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PHYLLOTAXIS IN BRYOPHYLLUM TUBIFLORUM: MORPHOGENETIC STUDIES AND COMPUTER SIMULATIONS

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SUMMARY

Bryophyllum tubiflorum exhibits several kinds of phyllotaxis patterns: pseudo-bijugate, imperfect pairs, transition helix, imperfect trios, tricussate and inverted helix. Assumptions were introduced and computed in a computer model that these changes in phyllotaxis are due either to changing rates of production of an inhibitor on the apical surface, or alternatively to changing apical size.

Observations on the structure of the apex supported the latter assumption. Mirror asymmetry in the position of the first two leaves was found necessary for the computer simulation, and was also confirmed by observation.

1. INTRODUCTION

Although there has been much discussion about theories of phyllotaxis, almost all botanists have accepted the axiom of HOFMEISTER (1868), namely that the place on the apex, where a new leaf arises, is fixed by the position of two or three older leaves that are nearest to the new leaf. In 1878 SCHWENDENER proposed a mechanical theory which stated that fields of tension arise around primordia and these in turn control the position of a new primordium. This theory is also the basis of SCHÜEPP's view (1966) on phyllotaxis.

In 1907 VAN ITERSON published his "Mathematische und mikroskopisch-anatomische Studien über Blattstellungen", in which he gave a mathematical treatment of "regular point systems" and applied it to phyllotaxis. He described regular point systems on cylinders, cones, etc., as well as different shapes of primordia, like circles and folioids. A recent resumé of van Iterson's ideas is provided by ERICKSON (1972).

Van Iterson assumed the following properties for regular systems of touching circles on cylinder surfaces (see *fig. 1*):

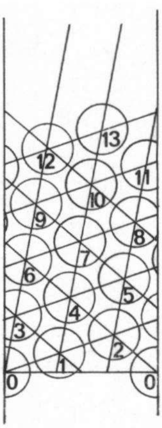
1. The radii of newly inserted primordia are the same as those of young primordia which are already present;
2. Each primordium is in contact with at least two older members (rule of Hofmeister);
3. The primordia are laid down in the largest gaps between te previous ones;
4. Small irregularities like the shape and position of new primordia can be eliminated during the continuous growth of the system.

The ideas of van Iterson are found again in somewhat modified form in the "first available space" theory of SNOW & SNOW (1931, 1933, 1934, 1947). According to this hypothesis each new leaf arises in the first available space on the apex above and between the existing leaves or other contact members in the top cycle, that means in the first space which attains both a necessary width and a necessary distance below the growing-point. The sequence, in which the several gaps are occupied by new leaves, depends on the position and shapes of those leaves only which border the gap.

In 1913 SCHOUTE proposed another theory, the diffusion theory which later was further elaborated by RICHARDS (1951) and RICHARDS & SCHWABE (1969). According to Schoute, contact hypotheses like those of van Iterson and Schwendener should be rejected because at first new organs never touch each other, only their spheres of influence might be touching. By starting from some shape, the primordia have to develop directly in their advanced shape.

Starting from the fact that the axiom of Hofmeister is right, Schoute stated the following theory (*fig. 2*):

1. Each apex contains a particular substance that inhibits the development of new leaves;
2. On each place of the apex, where the influence of this inhibitor reaches a low threshold, a new primordium will arise;
3. Each leaf centre goes directly into an inhibitor producing state, the inhibitor diffuses into the surrounding tissue;



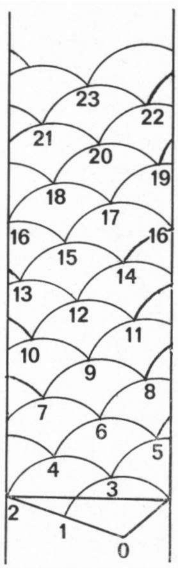


Fig. 1. Example of a 1+2+3 phyllotaxis pattern following van Iterson. The circles are primordia on a cylindrical apex.

Fig. 2. Example of a 1+2+3 phyllotaxis pattern following Schoute. The points are primordia on a cylindrical apex, while the circles represent the threshold concentration of an inhibitor, produced by the primordia.

4. From the topmost cells of the apex an inhibitor diffuses as well, so new leaves can arise only in a region under the tip of the apex.

Schoute, however, was not able to work out his theory on basis of geometrical constructions. To obtain more precise and realistic consequences of the theory computer simulation is needed. VEEN & LINDENMAYER (1973) have attempted to work out consequences of this theory on the computer.

The present paper applies some of this theory to the phyllotaxis patterns found in *Bryophyllum tubiflorum* Harv.. *Bryophyllum tubiflorum* has several kinds of phyllotaxis patterns, each of which are constant for at least some number of nodes: pseudo-bijugate, imperfect pairs, transition helix, imperfect trios, tricussate, and inverted helix (see *fig. 3* and *4*).

"Pseudo-bijugate" designates a pattern which deviates somewhat from the normal bijugate pattern. The transition pattern is a helix with a divergence angle of about 135°. "Tricussate" is a pattern in which the three leaves of a whorl are located 120° from each other and in which each whorl turns 60° with respect to the previous whorl. The inverted helix is characterized by its direction which is opposite to the direction of the transition helix.

In a very few cases the phyllotaxis becomes a tetracussate pattern directly after the starting decussate pattern.

Because of the different phyllotaxis patterns and of the rather short time needed for propagation (vegetative embryos are produced on the adult leaves) *Bryophyllum tubiflorum* is an interesting and useful plant for work on changing phyllotaxis patterns (see *fig. 5*).

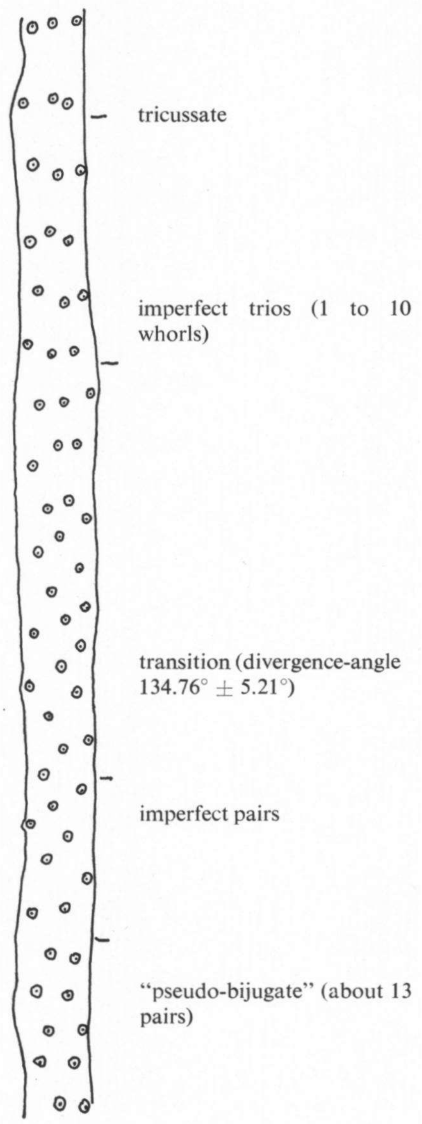
From computer simulations (Veen and Lindenmayer) it was predicted that the described transitions can be brought about by a 10-fold decrease in the production of the inhibitor (the size of the apex remaining constant), or by an increase of apex size during development by a factor of about 3/2 (the inhibitor production remaining constant).

Out of symmetry considerations a requirement was formulated by Veen and Lindenmayer which had to be fulfilled by the initial arrangement of the first two leaves in order that the bijugate and then the tricussate patterns be generated. Namely, one needs a starting condition without mirror symmetry. Otherwise, all subsequent stages will be mirror symmetric and this does not agree with the observed helical transition pattern. If one would start with one leaf the situation would always be mirror symmetric. If one starts with two leaves on different levels or of different sizes and 180° opposite they will still have mirror symmetry (*fig. 6*). Two leaves on the same level, but not 180° opposite, also have mirror symmetry (*fig. 6*).

Accordingly, one needs as minimum starting conditions:

a. Two initial leaves,
b. The leaves must be on different levels or on the same level with different production rates for the inhibitor,
c. The leaves must not be 180° opposite.

The two leaves in figure 6c just fulfil these requirements: the two initial leaves are not on the same level and are not 180° opposite. Alternately, they



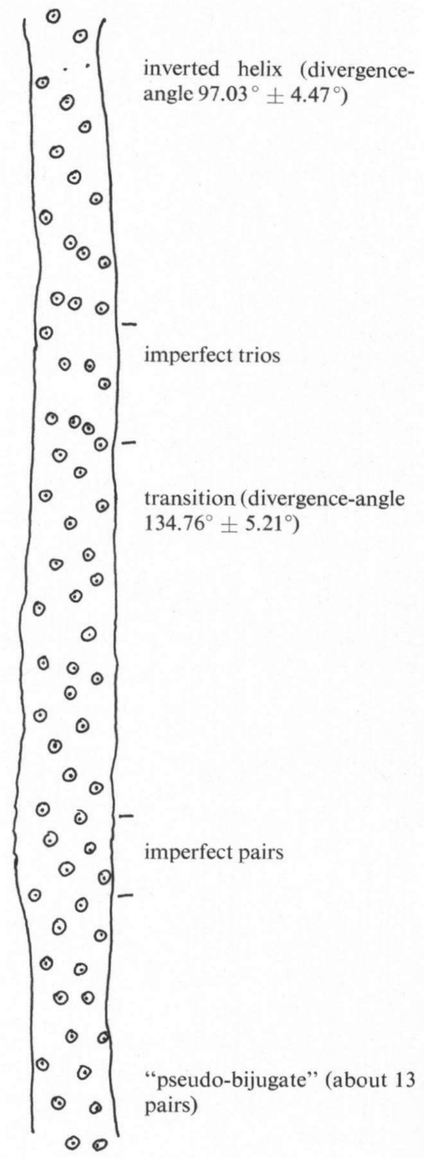


Fig. 3. Cortex stripe of *Bryophyllum tubiflorum* (tricussate).
Fig. 4. Cortex stripe of *Bryophyllum tubiflorum* (inverted helix).

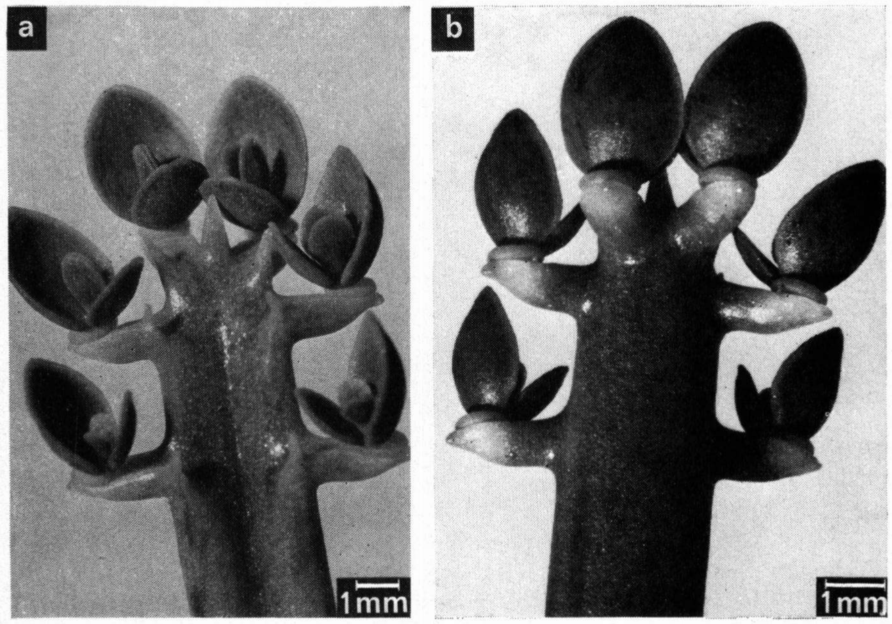


Fig. 5. Plantlets on a fully grown leaf of *Bryophyllum tubiflorum* (adaxial and abaxial view).

could be on the same level but have different rates of inhibitor production.

In this research the starting conditions for the generation of phyllotactic patterns have been studied on this plant, and the following questions have been asked:

Are the first two leaves on different levels; are the leaves on the same level but different in age; or are the leaves on the same level and of the same age but are the influences of the leaves different (is one leaf bigger than the other)? (See also *fig. 5*). Are the first two leaves 180° opposite or not? Furthermore, the surface areas of the apex in plants with different phyllotaxis patterns have been studied in order to find out whether the apex increases during development with a factor of about $^3/_2$ between the decussate (bijugate) and tricussate phases.

2. MATERIALS AND METHODS

2.1. Organisms

The crassulacean *Bryophyllum tubiflorum* Harv.* was obtained from the greenhouse of the Botanical Garden in Utrecht, from a clone (Bt 12) collected in 1939 in South Africa, and was used for most of the experiments. For one of the experiments *Kalanchoe daigremontiana* R. Hamet et Perr. de la Bâthie, obtained

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* The valid name of this species is *Kalanchoe tubiflora* (Harv.) R. Hamet. Since most authors of physiological literature use the synonym *B. tubiflorum* we also followed that usage.

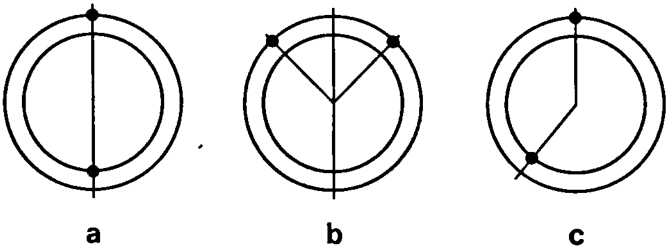


Fig. 6. Starting conditions for the first two leaves.
a. Two leaves on different levels and 180° opposite,
b. Two leaves on the same level but not 180° opposite,
c. Two leaves not on the same level and not 180° opposite.

from the same greenhouse was taken. This species has a constant decussate phyllotaxis.

For the same experiment the hybrid (B × 7) between *Bryophyllum tubiflorum* (Bt 7) and *Kalanchoe daigremontiana* (Bd 1), obtained from C. Gómez-Campo in Spain, was used. This species has the property that the leaves have nearly the shape of *Kalanchoe daigremontiana* and exactly the same phyllotaxis as *Bryophyllum tubiflorum.*

2.2. Preparation of cortex stripes

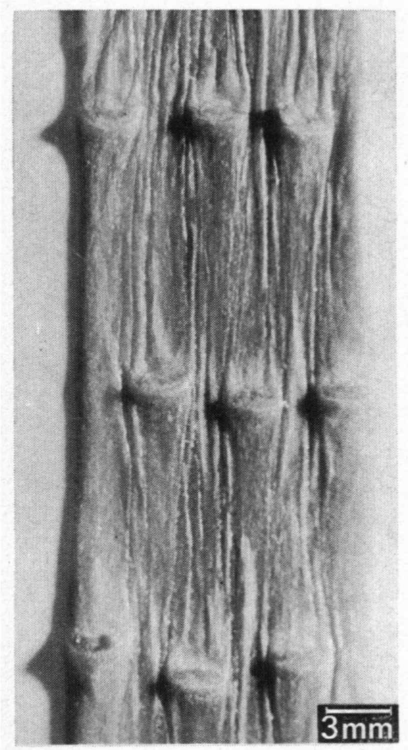
From cortex stripes prepared according to the method of C. Gómez-Campo it was possible to see the position of leaves of practically the whole plant. Cortex stripes are made by cutting a frozen stem along a vertical line and by separating the cortex from the stem. The central vascular bundles in the leaf scars were taken as references for the position of leaves (*fig. 7, 3* and *4*). The position of each central bundle was recorded on graph paper by pin pricks through them.

2.3. Elongation of internodes

Rather young plantlets and plantlets with a visible second leaf pair were put in complete darkness for 7 days. After 7 days the plantlets were observed under a stereomicroscope for internode elongation.

2.4. Preparations of sections through basal plate and first internode of plantlets

Transverse sections of 10 micron thickness were made of the plantlets of *Bryophyllum tubiflorum*, *Kalanchoe daigremontiana* and their hybrid on a microtome. The material was fixed in FAA (according to JENSEN 1962) and embedded in paraffin. The sections were stained in orcein that contained acetic acid (45%) and 2% orcein. Afterwards the sections were photographed. One photograph of the basal plate and one of the first internode are shown here (*figs. 8, 9a* and *9b*).



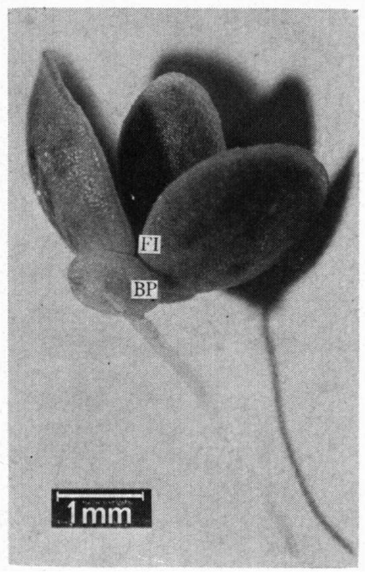
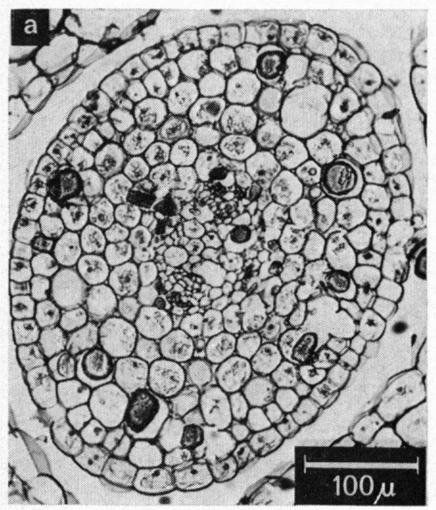


Fig. 7. Detail of a cortex stripe.
Fig. 8. Plantlet with basal plate (BP) and first internode (FI)



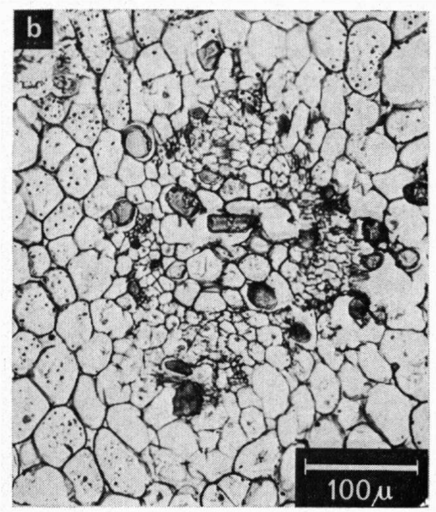


Fig. 9a. Transverse section through the first internode of *Bryophyllum tubiflorum*.
Fig. 9b. Transverse section through the basal plate of *Bryophyllum tubiflorum*.

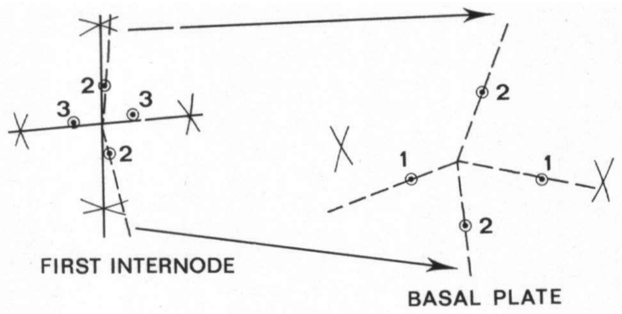


Fig. 10. Geometrical construction for the estimation of the centre of the first two leaf pairs.

2.5 Determination of divergence angles of primordia

To estimate the apical centre in the first phase of growth, one needs to find the centre between the second and the third leaf pairs (because of the different ages of the leaves of the first pair this pair cannot be used). If, as references for the leaf position the vascular bundles are taken, one can find the centre of the second and third pair in the transverse section of the first internode by drawing the middle-lines (*fig. 10*). The crossing point is the centre. Now it is possible to project this centre into the section through the plate with the four vascular bundles of the first two leaf pairs. From this centre it is possible to measure the angles between the first four leaves.

2.6. Preparation of apical sections

Transverse sections of 10 micron thickness were made of apices from *Bryophyllum tubiflorum* in the bijugate phase, in the transition zone, in the tricussate phase and in the inverted helix zone (*fig. 11a, b, c, d*). The material was taken from plants of nearly the same plastochron age. This was ascertained by measuring the lengths of the youngest visible primordia. The apices were fixed in Zenker fixation medium (according to JENSEN 1962) for five hr. and were embedded in paraffin (52 °C). The sections were stained in orcein and photographed. The orcein stains nuclear material. Afterwards the apical surface between the youngest visible primordia was measured with a planimeter.

3. RESULTS

3.1. Observations on cortex stripes (*fig. 3, 4, 7* and *12*)

On the cortex stripes only horizontal distances (divergence angles) can be meaningfully measured because in the case of vertical distances the internodes may be irregularly elongated. From *fig. 12*, in which the horizontal distances (angles) were plotted, it will be apparent that there is a gradual transition from the bijugate phase into the transition zone (see also GÓMEZ-CAMPO 1974).

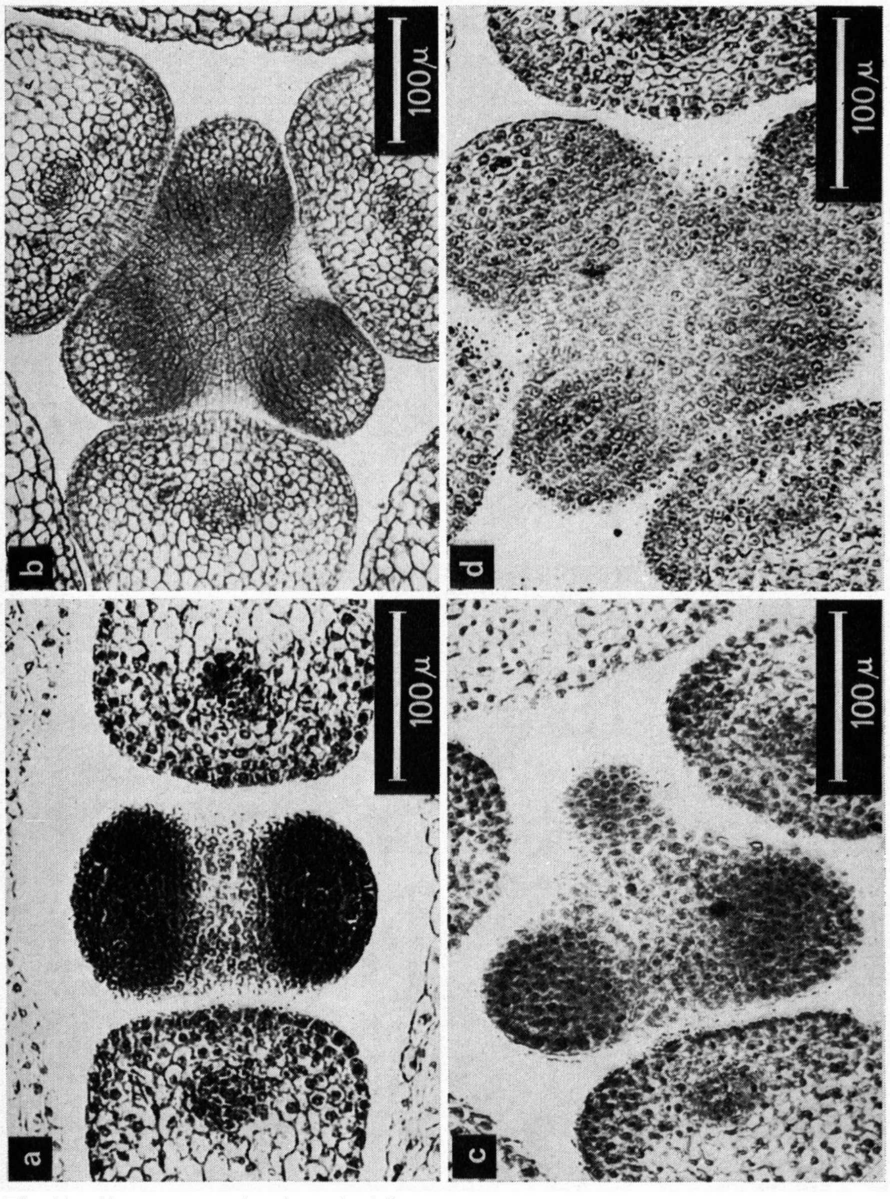


Fig. 11a. Transverse section through a bijugate apex of *Bryophyllum tubiflorum.*
Fig. 11b. Transverse section through a tricussate apex of *Bryophyllum tubiflorum.*
Fig. 11c. Transverse section through a transition apex of *Bryophyllum tubiflorum.*
Fig. 11d. Transverse section through an inverted helix apex of *Bryophyllum tubiflorum.*

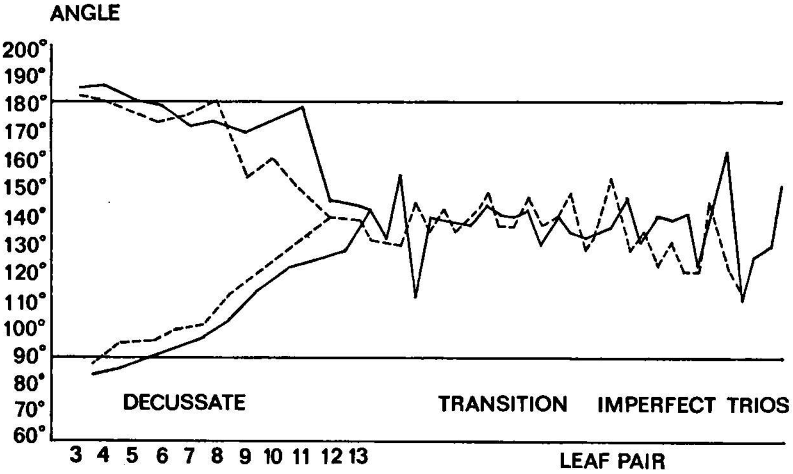
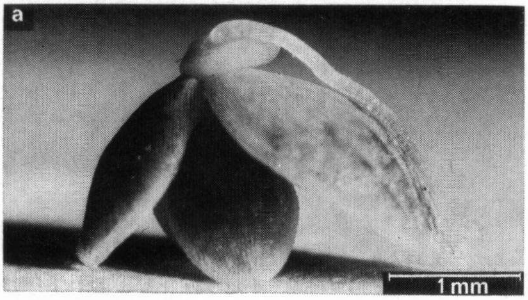
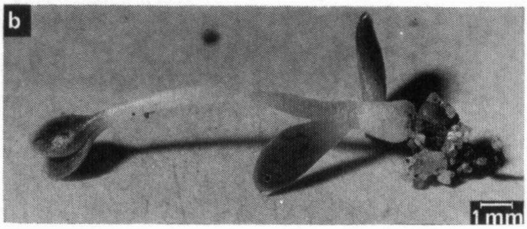


Fig. 12. Cortex stripe measurements of angles between leaf pairs for *Bryophyllum tubiflorum.*





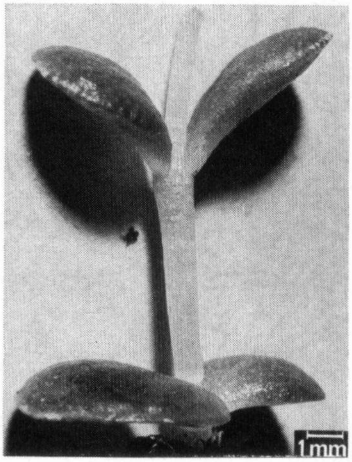


Fig. 13a. Normal plantlet of *Bryophyllum tubiflorum.*
Fig. 13b. Plantlet after 7 days of darkness of *Bryophyllum tubiflorum.*
Fig. 14. Elongation of the second leaf pair of *Bryophyllum tubiflorum.*

3.2. Elongation experiments on very young plantlets(*fig. 13*)

In all cases the basal plates of the plantlets which bear the first two leaves elongated on the side of the smaller leaf after 7 days of darkness. This means that the tissue of the plate on that side in younger. Indeed, the plate is growing in size during the development of the plantlet. Therefore, without the dark treatment the leaves of the first leaf pair are located on the same level on the plate, but are of different ages; furthermore, it is apparent from *fig. 14* that the leaves of the second leaf pair are exactly on the same level.

3.3. Measurements of angles between the first two leaf pairs (*table 1*)

The mean values of the angles, given in the table, were calculated both in a "one-sided" and "two-sided" manner. If the leaf pair lies not exactly 180° opposite, one can have asymmetry on two sides. "One-sided" mean values were obtained by translating each angle under 180° into its mirror value above 180°, and averaging over all values. "Two-sided" mean values were calculated by averaging the observed values directly. The standard deviation is rather large, due to the fact that exact transverse cutting is nearly impossible and that the position of the centre of a vascular bundle cannot be found exactly. Since the method used for the three kinds of plants was the same, and also the plantlets were nearly of the same size, it seemed possible to draw valid conclusions. Namely, it is clear that the mean angle between the first two leaves in *Bryophyllum tubiflorum* and in the hybrid differs from the angle between the first two leaves of *Kalanchoe daigremontiana.* The mean values indicate that the angle between the first two leaves in *Kalanchoe daigremontiana* deviates from 180° only by about 6°, in contrast with the ones in *Bryophyllum tubiflorum* and in the hybrid, which appear to deviate from 180° by about 14°. The latter angle exceeds considerably the value of the standard deviation.

3.4. Estimation of apical areas (*table 2*)

The pairwise ratios between the mean apical areas of the bijugate phase, transition helix, tricussate phase and inverted helix are as follows:

transition/bijugate = 1.580
tricussate/transition = 1.242
tricussate/bijugate = 1.966
inverted helix/tricussate = 1.257

The ratio of tricussate/bijugate apical areas (r_t^2/r_d^2, r = radius) agrees with the computer prediction, since $r_t/r_d = \sqrt{1.966} \approx 1.4$.

3.5. Computer simulations

As stated in the first chapter this research was carried out in connection with the computer simulations, obtained by Veen and Lindenmayer. In *fig. 15* one of their outputs has been printed. This simulation was based on the theory of Schoute, the diffusion theory; namely, that a particular substance, which is produced in the apex by the primordia, inhibits the development of new leaf primordia by diffusion through the apex.

Some of the assumptions for developing the theoretical model were:

1. The apex can be approximated by a cylinder and the processes leading to leaf initiation take place on the surface of the cylindrical section,

Table 1. *Angles between the first 2 leaf pairs.*

	Bryophyllum tub.				*Kalanchoe daigr.*				*Hybrid*			
	0	193	293	84	0	180	265	86	0	191	273	93
	0	182	269	82	0	180	269	89	0	156	233	53
	0	172	265	85	0	180	278	96	0	208	305	86
	0	195	287	96	0	188	279	90	0	185	242	54
	0	172	274	108	0	186	256	103	0	195	273	111
	0	160	278	89	0	184	202	91	0	200	259	105
	0	143	250	91	0	188	270	91	0	153	237	88
	0	180	277	76	0	191	207	106	0	191	278	73
	0	200	259	101	0	187	297	92	0	205	290	106
	0	232	311	130	0	180	255	75	0	196	286	86
	0	204	284	90	0	188	273	79	0	195	266	96
	0	150	268	88	0	203	270	100	0	155	266	91
	0	182	287	77	0	175	263	90	0	175	274	94
	0	200	286	86	0	196	285	92	0	192	284	112
	0	176	260	97	0	180	270	89	0	172	294	90
	0	177	292	94	0	160	257	75	0	165	264	74
	0	190	259	112	0	190	284	88	0	176	274	86
	0	194	265	85	0	184	261	106	0	184	296	91
	0	191	266	111	0	179	267	87	0	186	266	100
	0	203	291	87	0	191	279	99	0	171	248	68
	0	175	260	100	0	190	293	90	0	197	289	109
	0	176	268	77	0	180	268	80	0	182	271	69
	0	194	292	94	0	170	284	97	0	185	259	81
	0	200	261	77	0	171	233	76	0	219	291	111
	0	158	277	78	0	179	266	89	0	169	257	88
	0	200	275	78	0	180	266	88	0	181	262	78
	0	179	261	77	0	180	275	95				
	0	176	270	89	0	192	272	90				
	0	182	269	89	0	185	258	94				
	0	178	240	81	0	168	233	90				
	0	180	280	90	0	190	285	113				
	0	171	290	84	0	180	269	91				
	0	172	278	89	0	180	255	101				
	0	174	273	91	0	180	274	94				
	0	193	283	101	0	181	261	93				
					0	172	265	85				
					0	186	279	95				
					0	158	253	72				
					0	183	265	90				
					0	172	264	95				
					0	200	284	103				
	1	1	2	2	1	1	2	2	1	1	2	2
	first leafpair		second		first leafpair		second		first leafpair		second	
first leafpair	*mean value* one-sided 194.125° ± 11.428° two-sided 184.041° ± 18.383°				*mean value* one-sided 186.366° ± 6.903° two-sided 182.122° ± 9.147°				*mean value* one-sided 193.846° ± 9.396° two-sided 184.000° ± 16.248°			

Table 2. *The areas of apices (in microns) of Bryophyllum tubiflorum.*

decussate	transition helix	tricussate	inverted helix
65	117	121	220
51.3	93	105	171
58.8	98	125	165
90	118	132	121
56.3	121	135	111
50	91	86	145
36.3	120	111	152
89	130	97	113
68.8	86	91	170
71.3	81	143	176
77	96	150	192
67.5	97	102	143
65	97	98	130
51.3	87	128	158
73.8	85	115	
53.8	78	143	
71.3	116	138	
41.3	76	125	
53.8	96	121	
78.8	112	143	
63.8	86	132	
60		158	
56.3		132	
		101	
		115	
		118	
		128	
		128	
		116	
		136	
		133	
		135	
mean value 62.62 ± 12.96	mean value 99.20 ± 16.01	mean value 123.16 ± 17.28	mean value 154.79 ± 29.71

2. The cells are approximately of the same size and are arranged on a rectangular grid.
3. Every cell has 8 neighbours.

Veen and Lindenmayer used an equation for the diffusion of the inhibitor substance representing a digitized version of two-dimensional Fick's equation,

$$C_o^1 = C_o + \triangle C_o = \lambda \sum_{i=o}^{8} C_i + (1-9\lambda-D)\, C_o$$

where C_i = inhibitor concentration in the i-th cell, C_o = previous inhibitor concentration in center cell, C_o^1 = next inhibitor concentration in center cell, λ = diffusion-coefficient, D = decay-coefficient.

In this model the following values can be varied:

a. The width of the apex: number of cells along circumference of apex,

b. The leaf value: the concentration of inhibitor substance in the primordia (in figure 15 represented by black squares),
c. The threshold value: the value below which a new primordium arises,
d. The diffusion coefficient,
e. The decay coefficient: the inhibitor decays at a rate proportional to its concentration in every cell except the leaf primordia,
f. The growth rate: growth takes place by adding new rows at the top rim of the cylinder.

In figure 15 each number or letter represents a rounded-off value for the concentration of the inhibitor in a cell.

The program Blokco 28 for the computer simulations was written in ALGOL by A. H. Veen, while the computations were carried out on the Electrologica Philips X-8 computer at the University of Utrecht. Further information about the program can be found in VEEN & LINDENMAYER (1973).

In *fig. 15* a rather accurate approximation of the changing phyllotaxis of *Bryophyllum tubiflorum* has been obtained: the pattern starts with two almost opposite leaves of different leaf values (inhibitor producing factor), proceeds with a bijugate pattern and then goes over in a trijugate pattern via a helix. The parameters for this simulation were:

width	$W = 25$ cells
decay coefficient	$D = 0.15$ (15% per time step)
diffusion coefficient	$\lambda = 1/18$
threshold concentration	$T = 0.4$
growth rate	$G = 1/70$, after 70 computations of the concentrations of the inhibitor in each cell a new row was added.

The leaf value (L) was programmed to decrease gradually from 122 for the bijugate to 12 for the trijugate pattern. This tenfold change has been calculated to correspond to an increase of the width of the apex by a factor 3/2, if the leaf value should remain constant. L and T are relative concentrations of the hypothetical inhibitor and the simulation provides only values for L/T. Similarly, the simulation can only provide a ratio for D/λ. In figure 15 the width of the arrays is actually 50 symbols, rather than 25. This array size is merely the result of printing every symbol twice in order to make the graphical presentation more realistic (since the width of a printed symbol is 7/12 of its height).

Although this simulation is a good approximation of the phyllotaxis of *Bryophyllum tubiflorum*, it could still be improved. The transition zone, for instance, does not exactly correspond to observations (see *figs.* 3 and *4*). By attempting to vary the leaf values in a more continuous manner than in this simulation the results did not improve. More continuously means that after each growth step the leaf value was changed with the same factor (2 units per new line added). In the original simulation this was done at irregular intervals (see list of leaf values in *fig. 15*).

Although it is possible to improve the original simulation by varying the

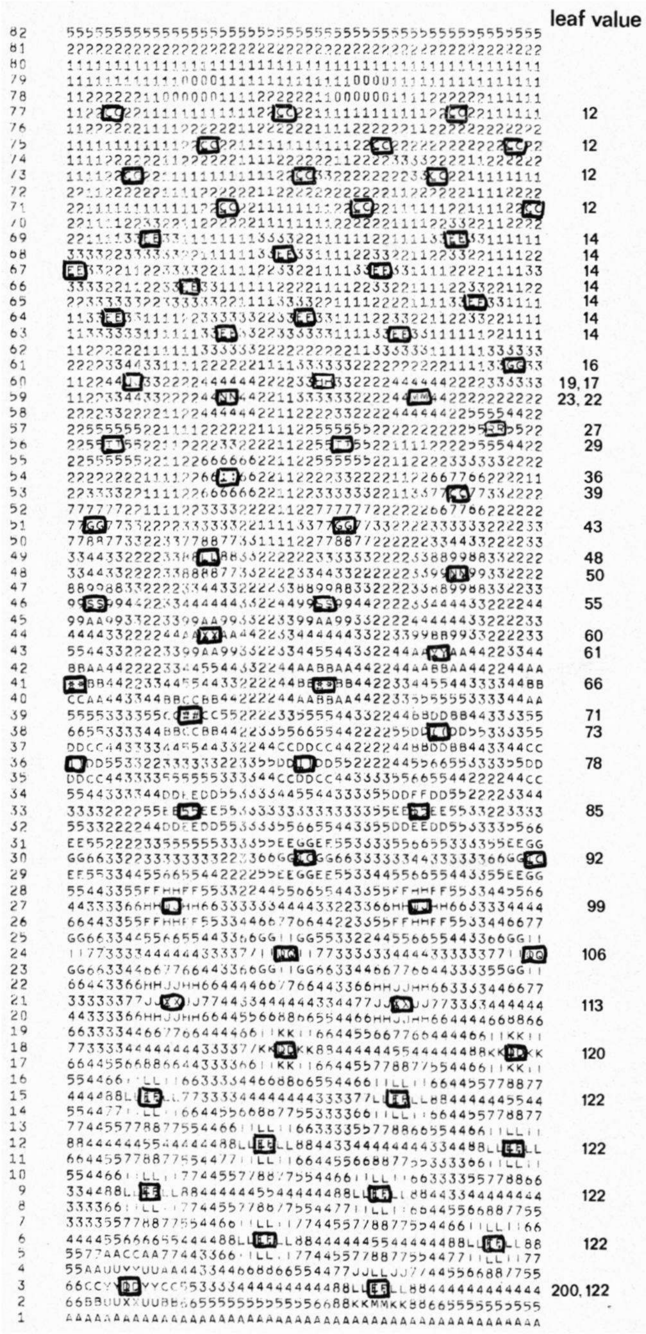
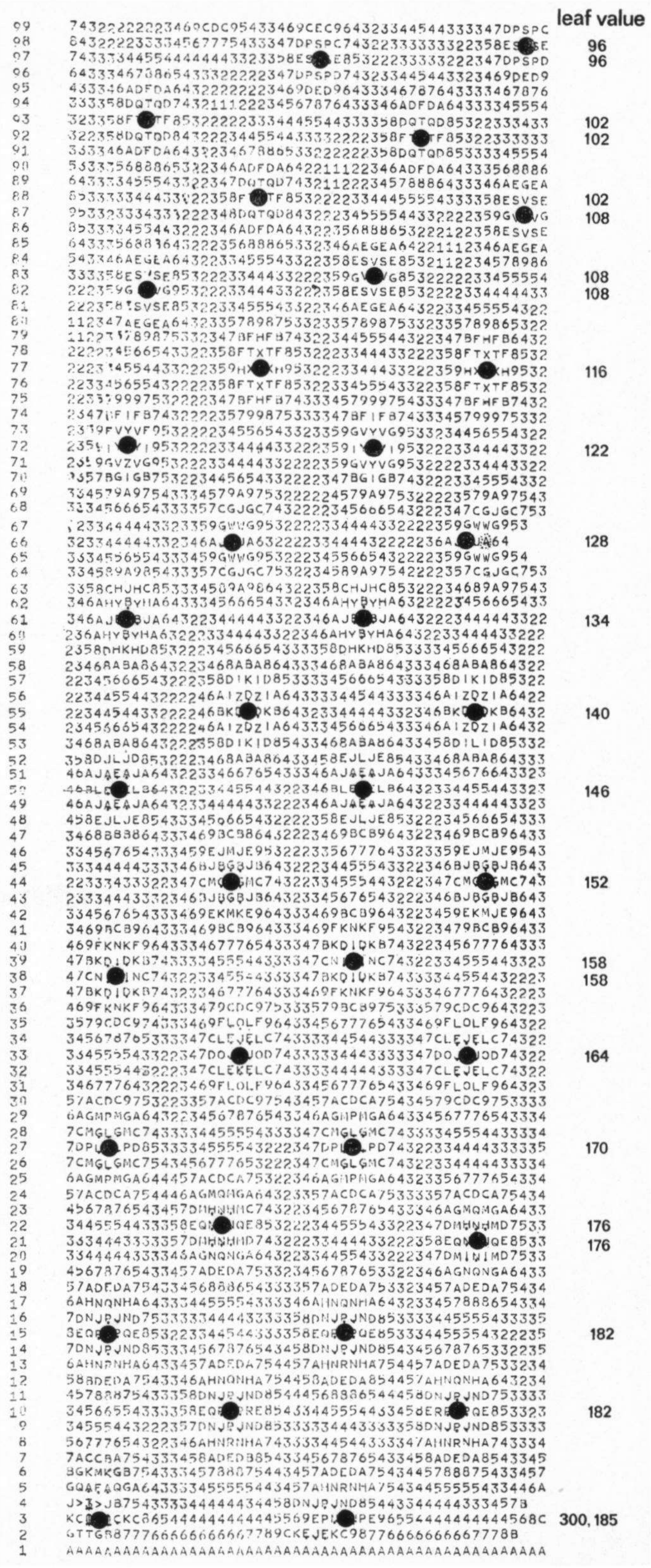


Fig. 15. Simulation of phyllotaxis of *Bryophyllum tubiflorum* by Veen and Lindenmayer. Each symbol standing for inhibitor concentration was printed double.
$W = 25$, $D = .15$, $\lambda = 1/18$, $T = .4$, $G = 1/70$, $L_1 = 200$, $L_2 = 122$, L decreases to 12.



```
                                                                leaf value
99   74322222223469CDC95433469CEC964323344544333347DPSPC
98   843222233334567775433347DPSPC743223333333322358ES●SE        96
97   743333445544444443323358ES●E853222333333222347DPSPD        96
96   643334673865433322222347DPSPD743233445443323469DED9
95   433346ADFDA64322222223469DED964333467876433333467876
94   333358DQTQD7432111222345678764333346ADFDA64333345554
93   323358F●TF853222223334445544333358DQTQD85322333433        102
92   322358DQTQD843222344554433332222358F●TF85322333333        102
91   333346ADFDA6432234678865332222222358DQTQD85333345554
90   533335688865322346ADFDA6422111222346ADFDA64333568886
89   643333455543322347DQTQD74321122234578886433346AEGEA
88   853333344433322358F●TF853222233444555543333358ESVSE        102
87   9533233343332222348DQTQD843222345555443322222359G●VG        108
86   853333455443222346ADFDA64322356888653222122358ESVSE
85   643335688364322235688865332346AEGEA6422111 2346AEGEA
84   543346AEGEA6432233455543322358ESVSE8532112234578986
83   333358ESVSE8532223344433222359G●VG853222223345554        108
82   222359G●VG953222334444332222358ESVSE853222334444433        108
81   222358ESVSE8532233455543322346AEGEA64322334555543 22
80   112347AEGEA6432335789875332335789875332335789865322
79   112235789875332347BFHFB7432234455544322347BFHFB6432
78   2222345665433222358FTXTF853222334443322 2358FTXTF8532
77   22233455443322222359HX●XH9532223344433222359HX●XH9532        116
76   223345655432222358FTXTF8532233455543322358FTXTF8532
75   2233799975322222347BFHFB7433345799975433347BFHFB7432
74   2347BFIFB743222235799875333347BFIFB7433345799975332
73   2339FVYVF95322223455654332 3359GVYVG953323445655432 2
72   2359IY●YI95322223344443322 2359IY●YI95322233444443322        122
71   2359GVZVG953222334444332222359GVYVG95322223344443322
70   2357BGIGB7532234456543322222347BGIGB7432223345554332
69   334579A97543334579A975322222 4579A975322223579A97543
68   3334566654333357CGJGC743222234566654322 2347CGJGC753
67   1233444443323359GWWG95322223344443322223 59GWWG953
66   3233444443322346AJ●JA632222334444322222 36AJ●JA64        128
65   3334556554333459GWWG953222345566543222 2359GWWG954
64   334589A985433357CGJGC753223458 9A97542222357CGJGC753
63   3358CHJHC85333458 9A98643223 58CHJHC8532223468 9A97543
62   346AHYBYHA643334566654332346AHYBYHA6322223456665433
61   346AJ●BJA643222344444332 2346AJ●BJA643222344444 43322        134
60   236AHYBYHA63222334444433 22346AHYBYHA643223344444332 22
59   2358DHKHD853222345666543333 58DHKHD853333345666543222
58   23468ABA864322346 8ABA864333468ABA864333468ABA864322
57   22345666543222358DIKID853333456665433333 58DIKID85322
56   22344554432222 46AIZDZIA6433334454433333 46AIZDZIA6422
55   2234454433 222246BKD●DKB643233444444332346BKD●DKB6432        140
54   23455665432222 46AIZDZIA643334566654333346AIZDZIA6432
53   3468ABA86432223 58DIKID85433468ABA86433458DILID85332
52   358DJLJD853222346 8ABA86433458EJLJE85433468ABA864333
51   46AJAEAJA64322334667654333346AJAEAJA643333456766 43323
50   46BLE●LB64322334455443322346BLE●LB6432334455443323        146
49   46AJAEAJA643223344444332 22346AJAEAJA643223344444 43323
48   458EJLJE8543334566654322223 58EJLJE853222345666 54333
47   3468BBB864333469BCB864322 23469BCB9643223469BCB96433
46   3345676543333459EJMJE953222335677764332 3359EJMJE9543
45   333444444333346BJBGBJB64322234455543322 346BJBGBJB643
44   22333433322347CMG●GMC743223345554432 22347CMG●GMC743        152
43   233344433322346BJBGBJB643233456765432 22346BJBGBJB643
42   3345676543333469EKMKE964333469BCB964322 3459EKMJE9643
41   3469BCB964333469BCB964333469FKNKF954322347 9BCB96433
40   469FKNKF96433346777654333347BKDIDKB74322345677 764333
39   47BKDIDKB7433333455544333347CN●NC743223345554 43323        158
38   47CN●NC74322334554433333 47BKDIDKB7433334455443 2223        158
37   47BKDIDKB74323346777643333469FKNKF96433346777643 2223
36   469FKNKF964333479CDC97533357 9BCB975333579CDC9643223
35   3579CDC974333469FLOLF9643345677765433469FLOLF964322
34   34567876533333 47CLEJELC74333344544333347CLEJELC74322
33   33455554332 2347DO●OD74333334444333334 7DO●OD74322        164
32   33455544322 2347CLEKELC7433333444443333347CLEJELC74322
31   3467776432223469FLOLF964334567776543 3469FLOLF964323
30   57ACDC9753223357ACDC97543457ACDCA75434579CDC9753333
29   6AGMPMGA64322345678765433 46AGMPMGA643345677765 43334
28   7CMGLGMC7433334455554333347CMGLGMC7433334555443 3334
27   7DPL●LPD85333345555432223 47DPL●LPD7432233444433 3335        170
26   7CMGLGMC75434567776532223 47CMGLGMC7432233444443 3334
25   6AGMPMGA644457ACDCA75322346AGMPMGA643233567776 54334
24   57ACDCA754446AGMQMGA64323357ACDCA75333357ACDCA75434
23   4567876543457DMHNHMC743223456787654333 46AGMQMGA6433
22   3445554433358EQN●NQE853222344555433 22347DMHNHMD7533        176
21   3334443333357DMHNHMD743222334444332 22358EQN●NQE8533        176
20   3344444333346AGNQNGA643223344554332 22347DMINIMD7533
19   45678765433457ADEDA753323456787653 322346AGNQNGA6433
18   57ADEDA75433456888654333 357ADEDA753323457ADEDA75434
17   6AHNQNHA64333445555433333 46AHNQNHA643233457888654334
16   7DNJPJND7533333444433333 58DNJPJND8533333445555433335
15   8EQ●PQE85322334454433333 58EQ●PQE8533344555543 22235        182
14   7DNJPJND853334567876543458DNJPJND854345678765332235
13   6AHNRNHA6433457ADEDA754457AHNRNHA754457ADEDA7533234
12   588DEDA7543346AHNQNHA754458ADEDA854457AHNQNHA643234
11   457888754333358DNJPJND85444568886544458DNJPJND753333
10   34566554333358EQ●RE854334455544345 8ER●QE853323        182
9    345554432223 57DNJPJND853333344433333 58DNJPJND853333
8    56777654322346AHNRNHA74333344544333347AHNRNHA743334
7    7ACCBA7543333458ADEDB8543345678765433458ADEDA8543345
6    8GKMKGB754333457888754434 57ADEDA7543445788875433457
5    GQAEAQGA64333345555544345 7AHNRNHA7543445555554 33446A
4    J>I>JB7543333444444434458DNJPJND854433444444333457B
3    KC●CKC8654444444444445569EP●PE9655444444444444568C        300, 185
2    GTTG887776666666666677 89CKEJEKC987776666666666677 78B
1    AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

Fig. 16. First column.

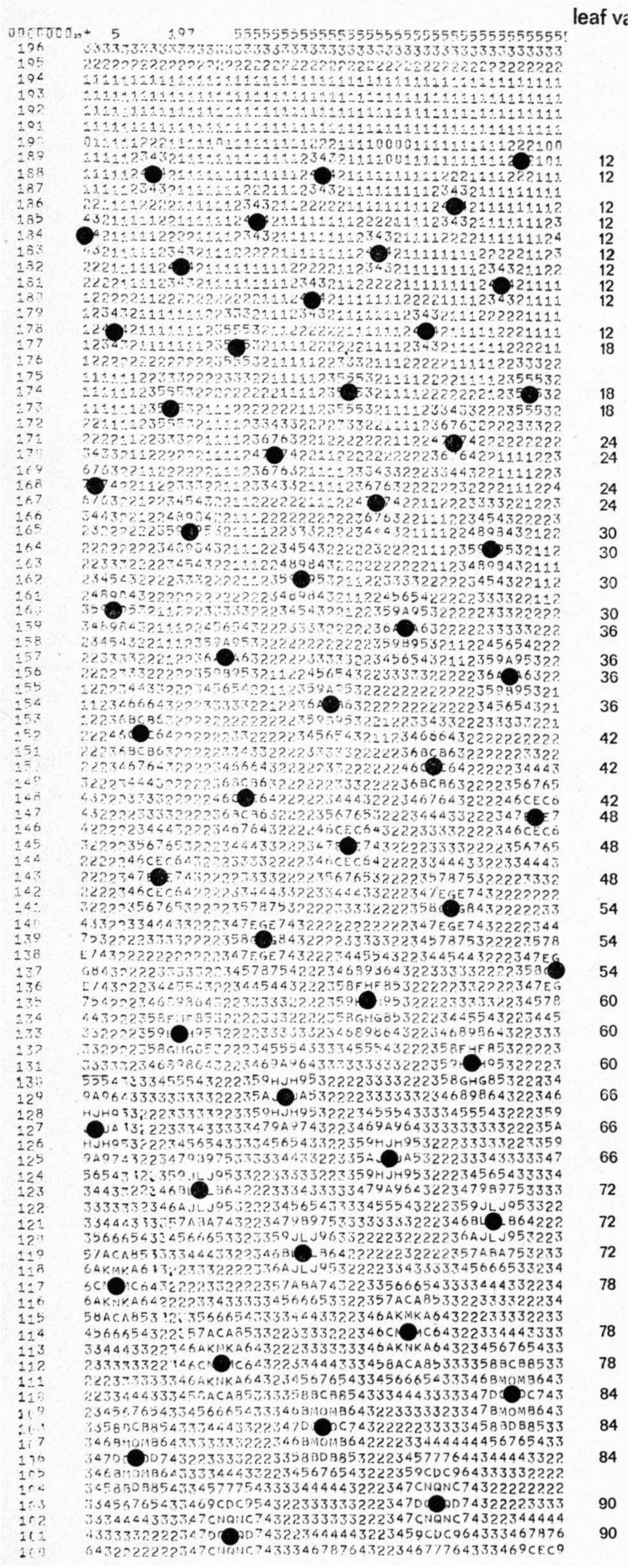


Fig. 16. Simulation of phyllotaxis of *Bryophyllum tubiflorum* with width 51 and asymmetryof 7°. W = 51, D = .019, $\lambda = 1/36$, T = .4, G = 1/70, $L_1 = 300$, $L_2 = 185$, L decreases to 12. Computation (corresponding to growth) begins at the bottom of the first column and ends at the top of the second column.

N.B. Due to an error in the printout, in line 66 the righthand leaf is shown in a faulty position. It should appear two spaces to the right.

Second column.

other variables, another shortcoming of the simulation is the small width used in the simulation, namely 25 cells. The smallest possible angle for asymmetry that can be reached is 14° (1/25 × 360°). If a bigger asymmetry angle was taken, for instance 28°, then the pseudo-bijugate pattern disappeared. According to the experimental results with a more or less variable asymmetry angle, it seems more useful to be able to vary the asymmetry and to change to smaller angles. Therefore, a width of 51 has been tried. Although the results are not final yet, because of the long computer time needed for such simulation, they are positive so far (*fig. 16*). This latter simulation is in better agreement with cortex stripes with respect to the zone between line 77 and 159. The transition helix in this case is more regular than in the previous simulation.

4. CONCLUSIONS

We can conclude from the results that several of the conditions which we assumed in the computer simulation are fulfilled. In the first place the mirror asymmetry is realised in the plants, and in the second place, the predicted increase of the apex size actually occurs during development in these plants.

In summary,

1. the first two leaves of the plants of *Bryophyllum tubiflorum* are not of the same age, thus they might exert different influences on the following primordia;
2. the first two leaves of the plants of *Bryophyllum tubiflorum* are not 180° opposite (mirror asymmetry follows from points 1 and 2);
3. the apical area increases during development with a factor 1.966 from bijugate to tricussate and the radius and circumference of the apex increase by a factor of about 1.4.

It is important to realize the consequences of these results. If the phyllotaxis in a plant is not constant, but changes into a different type, which is not simply a jugal multiple of the previous one, there have to be mirror asymmetry in the first starting leaves.

If the phyllotaxis changes into a higher phyllotaxis system, then under the diffusion hypothesis the apex has to increase, or the rate of the inhibitor production has to decrease, or the rate of inhibitor decay has to increase.

The cotyledons of a plant are mostly assumed to be the starting leaves of a phyllotaxis pattern. Monocotyledonous plants have mainly low phyllotaxis indices (alternate leaves), due to the single starting leaf, the single cotyledon, whose position is by necessity mirror symmetric. For dicotyledonous plants, however, the fully grown plants have often phyllotaxis patterns which are different from decussate, are of higher indices, and not multiples of decussate. In order to obtain a helix, the position of the cotyledons must be mirror asymmetric. Otherwise the cotyledons are not the starting leaves for phyllotaxis.

The irregular phyllotaxis patterns in *Bryophyllum tubiflorum* like the inverted helix and the tetracussate pattern represent further interesting problems. Tetracussate phyllotaxis can be considered as a jugal doubling of decussate, so the plantlet could be completely symmetric at the beginning, and from this pattern during development the apex circumference could increase two times. The observations fit this idea, because two plants were found with a tetracussate pattern, in which the decussate pattern changed directly into a tetracussate one.

Plants with an inverted helix are considered in *fig. 4* and *table 2*. These plants have imperfect trios different from those of the tricussate plants. Furthermore, it is obvious (*table 2*) that the apical area of the inverted helix is bigger than the area of the tricussate plants. It is possible that the apex in this case increases too quickly and too much in size before becoming constant in size, so that the tricussate phase would be passed over. Sometimes plants were found with this pattern in which a transitional tricussate phase was present too. According to our hypothesis this would mean that the apex increased after being constant for a while. This has still to be tested by computer simulation.

The results presented here can be said to be in accordance with the diffusion hypothesis for phyllotaxis, but they do not disprove the possibility of other hypotheses.

ACKNOWLEDGEMENTS

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The photograph for figure 11b was made by Maria Astrella Gómez-Campo, Instituto Nacional de Investigaciones Agronomicas, Madrid, and the rest by P. H. Hellendoorn.

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