ACTA AGROBOTANICA
Vol. XXIX, (1): 5-14, 1976

Influence of morphactin IT 3456 on the growth and anatomic structure of *Vicia faba* L. (*Faba vulgaris* Mnch.), variety 'Hangdown'*

MARIAN SMOLIŃSKI

Department of Plant Cytology and Cytochemistry, Institute of Plant Physiology and Cytology, University of Łódź, Poland

(Received: February 12, 1975)

Abstract

Plants growing from seeds treated with morphactin were small and changed, they did not flower. The apical buds of some seedlings died back. Disturbances of geotropism were observed in the shoots and roots. Inhibition of elongation growth was durable, whereas in the roots it lasted 7-10 days and their radial growth was distinctly stimulated in this period. The leaves reduced in size and deformed contained more stomata. The epidermis and mesophyll cells were reduced in size. The cambium did not form a closed ring. Primary and secondary xylem contained more vascular bundles than control plants. The mitotic activity of the pericycle cells was higher in the roots than in the shoots.

INTRODUCTION

In the foregoing and present decade the wide interest has been aroused by the group of growth regulators produced in the Merck Laboratories, West Germany. These compounds are fluorene derivatives. They have a modifying influence on the morphogenesis, therefore they have been called morphactins. The action of morphactins on the developmental processes in plants is rapid: they stimulate the development of lateral buds (branching of plants) and they cause dwarfism (Schneider 1964, 1970; Schneider et al. 1965; Mohr et al. 1967; Pieniążek and Saniewski 1969).

From among this group of growth regulators of particular interest is morphactin IT 3456 being a mixture of about 80 per cent of 2-chloro--9-hydroxyfluorene-(9)-carboxylic acid methyl ester and 20 per cent

* This work wos partly supported by Polish Academy of Sciences, Grant PAN-24.

9-hydroxyfluorene-(9)-carboxylic acid methyl ester and a small amount of 2,7-dichloro-9-hydroxyfluorene-(9)-carboxylic acid methyl ester (Mohr et al., 1967; Zalewska and Saniewski 1968).

The present paper is the continuation of work on the influence of morphactin on the activity of the cambium cells and on the differentiation of cells originating from the cambium.

MATERIAL AND METHODS

As material for the present studies served the young bean plants developed from seeds soaked for 24 h in an aqueous solution of morphactin IT 3456 of 5 or 30 ppm concentration and growing in pots. Moreover, plants were cultured, from seeds treated with morphactin, on garden beds. Anatomical observations were performed on longitudinal and cross sections of shoots and roots. Material macerated according to Jeffrey (Johansen 1940) was also used. The sections were stained with a mixture of acid fuchsin and malachite green. The presence of lignin in the cell walls was additionally checked by means of floroglucine with hydrochloric acid and the zinc chloriodine. The drawings were made with the use of a drawing apparatus. Germination energy was examined in 100 seeds soaked in an aqueous morphactin solution of 30 ppm concentration. The seeds were germinated in 10 separate samples each of them consisted of 10 seeds.

RESULTS

Morphological observations

Bean seeds soaked in aqueous solution of morphactin of 30 ppm concentration and these in distilled water (control showed a similar course of germination (Plate II — 3). Morphactin in the concentrations applied did not affect the germination (table 1).

The plants developing from seeds treated with morphactin showed a considerable inhibition of elongation growth. Their shoots were cro-

Table 1

Influence of morphactin on germination viability of field bean seeds

Number of seeds	Arithmetic means	
	Distilled water	Morphactin (30 ppm)
100	12.3±2.2	10.7±1.8

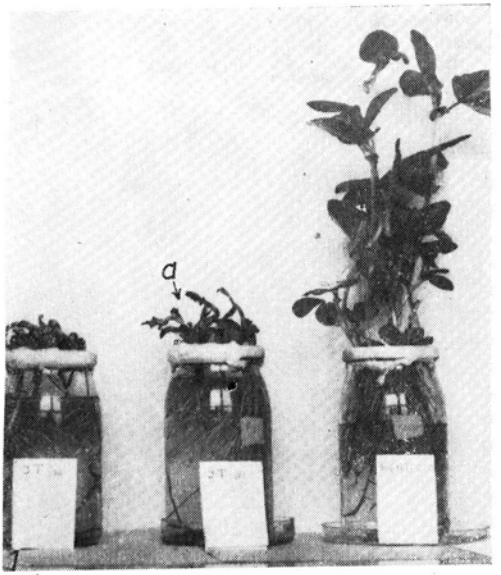
oked and the side buds in the lower nodes mostly developed into shoots with one elongated internode (Plate I — 1). The leaves of lateral shoots were reduced more than those of the main ones. The main and lateral shoots showed a more or less pronounced positive geotropism, whereas the roots — negative geotropism. The plants were dwarfed owing to the smaller number of internodes and their reduced length as compared with the controls. Elongation growth of the higher situated internodes was more inhibited than that of the lower ones. The lowest internodes (1-2) were of the same length or longer than those in the controls. The shoot apexes died in some of the seedlings. The lowest internodes (1-3) of the shoots, the hypocotyls and the upper segments of the roots, about 3 cm below the hypocotyl were thicker than those in control plants During the development of plants from seeds treated with morphactin solution of 5 or 30 ppm concentrations no distinct morphological differences were observed (Plate I — *1*). The leaves of the treated plants had smaller blades and were slightly creased (Plate I — *3b*). The plants in pots and on garden beds did not develop flower buds. The elongation growth inhibition of the shoots increased gradually during the experiment. The influence of morphactin IT 3456 was different as regards the root morphology. After germination the elongation growth of roots was weaker than that of control roots (Plate I — *2*). The inhibition of root growth was associated with their thickening. On the thickened segment of the main root no lateral roots appeared. The primary cortex showed longitudinal fissures. The inhibition of elongation growth of the roots was no more observed after 7-10 days, and the roots grew further like those of the control plants, forming the lateral roots. The root system of plants treated with morphactin was less developed (Plate I — *1*). The roots of plants growing on beds in the garden lacked root nodules or had but few, while the nodules appeared aboudantly on the roots of control plants.

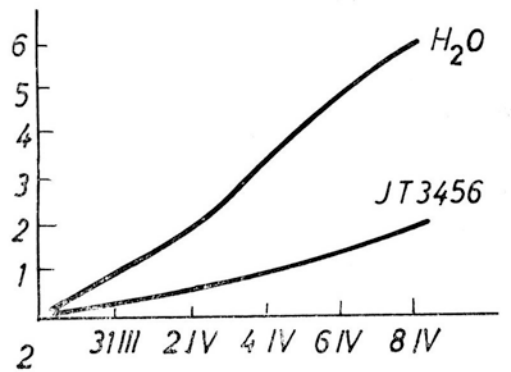
Anatomical structure

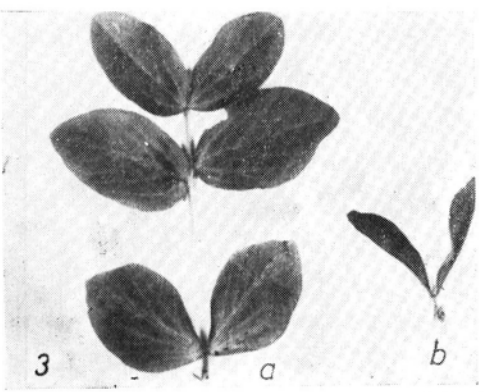
Shoot. The action of morphactin was expressed also in the anatomical structure. Figs *1* and *2* in Plate II show the structure of cross sections of shoots of control plants and those treated with morphactin in 5 ppm concentration. The drawings were made from the sections in the middle of the 2nd internode from the plant base. The anatomical structure of shoots from plants treated with morphactin in a 30 ppm concentration was similar to that shown in Plate II — 2. The control plants had a structure corresponding to that generally accepted (Metcalfe and Chalk 1950; Kaussmann 1963). The bean belongs to the group of plants characterizing a structure of *Helianthus* type (Kaussmann 1963).

In the shoots of bean plants exposed to the action of morphactin IT 3456 the changes were noticeable in the primary cortex cells and in the central cylinder. The cortex in cross sections was thicker than in the shoots of control plants (Plate II — *1, 2*). The higher volume of primary cortex was due to the larger dimensions and increasing number of cells between the epidermis and endodermis. The changes in the size and number of cells mostly involved the areas in the corners of the shoots (Plate III — *1, 2*). The vascular bundles and islets of sclerenchyma cells occurring in the primary cortex of the shoots were also larger than in the control plants at the corresponding levels (Plate III — *3, 4g*). Cambium in the vascular bundles situated in the primary cortex of plants treated with morphactin developed circularly (Plate III — *3, 4g*). The surface area of the root cylinder of shoots treated with morphactin was also higher than in shoots of control plants (Plate II — *1, 2*). The increase of the central cylinder was mainly due to a more intensive development of the vascular bundles and their larger number (Plate III — *3, 4d*; Plate IV — *1, 2d*). The cambium in the shoots did not form a closed ring like in the control plants. (Plate II — *1, 2f*; Plate III — *3, 4f*). In the plants treated with morphactin it developed in the form of separate arcs or rings encompassing the phloem of the vascular bundle (Plate III — *3, 4f*; Plate IV — *1, 2f*). The vessels differentiated in the vascular bundles next to the phloem. In this way the collateral vascular bundles resembled the concentric ones with phloem in the middle (Plate IV — *1, 2f*). In the primary and secondary xylem of the plants treated with morphactin mainly vessels differentiated forming compact tissues (Plate III — *3, 4d*). Besides, the vessels were shorter and narrower than those of the control plants (Plate IV — *3, 4*). The vessel walls showed net-like thickenings. The vessels of the secondary growth in shoots of plants treated with morphactin were more reduced than those in the primary xylem of the same plant. The parenchyma cells in the xylem of the vascular bundles of plants treated with morphactin formed groups, whereas in the xylem of control plants they appeared as single paratracheal and matetracheal cells. Moreover, the pericycle cells and those of the parenchyma bordering the pericycle became mitotically active. Additionally in the newly formed cell zone, cambium appeared fragmentarily. Vessels also differentiated here, still shorter than those in secondary growth of the vascular bundles (Plate III — *4j*). Phloem consisted of cells with dense contents and an elongated nucleus.

Lignification of the cell walls in the primary structure of plants treated with morphactin and of the controls did not differ as shown by the intensity of their staining. In the secondary growth of vascular bundles in plants treated with morphactin the groups of xylem cells with walls of various staining intensity were observed whereas in the secondary







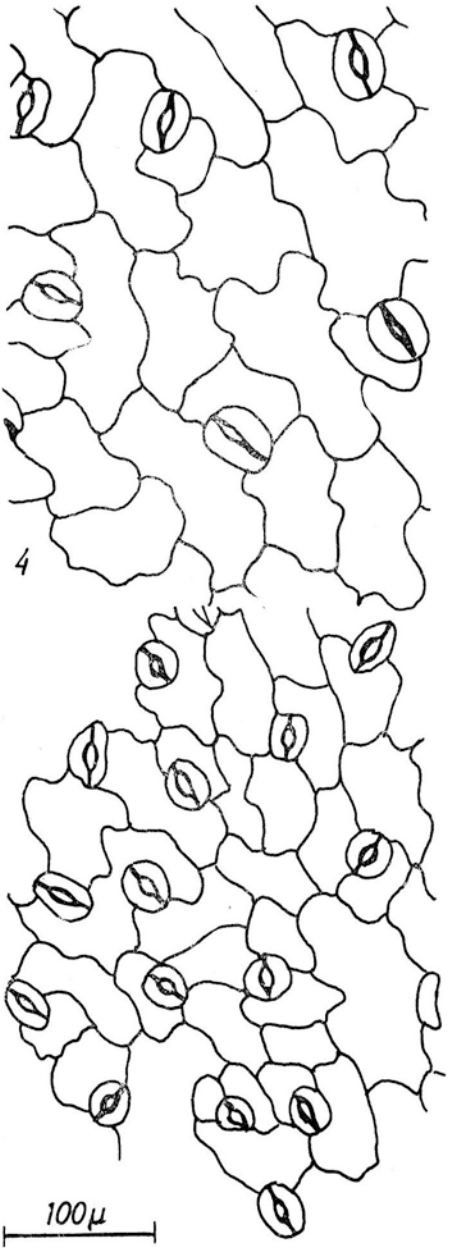


Plate I

1 — field bean control plants and plants treated with morphactin, *2* — elongation growth of roots of control plants and those treated with morphactin, *3a* — leaves of control plants, *3b* — leaves of plants treated with morphactin, *4* — epidermis from lower side of leaf of control plant, *5* — epidermis of lower side of leaf after treatment of plant with morphactin

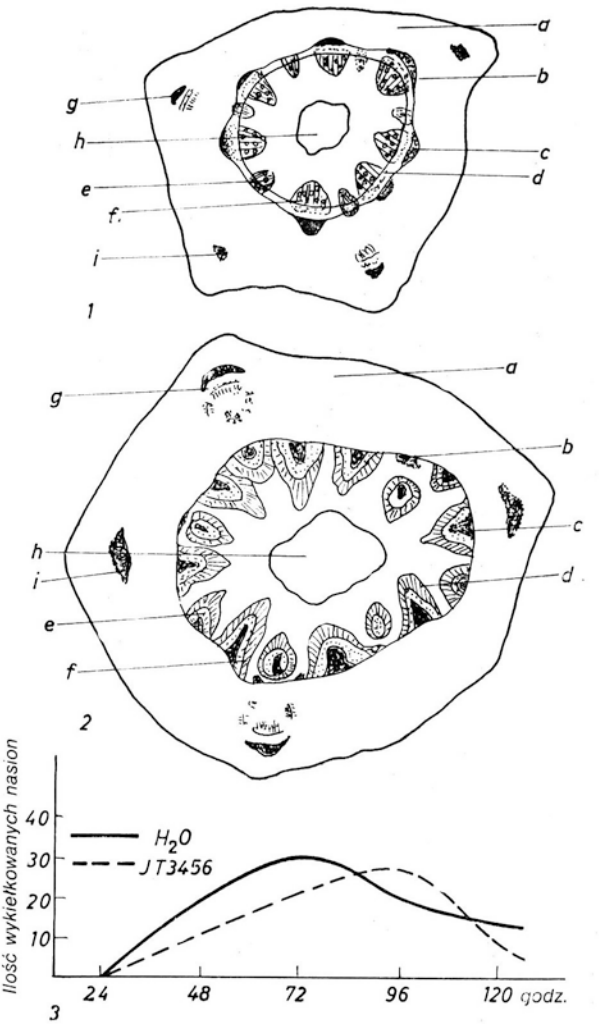


Plate II

1, *2* — cross sections from middle of 2nd internode of control plant shoot and plant treated with morphactin, *3* — germination of field bean seeds: treated with distilled water and with morphactin *a* — primary cortex, *b* — endoderm, *c* — fibres over phloem, *d* — xylem, *e* — phloem, *f* — cambium, *g* — vascular budle in primary cortex.

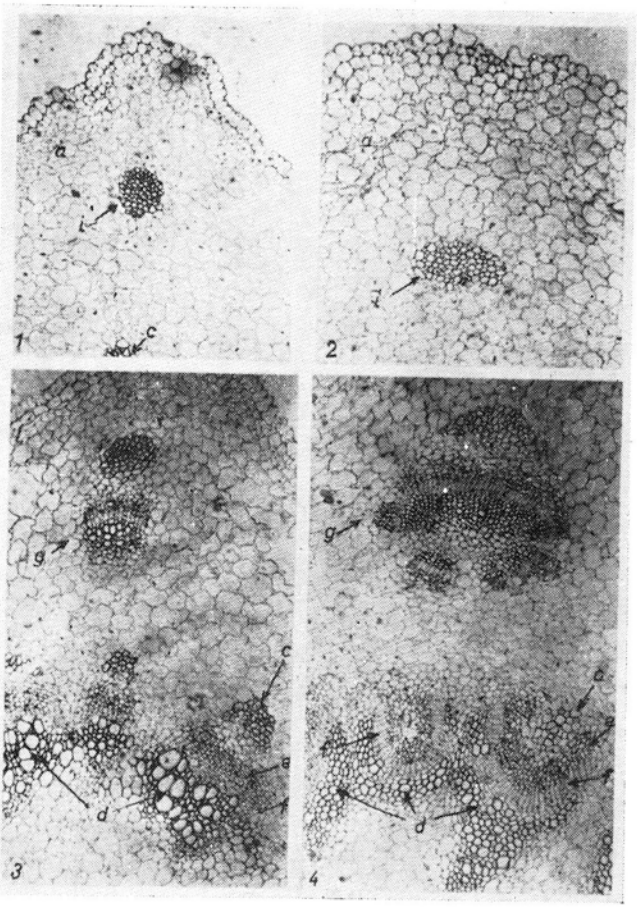
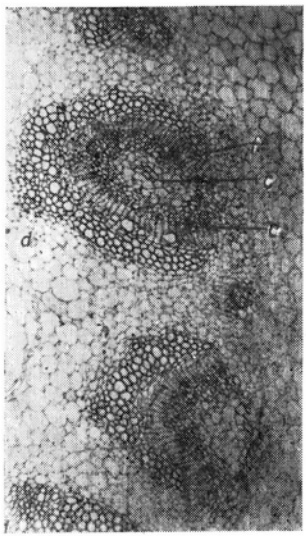
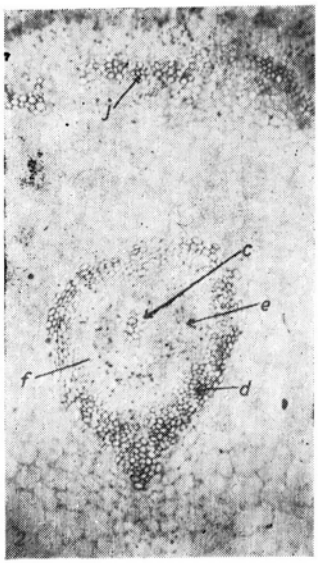


Plate III

1 — primary cortex in shoots of control plants at the level shown in Plate II, *1*; *2* — primary cortex in shoots of plants treated with morphactin at the level of Plate II. *2* (×70); *3* — fragment of primary cortex and central cylinder of shoots of control plants at the level of Plate II, *1*; *4* — analogous fragment of shoot of plant treated with morphactin at level of Plate II, *2* (×70), *a* — primary cortex parenchyma, *c* — fibres over phloem, *d* — xylem, *e* — phloem, *f* — cambial zone, *g* — vascular bundles in primary cortex





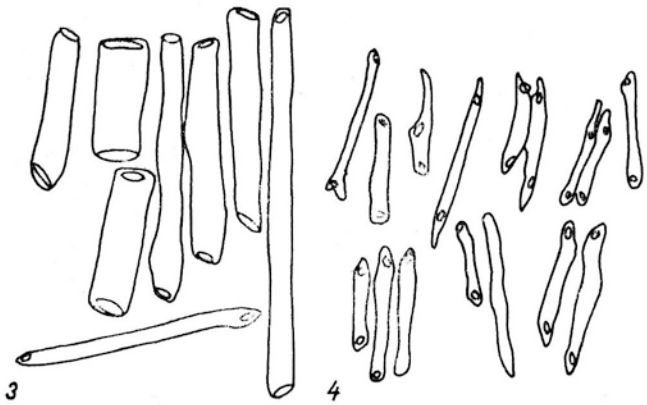


Plate IV

1, *2* — vascular bundles in shoots of plants treated with morphactin after 5 weeks of growth, *c* — fibres over phloem, *d* — xylem, *e* — phloem, *f* — cambium, *j* — differentiated vessels in pericyclic zone (1 — ×70, 2 — ×24), *3* — —vessels in xylem of control plants, *4* — vessels in xylem of plants treated with morphactin

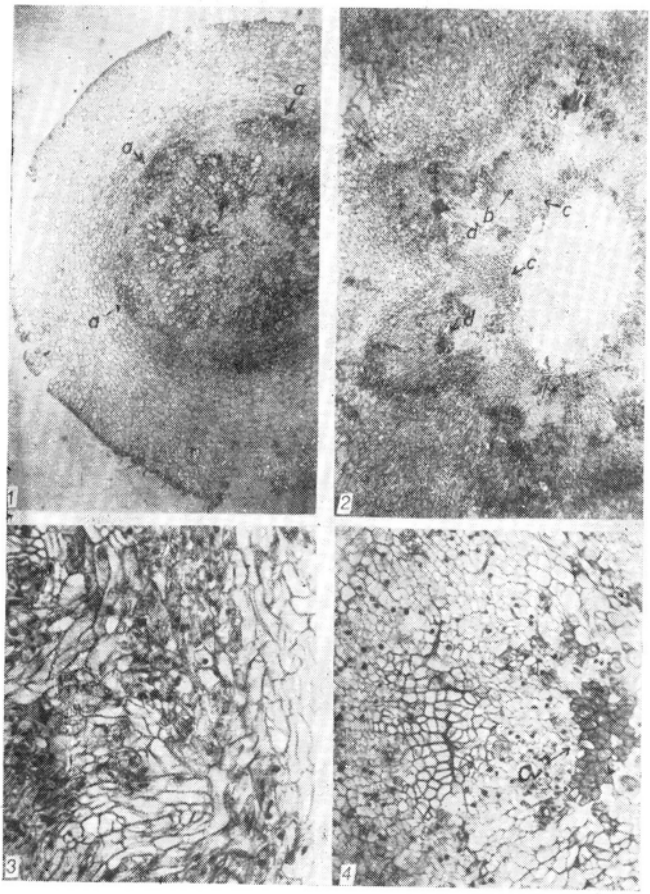
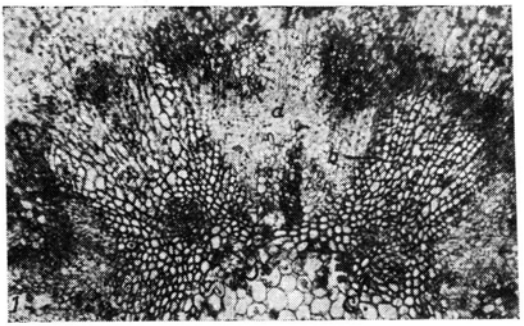


Plate V

1 — cross section of root of control plant, *2* — root of plant treated with morphactin, *a* — bast fibre, *c* — primary xylem, *b* — secondary xylem, *d* — cells of cambium between bundles of primary xylem (×24), *3* — longitudinal section through zone shown in photo *4*, *4* — cross section through zone formed as the result of mitotic activity of pericycle cells (×130)



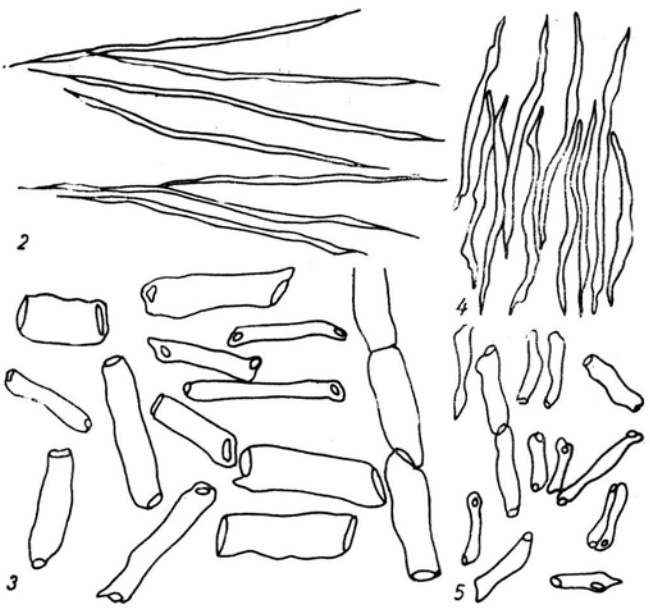


Plate VI

1 — cross section through xylem in roots of plants treated with morphactin (×70); macerated elements of root xylem: control sclerenchyma cells (*2*), vessels (*3*), sclerenchyma cells of plants treated with morphactin (*4*), vessels (*5*)

xylem of the control plants the cell walls stained uniformly. The differences in staining intensity of the cell walls suggest that this may, probably, be caused by a various lignin content in their walls.

Leaves. The surface area of leaves of plants grown from seeds treated with morphactin was reduced (Plate I — *3a,b*). The upper and lower epidermis of the leaves consisted of smaller cells and contained more stomata than did the leaves of control plants (Plate I — *4, 5*). The number of stomata per 1 mm² in the leaf epidermis of plants treated with morphactin and of the control ones was:

$$\text{upper epidermis} \quad \frac{40 \text{ — control}}{55 \text{ — after morphactin}}$$

$$\text{lower epidermis} \quad \frac{56 \text{ — control}}{109 \text{ — after morphactin}}$$

The mean number of hairs per 1 mm² was calculated, in the upper epidermis it was

$$\frac{14.5 \text{ — control,}}{29.9 \text{ — after morphactin.}}$$

The blade thickness was also reduced. Measurements showed:

$$\frac{483\ \mu\text{m — control}}{299\ \mu\text{m — after morphactin}}$$

The reduced thickness leaf blade resulted from the smaller dimensions of the palisade and spongy parenchyma cells.

Roots. The roots of the bean seedlings were tetra- and pentarchic in the thicgened zone of plants treated with morphactin (Plate V — *1, 2*). In this zone the primary cortex and the root cylinder were wider (Plate V — *1, 2*). The large volume of the primary cortex resulted from the increased volume of the cells and their larger number. Cambium formed in the roots a closed ring. It was most active at the protoxylem where a large number of vessels differentiated, forming a compact tissue but little differentiated as regards the diameter in the cross section (Plate VI — *1b*). The vessels of the secondary growth segment in the roots of plants treated with morphactin were shorter and narrower than those in the secondary xylem of control plants (Plate VI — *3, 5*). The cambium forming on the inner side of the phloem bundles was less active than over the protoxylem. Mainly parenchyma, some few vessels and sclerenchyma cells (Plate V — *1, 2d*) were found here. Cambium activity and differentiation of vessels in the xylem was opposite to that in the roots of control plants. Lignification of cell walls in the segment of secondary growth of xylem was nonuniform. Beside a group of cells with walls well saturated with lignin, others were poorly lignified, as

indicated by the intensity of wall staining by the reagents mentioned earlier. Sclerenchyma cells were also shorter than in the xylem of control plants (Plate VI — *2, 4*).

The pericycle cells at the sclerenchyma enclaves showed a high mitotic activity (Plate V — *2, 4a*). In the newly formed cell zone the vessels differentiated rather profusely. They were short, frequently deformed and net-like thickenings could be seen on their valls. These vessels ran in various directions (Plate III) and wall lignification was more nonuniform than in the xylem. Many cells showed very poor lignification.

DISCUSSION

The results of the present investigations indicate that morphactin IT 3456 applied to seeds produces marked changes in the development of seedlings. The lasting action of morphactin which did not subside in the course of the experiments and the greater modification in the morphological development of shoots than of roots suggest that morphactin is probably accumulated in the above-ground organs. This conclusion is confirmed by data from the literature: namely acropetal transport prevails in young seedlings (Schneider 1969) and translocation and accumulation of ^{14}C-morphactin in mitotically active meristem (Erdmann et al. 1967) is observed. The increase of morphactin content may be the cause of its more durable and deforming action. Such changes and the decrease of the number of internodes in the shoots, inhibition of their elongation growth, more pronounced towards the apex, dying back of the apical buds in some seedlings may be due to the accumulation of morphactin in the upper shoot zones.

Translocation of morphactin in acropetal direction would result in its diminished content in the roots so that their normal growth would be almost restored.

In bean seedlings growing from seeds treated with morphactin the lateral buds on the lower parts of the plant developed into short thickened shoots. It is known that development of lateral buds occurs when the dominance of the apex is weakened or abolished (Thiman 1937, 1939; Maciejewska-Potapczyk 1967). The mechanism of apical domination has not been so far elucidated. As result from the studies of Thimann (1937, 1939) auxin inhibits the growth of lateral buds and this effect is due to the concentration of the hormone flowing downwards from the apical bud. Schneider (1969) and Krelle and Libbert (1968) demonstrated that morphactin inhibits translocation of auxins along the vertical axis as well as in transversal direction. The apical domination in field bean seedlings is probably weakened by disturbances

in auxin transport due to morphactin. It cannot be ruled out, however, that abolition of the apical dominance might occur in some other way — by inhibition of synthesis or auxin inactivation.

The positive geotropism of shoots and negative of roots may also result from the disturbances in auxin distribution. It is known from the literature that geotropic and phototropic bending are attributed to different distribution and concentration of auxins in the plant organs.

The accelerated thickening of the lower shoot internodes, hypocotyls and roots (segments immediately below the hypocotyls) was caused by the enhanced cambial activity, by the mitotic activity of the pericycle cells and in some slight degree by hypertrophy of primary cortex cells. Similar results were also observed in other dicotyledonous plants (Saniewski et al. 1968; Smoliński et al. 1969). It is known at present that the mitotic activity of cambium and the cell differentiation are controlled by growth substances among which auxin plays a key role (Digby and Wareing 1966; Pieniążek and Saniewski 1968 a,b; Brown and Cormack 1937, quoted after Morey and Cronshaw 1968). In earlier studies interaction of morphactin with cytokinin and auxin was observed (Pieniążek and Saniewski 1968a, b; Smoliński et al. 1969, 1973). The stimulating influence of these substances on cambium and cell differentiation was particularly pronounced when the plants were simultaneously treated with morphactin, auxin and cytokinin (Pieniążek et al. 1970; Smoliński et al., unpublished). Thus, the interaction of morphactin with endogenous growth regulators, the disturbances in auxin distribution (Schneider 1969, 1970; Krelle and Libbert 1968) and accumulation of morphactin in active meristem may be the cause of abnormal cambium development, its nonuniform mitotic activity and quantitative and qualitative changes in the developing cells.

Similarly, the increased number of stomata and hairs in leaves of plants treated with morphactin may be due to the above named causes.

The lack of root nodules or their low number on the roots of seedlings from seeds treated with morphactin is probably the immediate result of the inhibitory effect of morphactin on bacterial development. It is known that bacteria produce organic compounds from carbohydrates obtained from higher plants. Thus the carbohydrate level in the plant influences the development of the root nodules. In view of the conciderable reduction of all above-ground organs it would seem that plants exposed to the action of morphactin are poorer in carbohydrates, this causing a weak development of bacterial symbiosis and reduction of the nodules.

The authors is most grateful to Professor M. Olszewska and Professor E. Mikulska for discussing the results and valuable advice.

REFERENCES

Digby J., Wareing P. F., 1966. The effect of applied growth hormones on cambial division and differentiation of the cambial derivatives Ann. Bot. N. S. 30: 539-548.

Erdmann D., Mohr G., Schneider G., 1967. Untersuchungen mit einem ^{14}C-markierten Morphactin (Fluorenol). Vortrag VI Inter. Pflanzenschutzkongr. Wien 1966. Marck-Bl. 17: 11-20.

Kaussmann B., 1963. Pflanzenanatomie unter besonderer Berüchsichtigung der Kultur-und Nutzpflanzen. VEB Gustv Fischer Verlag. Jena.

Krelle E., Libbert E., 1968. Inhibition of the polar auksin transport by a morphactin Planta 80: 317-320.

Johansen D. A., 1940. Plant microtechnique. McGraw — Hill Book Company. New Jork a. London.

Maciejewska-Potapczyk W., 1967. Substancje wzrostowe. PWRiL. Warszawa.

Metcalfe C. R., Chalk L., 1950. Anatomy of the Dicotyledons. Oxford. At the Clarendon Press.

Mohr G., Erdmann D., Scheinder G., 1967. Morphaktine eine neue Klasse von Pflanzenwachstumregulatoren. Vortr. VI. Intern. Pflanzenschutzkongres. Wien 1960. Marck — Bl. 17: 1-9.

Morey P. R., Cronshaw J., 1968. The effect of plant growth substances on the development of tension wood in horizontally inclined stems of *Acer rubrum* seedlings. Protoplasma 65: 379-391.

Fieniążek J., Saniewski M., 1968a. The synergistic effect of benzyladenine and morphaction on cambial activity in apple shoots. Bull. Acad Polon. Sci. Ser. Biol. 16: 381-384

Pieniążek J., Saniewski M., 1968b. Hormone control of cambial activity and xylem differentiation in apple shoots. Acta Agrobotanica 21: 113-129.

Fieniążek J., Saniewski M., 1969. Nowe dane o morfaktynach i możliwościach ich zastosowania w rolnictwie i ogrodnictwie. Postępy Nauk Rolniczych 3/4 (117).

Pieniążek J., Smoliński M., Saniewski M., 1970. Induced structural changes in anatomy of apple shoots after treatment with morphactin (IT 3456) and other growth regulators (NAA, GA, BA). Acta Agrobotanica 23: 387-396.

Saniewski M., Smoliński M., Pieniążek J., 1968. The effect of morphactin on the anatomical structures of *Pisum sativum* L. and *Dolichos lablab* L. roots Bull. Acad. Polon. Sci. Ser. Sci. Biol. 16: 513-515.

Schneider G., 1964. Eine neue Gruppe von synthetischen Pflanzenwachstumregulatoren. Naturwissenschaften 51: 46-417.

Schneider G., Erdmann D., Lust S., Mohr S., Niethammer K., 1965. Morphactins a novel group of plant growth regulators. Nature 208: 1013.

Schneider G., 1969. Morphaktine, Wirkung auf Entvicklung und Wachstum von höheren Pflanzen. Dtsch. Bot. Ges. Neue Folge 3: 19-31.

Schneider G., 1970. Morphactins: Physiology and Performance Ann. Rev. Plant Physiol. 21: 499-536.

Smoliński M., Saniewski M., Pieniążek J., 1969. The changes in anatomical structures in *Pisum sativum* L. seedlings after treatment with morphactin (IT 3456) and benzyladenine. Acta Soc. Bot. Polon. 38: 303-308.

Smoliński M., Saniewski M., Pieniążek J., 1973. The effect of growth

regulators on cambial activity and xylem differentiation in shoots *Sorbus aucuparia* L. (praca nie publikowana).

Smoliński M., Saniewski M., Pieniążek J., 1973. Morphological and anatomical changes in the roots of apple seedlings treated with morphactin (IT 3456) and a-naphtalene acetic acid NAA. Biologia Plantarum (Praha) 16 (3): 227-229.

Thimann K. V., 1937. On the nature of inhibitions caused by auxin Amer. Jour. Bot. 24: 407-412:

Thimann K. V., 1939. Auxin and the inhibition of plant growth. Biol. Rev. 14: 314-337.

Zalewska J., Saniewski M., 1968. Wpływ morfaktyn na aktywność enzymów związanych z utlenianiem kwasu 3-indolilooctowego (IAA) w siewkach grochu. Zesz. Nauk. U. Łódzkiego, S. II, 30: 101-108.

Wpływ morfaktyny IT 3456 na wzrost i budowę anatomiczną *Vicia faba* L. (*Faba vulgaris* Mnch.) odm. 'Hangdown'

Streszczenie

Zbadano wpływ morfaktyny IT 3456 na rozwój siewek bobu. Morfaktyna została zaaplikowana przed wysiewem nasion. Morfaktyna nie wpływała na zmianę energii kiełkowania.

Działanie morfaktyny w łodygach było nie przemijające i bardziej modyfikujące niż w korzeniach. Rośliny były małe, pokrzywione, rozgałęziające się i nie zakwitały. U niektórych zamierały wierzchołkowe pąki pędów głównych. Łodygi wykazywały geotropizm dodatni, korzenie — ujemny.

Korzenie, pochodzące z nasion potraktowanych morfaktyną, wykazywały przez 7-10 dni mniejszy wzrost wydłużeniowy, a wyraźnie większy wzrost radialny niż korzenie roślin kontrolnych. Na zgrubiałych odcinkach korzeni głównych nie było korzeni bocznych i w korze pierwotnej tworzyły się podłużne, szczelinowate pęknięcia. Gdy wzrost elongacyjny został wznowiony, wtedy tworzyły się korzenie boczne, a średnica korzenia głównego na tym poziomie nie różniła się wielkością od średnicy korzenia roślin kontrolnych. Poza tym na korzeniach siewek po morfaktynie występowały nieliczne brodawki korzeniowe lub w ogóle ich nie było.

Liście roślin były zmniejszone, zniekształcone, zawierały więcej szparek i włosków na 1 mm² powierzchni, a komórki opidermy i mezofilu były mniejsze niż w liściach roślin kontrolnych.

Dolne międzywęźla łodyg, hypokotyle i górne odcinki korzeni miały grubszą korę pierwotną i silniej rozwinięty walec osiowy niż rośliny kontrolne.

Wzrost radialny kory pierwotnej spowodowany był zwiększoną ilością komórek między epidermą i endodermą i większymi wymiarami komórek, a wzrost walca osiowego — zwiększoną aktywnością kambium i komórek perycyklu.

W łodygach roślin potraktowanych morfaktyną kambium nie tworzyło zamkniętego pierścienia. Kambium wiązkowe wykształcało się w postaci łuków lub pierścieni obejmujących floem i w ten sposób wiązki kolateralne upodobniały się do wiązek leptocentrycznych.

Drewno pierwotne i wtórne łodyg i korzeni wykazywało wyraźnie więcej na-

czyń w porównaniu z kontrolą; naczynia i komórki sklerenchymatyczne były krótsze od analogicznych komórek drewna roślin kontrolnych.

Komórki perycyklu w łodygach i korzeniach były aktywne mitotycznie; w korzeniach aktywność mitotyczna komórek perycyklu była większa niż w łodygach. W nowo utworzonej warstwie powstałej z komórek perycyklu różnicowały się naczynia krótkie, często zdeformowane z siatkowatymi zgrubieniami ścian.

Lignifikacja komórek drewna wtórnego i komórek w strefie perycyklicznej była nierównomierna.