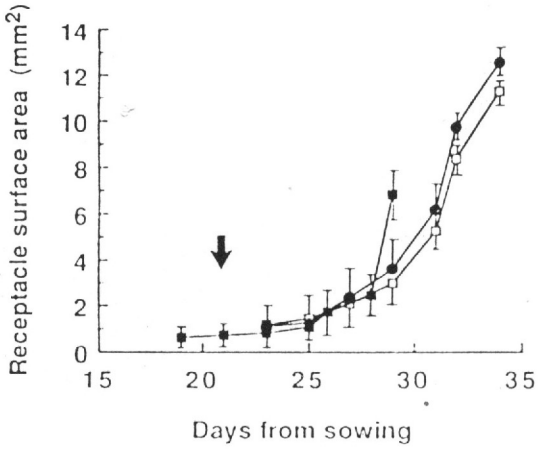

FIG. 3.—*Changes in the surface area of the receptacle during the experimental period from FS 2 to FS 5 (19 to 29 DFS in SD and 23 to 32 DFS in LD). The arrow indicates the day of the first application of N^6-benzyladenine (BA). Each point is the mean of five values. Vertical bars = ± S. E. . ■: SD; □: LD; ●: LD + BA.*

ting the cell number and volume of the surface layer and sub-surface meristem of the receptacle are presented in Table 1.

There is a continuous increase in surface area varying from 0.67 mm^2 at FS 2, to 6.80 mm^2 at FS 5 in SD and from 1.10 mm^2 at FS 2 to 12.50 mm^2 at FS 5 in plants growing in a LD photoperiod and treated with N^6-benzyladenine (LD + BA). The maximum growth rates were 1.68 mm^2 day^{-1} in SD plants, reached between 26 and 29 DFS and 1.78 mm^2 day^{-1} in LD + BA plants, reached between 31 and 34 DFS (Table 1).

The calculated volume for the first layer of cells covering the receptacle surface, showed a significant increase between FS 4 and 5 (Table 1), coinciding with the stage where the receptacle attains its maximum rate of expansion. As these growth changes occured, the dome became flat and the flanks became rounded. It was in this period, i.e. between FS 4 and 5 when a considerable increase occured in the number of calculated cells in the SL. The increase ranged from 92 x 10^3 cells in SD to 200 x 10^3 and 236 x 10^3 cells in LD and LD + BA respectively (Table 1). The cell volume which was decreasing before FS 3, remained nearly constant throughout this period this period in all the three treatments (Table 1). The same pattern was followed in the SSM with values ranging between 204 x 10^3 cells for SD and 564 x 10^3 and 711 x 10^3 cells in LD and LD +BA respectively (Table 1). The volume of the cells remained nearly constant during the expansion of the bare receptacle (Table 1) suggesting that during the expansion period, the tissue of the receptacle surface has a high rate of cell division.

The 3-D representation of the morphological changes in the receptacle surface is presented in Figure 4. It is then shown that the surface elongates and expands in area as it ages. This is due to increased cell number but not to cell enlargement

TABLE 1

Quantification (absolute values) of the main components of the receptacle tissue.

Days from sowing	Floral stage	Sub-surface meristem			Surface layer			
		Total vol. ($mm^3 . 10^3$)	Cell vol. ($\mu m^3 . 10^{-3}$)	Cell N.°	Total vol. ($mm^3 . 10^3$)	Cell vol. ($\mu m^3 . 10^{-3}$)	Cell N.°	Surface (mm^2)
SHORT DAY								
19	2	8.1	2.10	3.850	6.0	2.04	2.940	0.67
21	3	15.0	0.78	19.200	6.1	0.61	10.000	0.74
25	4	33.1	0.71	46.470	8.5	0.52	16.340	1.10
29	5	130.0	0.64	204.080	40.9	0.44	92.900	6.80
LONG DAY								
23	2	9.1	2.13	4.260	7.1	2.23	3.010	1.20
25	3	18.0	0.80	22.400	8.1	0.63	12.820	1.43
29	4	36.7	0.72	51.000	20.6	0.56	36.400	3.04
34	5	367.0	0.65	564.600	90.2	0.45	200.200	11.30
LONG DAY +B ENZYLADENINE								
23	2	9.0	2.09	4.250	8.9	2.05	4.300	1.10
25	3	17.0	0.79	21.150	10.5	0.64	16.410	1.30
29	4	43.1	0.71	71.000	24.3	0.49	50.210	3.60
34	5	448.0	0.63	711.100	97.1	0.41	236.200	12.50

(Table 1). The nearly constant cell volume in the receptacle surface layer during its early growth could be because cell division is occuring simultaneously with surface expansion. Support for this comes from the constant increase in the cell number in the first cell layer while the cell volume remained nearly constant during this period (Table 1). It is also shown in Figure 4 that the receptacle surface retains a dome-shaped centre during all the early stages of growth until the initiation of floret primordia (FS 5 in Figure 4-3) and that the central area begins to flatten towards the centre to form the generative zone described by Palmer and Steer (1985), suggesting that the sub-surface retains some capacity for vertical growth until floret primordia development commences.

The application of N^6-benzyladenine produced a significant increase in the receptacle surface area, the volume of the receptacle tissue, and the total number of cells (Figure 3, Table 1). This was produced without any reduction in the average cell size in the receptacle surface, by comparison with the LD control. This means that the effect of N^6-benzyladenine on receptacle growth is by direct promotion of the rate of cell division (Skoog and Armstrong, 1970; Letham, 1978), cell size being unchanged. However it is interesting

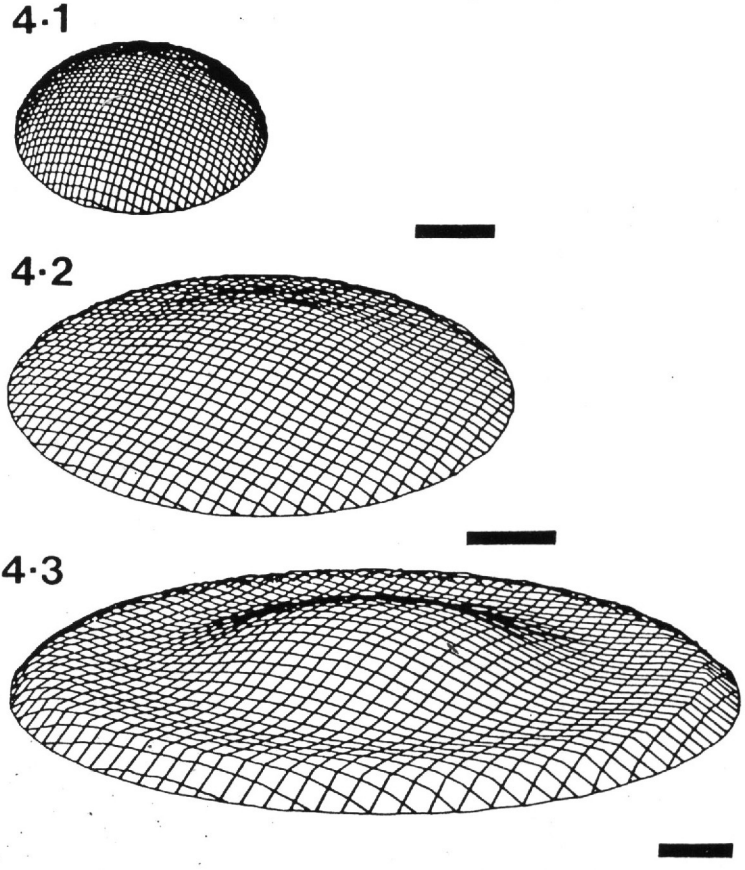

FIG. 4.—*Computer-aided reconstruction of the receptacle surface in plants grown under a LD photoperiod for three stages of its development. 4-1: FS 3; 4-2: FS 4; 4-3: FS 5. Showing the characteristic central dome-shaped structure of the receptacle during its growth. Bar = 200 μm.*

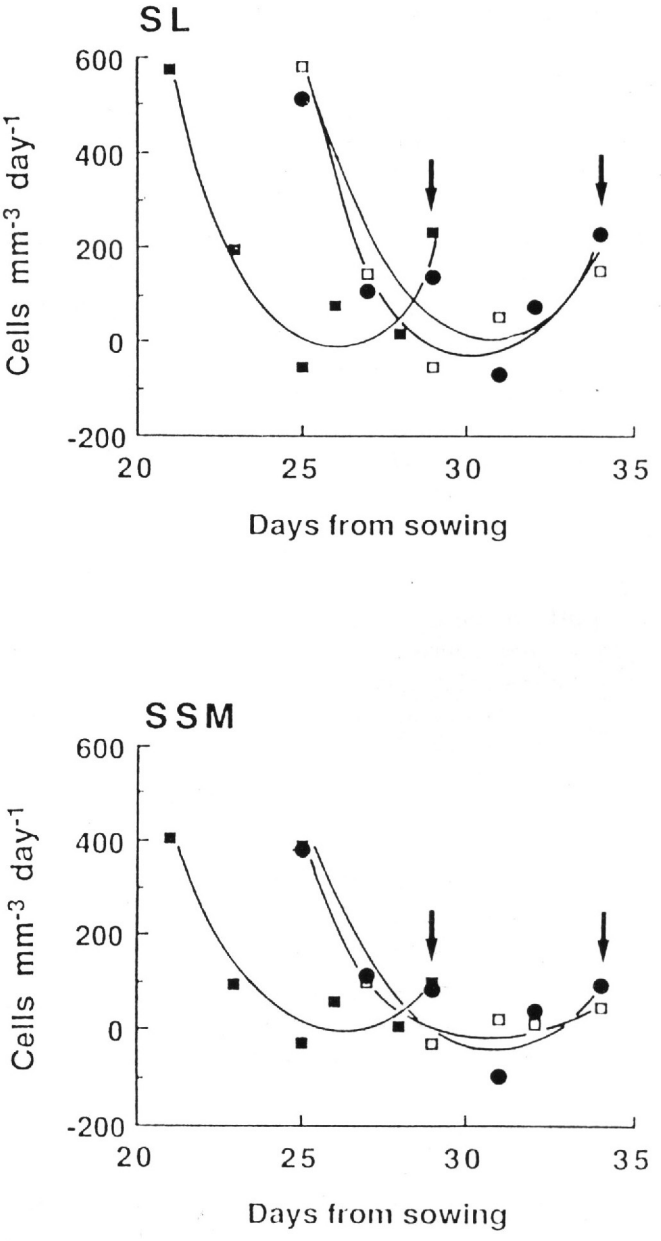

FIG. 5.—*Dynamics of the mean cell population (cells mm^3 day^{-1}) in the surface layer (SL) or the sub-surface meristem (SSM) of the receptacle for the three treatments during 15 days of floral development (from FS 2 to FS 5). The rate of increase of the cell population both in the SL and SSM between 26-28 DFS in SD and 29-33 DFS in LD, indicates the reactivation of cellular activity, coincident with the appearance of the first set of floret primordia at the rim of the recetacle. Arrows show the beginning of FS 5.*
■: *SD;* □: *LD;* ●: *LD +B A.*

to note that this BA-induced rate of cell activity did not produce a significant difference in the rate of floral development with control plants even though in 25% of the cases, an increment in the number of the parastichies at the receptacle rim was observed (Table 2).

A proposed model of the dynamics of floret primordia generation

It is known that at FS 5 there is a high frequency of periclinal cell divisions accounting for the generation of floret primordia at the receptacle rim (J. H. Palmer personal communication). The observed increase in the rate of cell production at the beginning of FS 5 (32-35 DFS, Figure 5) both in the SL or in the SSM, suggests that there is an increase in the meristematic activity of all the apical tissue at this time. Before flowering has been determined, one of the main events in the shoot apex is that the cell cycle becomes pseudo-asynchronous. Cell synchronism during floral evocation has been described as a prerequisite to attain the initial state of primordia formation (Bernier, 1971; Lyndon, 1977; Grose and Lyndon, 1984). This phenomenon could account for the morphogenetic response of the receptacle at the time of floret primordia development. The creation of a bulging rim during the period between FS 4 and 5 (Figure 4-3), could then indicate that probably the cell population of the whole rim has reached synchrony. While the surface is expanding and the florets are differentiating, this synchronism could be displaced concentrically towards the receptacle centre. The synchronism in the cell cycle time of the cell population of the receptacle surface could then be moving inwards as ring "fields" of meristematic activity, configurating the generative front proposed by Palmer and Steer (1985) and hence concentric rings of florets would be differentiating consecutively (Figure 6). The radial expansion of the receptacle surface and the dynamics of floret primordia differentiation could

TABLE 2

Age (days from sowing) to attain FS 1 and FS 5 in the different treatments and number of row parastichies observed at maturity.

Treatment	Attainment of FS 1 (DFS)		Attainment of FS 5 (DFS)		Number of rim rows at maturity	
					Pair n.°	%
Short day	16	(1) (*)	30	(3)	34/55	100
Long day	19	(2)	36	(2)	55/89	100
Long day + BA	18	(2)	34	(2)	55/89	75
					89/144	25

(*) Standard error.

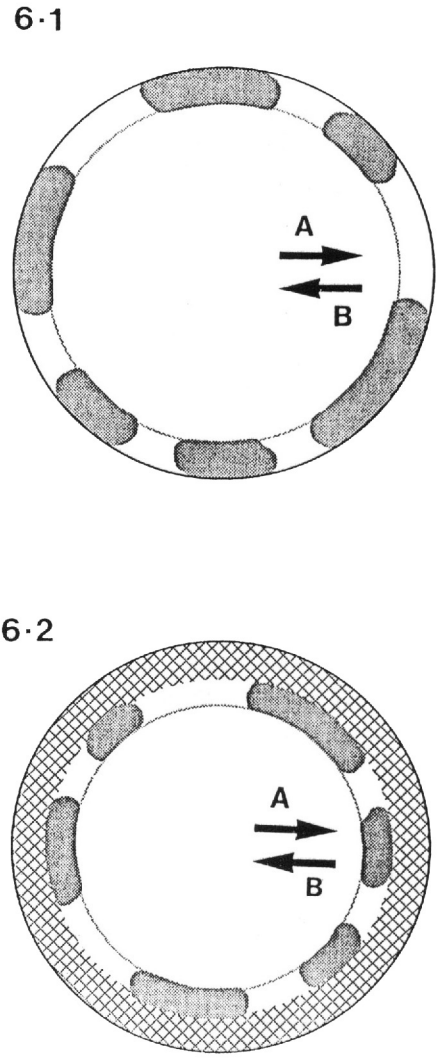

FIG. 6.—*Schematic representation of the proposed mechanism of floret differentiation in the sunflower receptacle. 6-1: Starting in FS 5, "fields" of floret primordia are differentiated at the receptacle rim (grey areas). At that time cell division synchronism occurs in nearly all the circumference to create a toroidal rim (See Figure 3-3). 6-2: While the whole peripheral "ring" has become differentiated (crossed hatched area), a new concentric ring starts to differentiate centripetally and the process is repeated until all the receptacle surface has been covered by florets, the term ring this model partially replaces the generative front definition used by Palmer and Steer (1985). A: Direction of receptacle surface expansion; B: direction of ring displacement.*

then be assumed as the result of an "oscillating growth" which probably starts before FS 4 and continues until all the receptacle surface has been differentiated in floret primordia (FS 8) (See Figure 6 for a more detailed explanation). The increase in cell division rate before FS 5, the degree of synchrony in the cell division cycle and the oscillating surface expansion of the bare surface of the receptacle, could be important in allowing a new set of primordia to be set up.

CONCLUSIONS

The results presented in this paper describe the global dynamics of surface expansion in the receptacle of sunflower. The information provided here can then be used to generate a dynamic model for the receptacle surface expansion with time. The model proposed of surface expansion and floret generation deals with the global dynamics of surface expansion. Nevertheless a more detailed information is needed to assess the kinetics of local cell growth and development on the surface. This will enable to determine locally the dynamics of surface expansion and its direct influence on floret organogenesis.

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