In assessing the effects of the tides on intertidal seaweed populations, cloudless days which occurred towards the end of a series of such tides were selected. Diamond Head reef on Oahu Island (Hawaii) was selected as the study area and 3 species of *Hypnea* were used as research material (Table II).

Table II. Percentages of *Hypnea* thalli with killed and bleached apices during mid-day 0.0 cm and 'minus' low tides

Species	Date	Distance seaward from shore (m)			
		0	5	10	15
H. cervicornis	10. 3. 1973	100±0	67±6	53±4	0±0
	6. 5. 1973	100±0	69±8	48±3	0±0
H. chordacea	10. 3. 1973	–	100±0	56±4	7±2
	6. 5. 1973	–	83±5	60±6	0±0
H. nidifica	10. 3. 1973	–	25±3	8±1	0±0
	6. 5. 1973	–	33±4	19±2	0±0

–, No thalli grew in the area.

Table III. Percentages of *Hypnea* thalli with killed and bleached apices during early morning 0.0 cm and 'minus' low tides

Species	Date	Distance seaward from shore (m)			
		0	5	10	15
H. cervicornis	28. 4. 1973	4±0.5	0±0	0±0	0±0
	29. 7. 1973	0±0	3±0.5	0±0	0±0
H. chordacea	28. 6. 1973	–	18±2	0±0	0±0
	29. 7. 1973	–	8±1	5±1	0±0
H. nidifica	28. 6. 1973	–	7±2	0±0	0±0
	29. 7. 1973	–	0±0	0±0	0±0

–, No thalli grew in the area.

Sampling was done along a transect across the intertidal belt. A 45 cm diameter brass sampling ring was randomly tossed 6 times at each of 0, 5, 10 and 15 m seaward from a gently sloping shore. Where the ring fell, thalli of the selected species showing signs of killing through tide-induced emersion were counted. The affected plants had bleached, killed and disintegrated tips. The percentages of affected *Hypnea* plants were subsequently calculated and the data tabulated (Table II).

The procedure was repeated on selected dates (Table III) when the intertidal seaweeds were emersed during early morning – (06.00 to 09.00 h) 'zero' and 'minus' low tides, i. e., when the light intensity, temperature and desiccation would be expected to be less destructive.

Results and discussion. The data obtained (Table I) show that, in the Hawaiian Islands, there is definite seasonality in tidal behaviour. *Hypnea* thalli showed considerable damage (Table II) during the days with mid-day 'zero' and minus low tides. More bleaching and killing of the thalli was observed for *H. cervicornis* and *H. chordacea* which grow higher up intertidally than for *H. nidifica* which is more subtidal. On the days with early morning low tides (Table III), algal killing by tide-induced emersion was negligible.

From the literature[5] and from these results, one can predict that the season when intertidal seaweed populations in Hawaii would be minimal is towards the end of the seasons (June and July) with the highest frequency of mid-day 'zero' and 'minus' low tides. During August to November, when such tides are non-existent, intertidal seaweed populations would be expected to increase steadily to a maximum. Indeed the seasonal standing crop variations of the above species of *Hypnea* show this predicted pattern[7]. DEWREEDE's[8] findings on the seasonal variations of *Sargassum* populations in Hawaii (showing maxima during November and December and minima in May to July) also seem to fit this predicted pattern. These results suggest that in our attempts to determine the causal factors for seasonal changes in seaweed populations in the tropics, tidal behaviour is one of the most important factors to be examined.

[7] K. E. MSHIGENI, Ph. D. dissertation, Univ. Hawaii (1974).
[8] R. E. DEWREEDE, Ph. D. dissertation, Univ. Hawaii (1973).

Nuclear Fusion and Irregular Cytokinesis in Binucleate and Tetraploid Cells of *Vicia faba* after Caffeine Treatment

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Summary. By means of 3 h treatment with 0.2% caffeine solution, binucleate and tetraploid cells were obtained in the lateral root meristem of *Vicia faba*. During recovery changing rates of fused interphases were noticed. Cell walls were formed in the equatorial plane of the preceeding division of binucleate and tetraploid cells at interphase and in the course of bimitosis or 4*n*-mitosis at prophase or metaphase; during bitelophase a constriction of the fused nuclei could be seen. The conclusion is that the basic requirements of cytokinesis are not affected by caffeine.

The effect of caffeine on cytokinesis[1] leads to binucleate cells that divide synchronously[2], or to tetraploid cells[3]. During division of binucleate cells, there may be formed tetraploid nuclei by fusions[3,4]. In the present paper, the further development of these cells is described.

Materials and methods. Vicia faba seedlings were grown in moist sand and, after cutting off the main root up to 3 cm, they were transferred into aerated (3 ml/sec) Hoagland's general solution No. 3 in permanent light at 25 ± 1 °C. Lateral roots were treated 3 h with 0.2% caffeine solution (DAB 7, Merck, Darmstadt) in distilled water and fixed at different times during recovery[3]. Bimitoses and 4 *n*-mitoses were studied at Feulgen stained squash preparations.

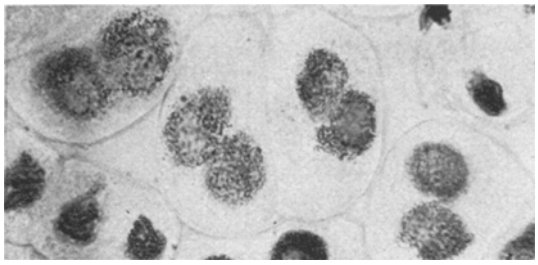

Fig. 1. Fusion stages of interphase nuclei. 2 h recovery. ×600.

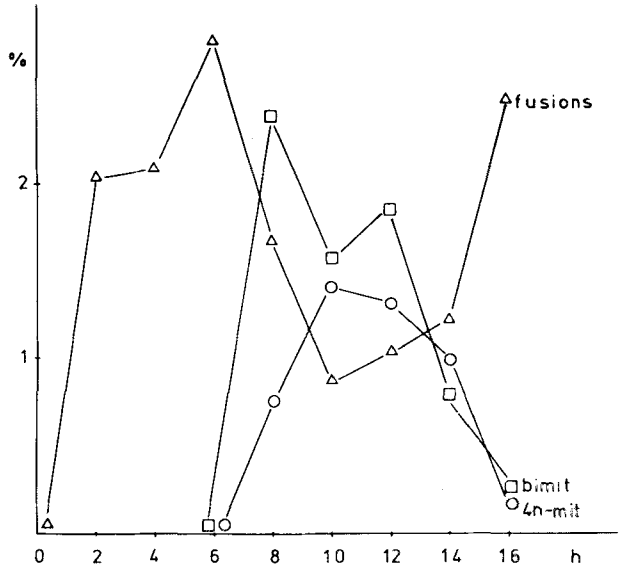

Fig. 2. Fusions, bimitoses and 4 *n*-mitoses dependent on time of recovery after 3 h caffeine treatment. Abscissa: h after the end of caffeine treatment.

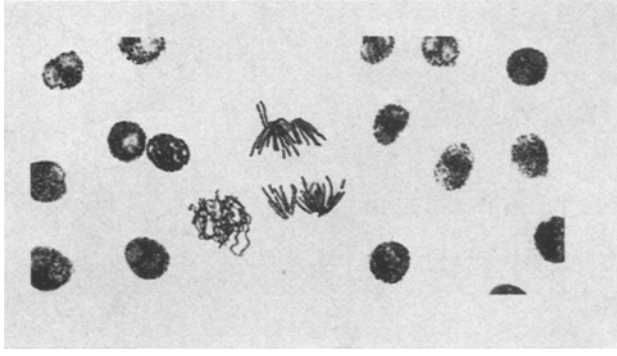

Fig. 3. Beginning fusion at bianaphase. 10 h recovery. ×350.

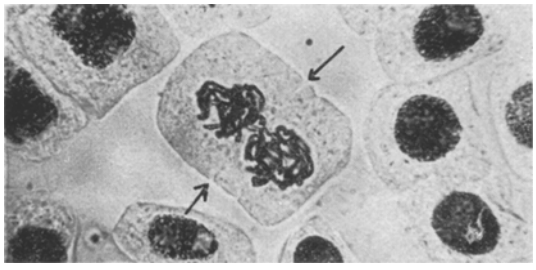

Fig. 4. Cell wall at bimetaphase. 10 h recovery. ×880.

Results and discussion. 2 h after the caffeine treatment the first fusions of interphase nuclei can be observed (Figure 1). After that there is a maximum at $t = 6$, a minimum at $t = 10$, then the percentage of fused interphase nuclei is increasing (Figure 2). There is a strong negative correlation between fused interphases and 4 *n*-mitoses, while there is no correlation between interphase fusions and bimitoses. Most of the tetraploid cells seem to have their origin in fused interphase nuclei, but there is no direct connexion between fused interphases and bimitoses. The increasing rate of fusions between $t = 14$ and $t = 16$ can be explained by nuclear fusions after parallel bimitosis[6]. The time interval between the appearance of corresponding fusion rates (e.g. 50% of the first maximum) in the slope of the graph is about 13 h and equivalent to the mitotic cycle time of *Vicia faba* under the given conditions[5]. The binucleate and tetraploid cells induced by the caffeine treatment enter mitosis about 8 h after the end of treatment[3]. In roots of *Allium*, González-Fernández et al.[6] distinguish between coaxial and parallel bimitoses. Both types were found in roots of *Vicia*, too, but no perpendicular bimitoses[6]. The further development of the cells is contingent on the position of the spindle axes. After the first division coaxial bimitoses form a $2n - 2n + 2n - 2n$ complex that may become $2n - 4n - 2n$ by fusion (cp. for *Allium*, González-Fernández et al.[6]). Parallel bimitoses may be regarded as tetraploid mitoses from the beginning, when the spindle axes are close enough to each other (Figure 3), or there may occur fusions at anaphase or telophase resulting in two $4n$-nuclei.

[1] B. Kihlman and A. Levan, Hereditas *35*, 109 (1949).
[2] G. Giménez-Martín, A. González-Fernández and J. F. López-Sáez, J. Cell Biol. *26*, 305 (1965).
[3] W. Röper, Arzneimittelforsch. (Drug Res.), in press.
[4] G. Giménez-Martín, J. F. López-Sáez, P. Moreno and A. González-Fernández, Chromosoma *25*, 282 (1968).
[5] W. Röper, Biol. Zbl., in press.
[6] A. González-Fernández, J. F. López-Sáez and G. Giménez-Martín, Expl Cell Res. *43*, 255 (1966).

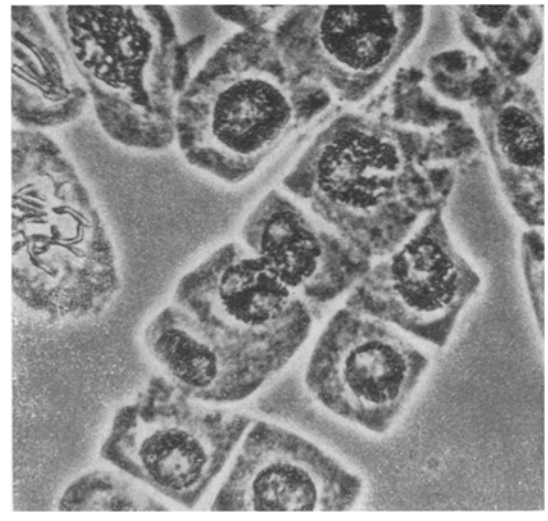

Fig. 5. Irregular cytokinesis at bitelophase after fusion. 11 h recovery. ×800.

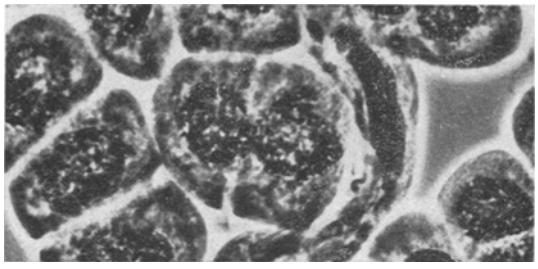

Fig. 6. Cell wall formation at 4 *n*-interphase (fused nuclei). 2 h recovery. ×830.

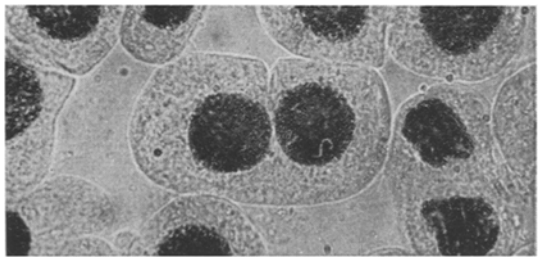

Fig. 7. Incomplete cell wall at bi-interphase. 6 h recovery. ×660.

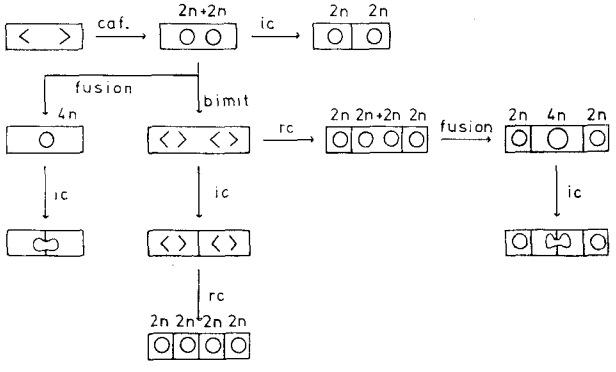

Fig. 8. Scheme showing the possibilities of development of binucleate cells. caf, caffeine treatment; bimit, bimitosis; ic, irregular cytokinesis; rc, regular cytokinesis.

In some cells, a cytokinesis could be stated as well between the nuclei of binucleate cells (Figure 4) and – in most cases – constricting the tetraploid nucleus of the $2n - 4n - 2n$ complex (Figure 5). This may even happen before bitelophase has finished. The cell wall was always formed in the equatorial plane of the preceeding division disturbed by caffeine. In tetraploid cells a beginning formation of a cell wall at interphase or during mitosis could be observed, too (Figure 6); as these nuclei are supposed to have their origin in a fusion of two nuclei at bi-interphase or biprophase, the constrictions happen in the former equatorial plane, too; in contrast to irregular cytokinesis at bitelophase no complete constrictions were observed in these cases. GONZÁLEZ-FERNÁNDEZ et al.[7] concluded from cytokinesis during prophase in roots treated with ethidium-bromide that cytokinesis is independent of RNA-synthesis, as soon as the nucleus has entered prophase. This means that the fundamental requirements of cytokinesis have been fulfilled during interphase. Caffeine inhibits the process of cytokinesis; on condition that it does no affect the basic requirements of cytokinesis, it would be possible that after subsidence of caffeine effects cytokinesis may be concluded during recovery at the original place, even leading to nuclear constrictions. In these cases the effect of caffeine may be regarded as delay. As cytokinesis was observed during biprophase and bimetaphase, during bi-interphase it seemed possible, too, and could be observed in a few cells (Figure 7). In all cases mentioned (Figure 8), the binucleate population would be decreased uncontrollably, and difficulties may arise in the determination of mitotic cycle time by means of caffeine treatment[2]. Further investigations will be carried out in order to study the mechanisms of cell wall formation in caffeine-treated cells.

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Distribution of Chlorophyllase Activity and Levels of Chlorophylls a and b in Sandal (*Santalum album* L.) Affected by Spike Disease

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Summary. Compared to healthy specimens, the levels of chlorophylls a and b and the activity of chlorophyllase towards the two pigments remained lower in the leaves of spiked sandal at all stages of leaf development, except in the senescing diseased leaves where the chlorophyllase activity showed a steep rise.

One of the characteristic symptoms in sandal affected by spike disease[1], caused by mycoplasma-like organisms (MLO)[2–4], is leaf chlorosis. A defective translocation of iron from the roots to aerial parts was earlier considered as a cause for the chlorosis in the spiked sandal[5]. In photosynthesis, the role of chlorophyll a is fundamental while that of chlorophyll b is of an accessory nature[6], and the level of chlorophyll in the tissue is dependent on chlorophyllase activity. In respect of the chlorosis in some virus-infected plants, increased hydrolysis of chlorophyll due to an increase in chlorophyllase activity[7–9], was considered as the main cause for chlorosis. Although the chlorophylls and chlorophyllase were investigated in these plants, the distribution of the activity of the enzyme towards the two pigments was not studied. Further, information on the nature of changes caused in the chlorophylls and chlorophyllase activity in plants infected by MLO is lacking. Hence a study of chlorophylls a and b and the distribution of chlorophyllase activity towards the two pigments in healthy and spiked sandal trees was