problem, we have to pay more attention to the algal part of the lichen than we were apt to do.

It was already a well-known fact that many lichenic acids consist of a lichenic acid s.s. (e.g. lecanoric acid) esterified with erythritol, which was already known as the metabolic product of the alga. Here we have an indication that some other substances as well may have an algal origin.

In some preliminary experiments we added apatococcin to cultures of some of our fungi on media poor in nutritive substances. No reaction was observed, the fungus being apparently unable to use this substance in its metabolism.

Conclusion.

The fungal symbionts in lichenized algal covers can be cultivated with more succes than true lichen fungi. Their great similarity to the latter makes it probable that they are related to certain true lichen fungi and that this alga-fungus symbiosis is comparable to the lichen symbiosis. In consequence they form an excellent object for the study of this symbiosis. The fungi are unable to fix atmospheric nitrogen. They cannot develop without aneurin, which they obtain, in nature, from their algal partner. In none of the cultures on various media, the presence of lichenic acids or similar products could be detected. On the contrary, it appeared that the alga Apatococcus is the producer of a remarkable metabolic product, called apatococcin, with the tentative formula $C_{23}H_{45}O_4N$. Some chemical properties of this substance are described. A relationship with certain lichenic acids is suggested. The investigation is continued.

I want to thank Prof. Dr. G. VAN ITERSON for his valuable help and for allowing me to make use of the unpublished work of VAN DE SANDE and Prof. Dr. F. KÖGL who kindly gave some critical remarks as to my work on the chemical constitution of apatococcin.

LITERATURE.

ASAHINA, Y., Fortschr. Chem. Org. Naturstoffe **2**, 27 (1939).
JAAG, O., Thèse Geneve. (1929).
MÖLLER, A., Thesis Münster i. W. (1887).
RAISTRICK, H., Ann. Rev. Biochem. **10**, 571 (1941).
SCHMID, G., Flora **128**, 211 (1933).
THOMAS, E. A., Beitr. Krypt. Flora. Schweiz. **9**, H. 1 (1939).
TOBLER, F., Ber. D. Bot. Ges. **27**, 421 (1909).
WERNER, R. G., Thèse Paris Mulhouse. (1927).

Botany. — *On the influence of Colchicin upon the anthers of Carthamus tinctorius L.* By Miss J. M. KRIJTHE (from the Laboratory of Genetics, Agricultural Institute, Wageningen). (Communicated by Prof. L. G. M. BAAS BECKING.)

(Communicated at the meeting of February 28, 1942.)

Although the literature on the influence of colchicin on living matter is voluminous, only a few papers deal with the effects of this substance upon flowers and inflorescences. Some of these papers only mention morphological characteristics such as pollen- or stomatal size, from which measurements often deductions are drawn as to tetra- or polyploidy of the material, often without cytological control.

Adequate cytological research has been published by LEVAN (1939), WALKER (1938), DERMEN (1938) and SATÔ (1939) — all on monocotyledons. The above authors followed — with minor variations — the following procedure; the entire inflorescence was treated for 5—6 days with a colchicin-solution of 0.1—1 %. Attention was almost exclusively directed towards changes in nuclear division, to wit: the absence of the spindle and chromosome-pairing (the chromosomes, however, dividing), with the subsequent absence of cell-division, by which absence abnormal large cells appear. These cells either show a large, tetraploid nucleus or several small nuclei.

This may be demonstrated not only with pollen grains, but also with unicellular staminal hairs. SATÔ mentions the appearance of irregular and incomplete cell-walls, without detailed description of their nature.

Material.

The present paper deals chiefly with phenomena observed in the inflorescences of the safflower (Carthamus tinctorius L.).

The safflower, a composite belonging to the Cynareae, appeared to be a favourable object because of its short vegetation-period (3—4 months), its profuse flowering (30 inflorescences per plant) and the relatively small number of chromosomes (haploid 12).

Method.

It was originally attempted to obtain tetraploid plants by the treatment of seeds and young seedlings with colchicin. As this proved to be unsuccessful (only two pairs of leaves developing subsequent to the treatment showing effects), young inflorescences (3—5 mm cross-section) were used.

The involucre was pushed aside by means of pincers, after which the cavity above the individual flowerets was filled with a colchicin-agar (0.4—0.8 % colchicin), or an aqueous solution of colchicin (10 drops aqueous 0.2 % solution) was applied for three consecutive days. Controls received 1 % agar, or water.

The involucre closed after treatment. The controls showed normal growth. The effects described seem, therefore, due to the colchicin applied.

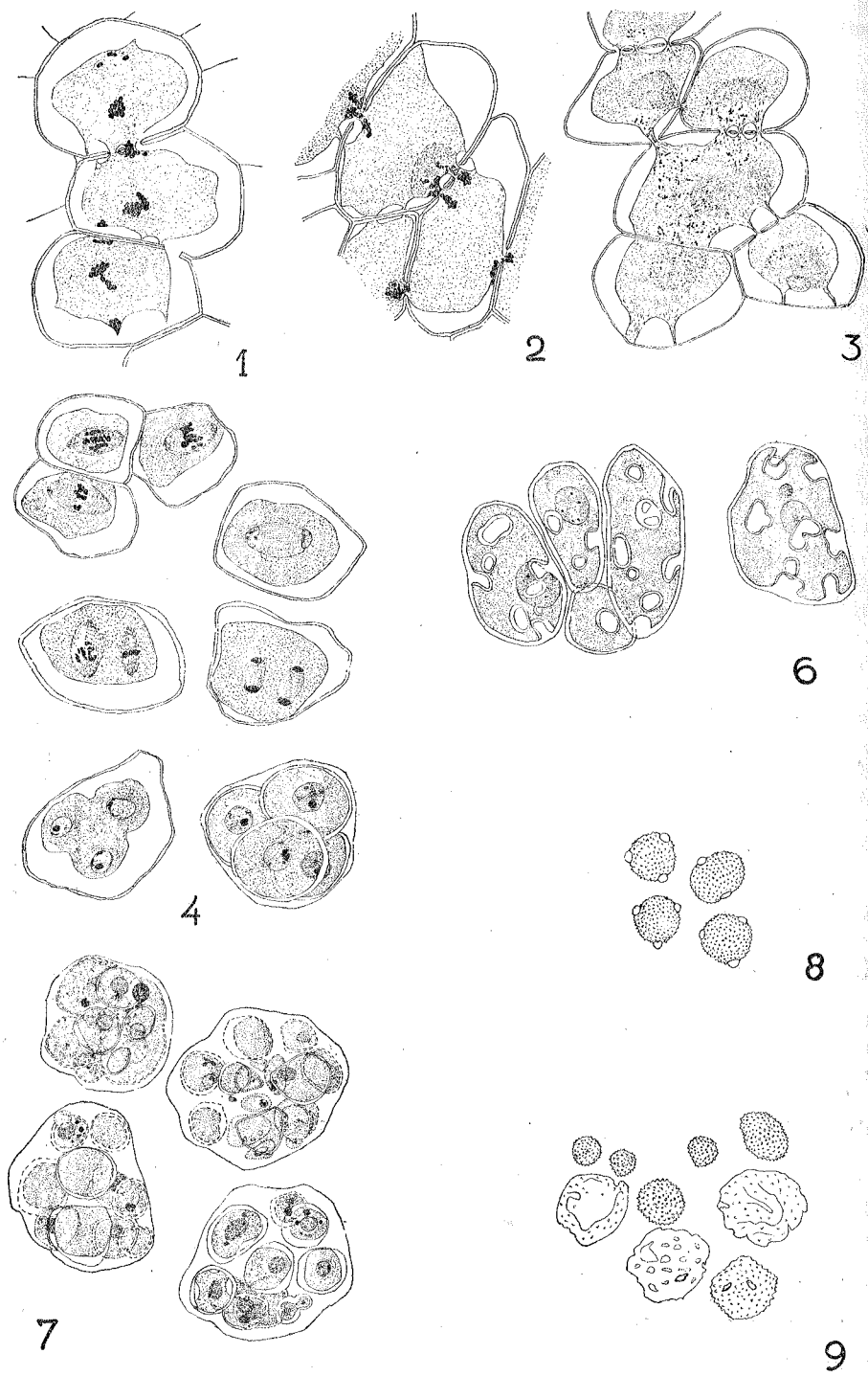
The treated inflorescences were enclosed for three days in parchment bags, to prevent dessication. The fixation of the flowerets took place either in NAVASHIN's or CARNOY's fluid, between 7.30 and 11.30 a.m. The sections were cut to a thickness of 10 μ and stained with HEIDENHAIN-haematoxylin or with gentian-violet.

Results.

1. *Morphological changes.*

Already after one week a broadening of the entire inflorescence could be observed.

The normal inflorescence is pyriform, the treated inflorescence showed a flat apex and a sudden transition towards the petiole. The tips of the involucral leaves curled inwards, instead of remaining in their vertical position.



LEGEND TO FIGURES.

Fig. 1. Archespore in division, chromosomes within protoplasm, partly in the plasmodesms. 500 ×.

„ 2. As fig. 1 chromosomes all in plasmodesms. 500 ×.

„ 3. Chromosome-fragments passing through openings in the wall. 500 ×.

„ 4. Normal pollen-formation. Four nuclei are present prior to wall-formation. Spindles apparent. 500 ×.

„ 5. Theca, longitudinal, showing plasmatic connections between pollen mother cells. Note lobed nuclei in tapetum. 500 ×.

„ 6. Pollen mother cells showing wall-intrusion. Protoplasm retracted from wall. 500 ×.

„ 7. Pollen mother cells showing various number of pollen grains. 500 ×.

„ 8. Normal pollen grains. 135 ×.

„ 9. Treated pollen grains. 135 ×.

Further development showed a progressive unfolding of the involucre, showing the flowerets at the base of the inflorescence. The untreated inflorescence remained covered. Treated flowers were 4—5 weeks late in bloom. Untreated flowers show the protrusion of the corollar tube from their involucre, while the pointed petals are in a horizontal position. The floweret reaches a length of 20—25 mm. The long slender gynaecium shows two yellow stigmata, densely covered by stiff hairs, pointing downwards. The pollen-grains are round, with four pores, bright yellow in colour and homogeneous in size. The yellow corolla becomes orange after flowering.

Treated flowers reach a length of ± 10 mm, do not protrude outside the involucre. The corollar tube is strongly wrinkled, while the petals are ribbon-shaped, with a blunt apex. The petals do not open during flowering. The contents of anthers seem partly desiccated and the short, heavy gynaecium carries a single, heavy, stigma, showing irrigular hairs, pointing in all directions. With the unaided eye the stigma shows a woolly effect. The pollen is irrigular in size, dark in colour and with a variable number of pores. No seed-formation takes place. Treated buds are shorter and are shaped as an inverted cone, while the controls show buds which are long and slender and of a conical shape. During the period of flowering treated flowerets are dark orange, much darker than controls after their period of flowering.

2. *Cytology.*

Dependent upon the stage of development the colchicin shows different effects. The division of the archespore is influenced in another way as the reduction-division. In all divisions the absence of a spindle, as observed by other authors, was apparent.

The chromatin seems scattered throughout the protoplasm in an arbitrary way. Sometimes the chromosomes are packed into dense clusters, some sections showed a number of small darkly stained granules; possibly chromosome-fragments. As far as could be ascertained, the number of chromosomes remained normal, while the resting nuclei showed no aberrant size. Controls show the normal scheme of division, the spindle being clearly visible. The occurrence of large, lobed nuclei in the tapetum was also observed in the controls and seems, therefore, to be a normal phenomenon.

The above phenomena are in harmony with the findings of other authors. The literature, however, seems silent on the following point; the influence of colchicin upon the cell wall.

The pollen mother cells show openings in their walls at the points of contact with neighbouring cells. Where the section was made centrally through such an opening, smaller or wider protoplasmic strands could be observed, connecting the plasma of the adjacent cells. Very young, not yet thickened walls already show these pores or pits, which are much wider than those known of the plasmodesms. In some cases all of the pollen mother cells over the entire length of the anther are joined by strands of protoplasm. Untreated anthers never showed these connections. Moreover, the shape of the wall in the neighbourhood of the pit and, the topography of the protoplasm in this region, showed that the structures are no artefacts.

Colchicin showed another influence upon the pollen mother cells already formed. Here the cells are often rounded, while the wall often grows to such dimension that hardly any lumen is left. The dimensions of the cells show them to be pollen-mother cells and not pollengrains. Apart from this phenomenon the cell wall may become thickened at localized spots, which seem scattered over the surface of the pollen mother cell. The substance which is deposited in the above cases seems to be callose. Reso-blue gave a beautiful colour, while no birefringence in polarized light could be observed.

The formation of these "wall-intrusions" seems to bear no connection to cell division. Their arbitrary distribution seems to corroborate this. In some cases in a single theca the mother cells formed apparently normal tetrads at one end, while at the other end the cells showed wall-intrusion.

Reduction division under the influence of colchicin shows that, instead of four pollen grains, 10—17 pollen grains appear from a single pollen mother cell. Most of the grains showed the presence of a nucleus, the smallest grains excepted. In these too little chromatin was probably present.

The "warty" appearance of the normal pollen is less evident in the "treated" grains. Pores are indicated in the larger ones, which are often furrowed. Pores seem to be entirely absent from the grains of normal and subnormal size.

Discussion.

Pollen-formation in Monocotyledons involves the formation of a wall after the heterotypic division (dyad) as well as after the homoiotypic division (tetrad). In the Dicotyledons pollen formation is simultaneous. After the termination of the entire reduction division, the four nuclei are situated at the apices of a tetraedron, the wall is formed by infolding of the wall of the pollen mother cell. In the Monocotyledons the reaction is nuclear, in the Dicotyledons it is plasmatic.

The difference in reaction between Carthamus and Allium (LEVAN) might be ascribed to the above facts. The formation of pollen mother cells in Carthamus with irregular wall-intrusions might be a link in the process, terminating in the formation of supernumerary pollen grains.

In regard to the formation of the cell-wall intrusion many instances are known where

such abnormal phenomena occur. Orchid mycorrhiza causes abnormal thickening of the walls of the host-cells (BURGEFF, 1932).

In old algal cultures wall-thickening has been observed (KÜSTER, 1935). MICHAELIS (1926) obtained supernumerary pollen grains from pollen mother cells by cold-shock (of 20° C). Complete walls were always formed, however, in this case.

Structures, analogous to those observed in Carthamus anthers are, of course, sieve tubes and storage cells in endosperms. In these cases, however, the plasmodesms are much narrower. Vascular wound tissue (TIMMEL, 1927) and centrifuged Spirogya filaments (VAN WISSELINGH, 1903, 1909) show incomplete walls.

A striking resemblance of the structures observed with the intercellular plasmatic connections of Rhodophyceae (JUNGERS, 1933) cannot be denied.

Only a few references were found in the literature pertaining to the influence of chemicals upon wall-formation. NĚMEC (1904) observed, in the roots of Vicia faba, incomplete wall-formation with concomitant absence of the spindle after treatment with chloral-hydrate. NAVASHIN (1938) treated seeds of various plants with sublimated acenapthene. He observed the formation of new cell-walls within the old wall, dividing the cell into smaller cells, some of them anucleate. As acenapthene seems to assert a similar (although weaker) influence as colchicin upon plant-cells, this result seems interesting.

SUMMARY.

Young inflorescences of Carthamus tinctorius L. were treated for three consecutive days with colchicin-agar (0.4—0.8 % colchicin) or a solution of colchicin 0.2 %. Dependent upon the stage of development of the cells the reaction was different. In all stages of the development of the archespore the spindle remained absent. Pollen mother cells during their formation communicated by means of protoplasmic strands running through large openings in the walls. Mature pollen mother cells showed, at arbitrary places of their walls, callose intrusions. By these intrusions the mothercells are divided into 10—17 pollen grains.

LITERATURE.

BURGEFF, H., 1932. Saprophytismus und Symbiose. Jena.

DERMEN, H., 1938. A cytological analysis of polypoidy induced by colchicine and by extremes of temperature. Journ. of Hered. **29**, 6, 211.

JUNGERS, V., 1933. Recherches sur les plasmodesmes chez les végétaux II. Les synapsis des algues rouges. La Cellule **42**, 7.

KÜSTER, E., 1925. Pathologische Pflanzenanatomie 3. Aufl. Jena.

————, 1935. Die Pflanzenzelle. Jena.

LEVAN, A., 1939. The effect of colchicine on meiosis in Allium. Hereditas **25**, 1, 9.

MICHAELIS, P., 1926. Ueber den Einfluss der Kälte auf die Reduktionsteilung von Epilobium. Planta **1**, 5, 569.

NAVASHIN, M., 1938. Influence of acenapthene on the division of cells and nuclei. Compt. Rend. (Doklady) Acad. Sci. U.R.S.S. **19**, 193.

NĚMEC, B., 1904. Ueber die Einwirkung des Chloralhydrates auf die Kern- und Zellteilung. Jahrb. f. wiss. Bot. **39**, 645.

SATÔ, D., 1939. The effect of colchicine on meiosis in Aloinae. Bot. Mag. Tokyo **53**, (629) 200.

TIMMEL, H., 1927. Ueber die Bildung anomaler Tracheïden im Phloem. Flora **122**, 203.

WALKER, RUTH I., 1938. The effect of colchicine on microspore-mothercells and microspores of Tradescantia paludosa. Amer. Journ. Bot. **25**, 4, 280.

WISSELINGH, C. VAN, 1903. Ueber abnormale Kernteilung. 5. Beitrag zur Kenntnis der Karyokinese. Bot. Zeit. **61**, 201.

————, 1909. Zur Physiologie der Spirogyra-Zelle. Beih. Bot. Zbl. **24**, Abt. I, 133.