

Induced structural changes in anatomy of apple shoots after treatment with morphactin IT 3456 and other growth regulators (NAA, GA, BA)

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INTRODUCTION

We have reported previously (Pieniążek and Saniewski 1968a) that apple shoots treated in the fall with morphactin (IT 3456) and benzyladenine (BA) showed considerable thickenings under the buds. It was then supposed that a small amount of endogenous auxin was responsible for the synergism with exogenously supplied BA in cambial reactivation, and the presence of morphactin enhanced the stimulation of cambial activity. The newly produced xylem was however distorted probably interfering thus with normal flow of nutrients and growth regulators.

In the present study the experiment was extended to summer and early spring treatment, and the induced structural changes in the anatomy of apple shoots were studied in more detail.

MATERIAL AND METHODS

Experiment A. Four year old Antonovka cultivar trees growing in the Orchard of the Institute of Pomology in Skierniewice were used. The current year shoots were decapitated, the leaves and buds removed on the distance of about 30 cm, and the following growth regulators applied in water solution to the stem as described previously (Pieniążek and Saniewski 1968b).

The same concentrations were used in mixtures of the growth regulators*.

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|-------------------------|--------------|
| 1. Control (water only) | 2. NAA 20 |
| 3. BA 70 | 4. GA 50 |
| 5. IT 3456 30 | 6. NAA+IT |
| 7. NAA+BA | 8. NAA+BA+IT |

Abbreviations: NAA — naphthalene-acetic acid, GA — gibberellic acid, BA — benzyladenine, IT 3456 — morphactin. The concentrations are given in mg/l.

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|---------------|--------------|
| 9. GA+IT | 10. NAA+GA |
| 11. NAA+GA+IT | 12. BA+IT |
| 13. BA+GA | 14. BA+GA+IT |

There were 6 shoots in each treatment, and 3 ml of solution were given per shoot. The treatments were made on July 20, 1967 and July 5, 1968. After 24 days the shoots were collected and put in 70% ethanol for anatomical examination (8 cm long section of decapitated shoot). Some shoots were also collected on the day the experiment started. These besides water treated shoots were used as checks.

Experiment B. Chilled Antonovka one year old seedlings were brought at the beginning of March to the heated greenhouse. The buds were all dormant when treatment was made on March 11, 1969.

IT 3456 (30 mg/l) was used, and one decapitated root was soaked in 3 ml of the above solution for a week. The other roots of the seedling were left untreated. Ten seedlings per treatment were used. The shoots were collected on April 29, 1969 and preserved in 70% ethanol.

Transverse, longitudinal tangential and radial sections from control and treated plants in both experiments were made about 8 cm, below the place of decapitation.

The maceration of wood was made according to the method of Jeffrey's (given in: D. A. Johansen „Plant Microtechnique, 1940 p. 104). Lignin was detected after staining with malachite green and acid fuchsin, phloroglucine in HCl and with iodine in zinc chloride. All drawings were made using the same magnification.

RESULTS

At the time the Experiment A was set up the cambium was still active. After decapitation and the removal of leaves and buds all cambial activity ceased, and no new growth increment was found in the shoots treated only with water (control). However, the last two or three layers of cells were seemingly better lignified than in the shoots collected on the day the experiment began (Fig. 1).

The anatomical examination of the shoots treated with NAA, GA, BA and the mixture of NAA plus GA, NAA plus BA and BA plus GA gave similar results to those reported previously (Pieniążek and Saniewski 1968b): NAA induced the formation of a few layers of xylem (2—4), whereas GA and BA applied together or separately did not support cambial activity. However, in the shoots treated with the mixture of NAA plus BA and NAA plus GA the synergistic effect of these growth regulators on cambial division was found. A wide new ring of xylem was produced. After maceration it was found to contain

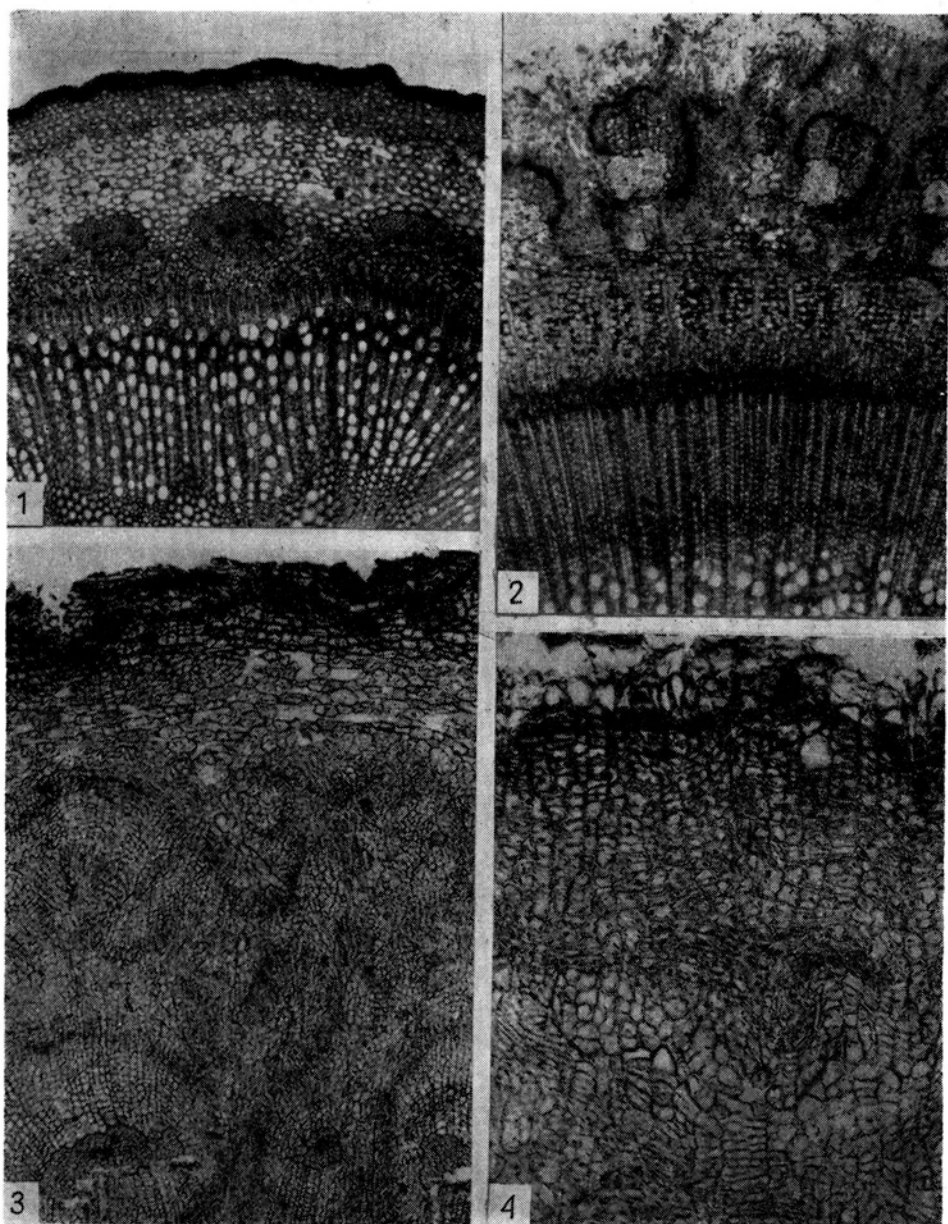


Fig. 1-4. Experiment A. The apple shoot sections
1 — Control (water only). Magn. $\times 56$; 2 — NAA + BA + IT 3456. Magn. $\times 56$; 3 — Detail of the cortex from fig. 2 (transverse section); 4 — Transverse section of the cortex (treatment like in fig. 2). Magn. $\times 145$.

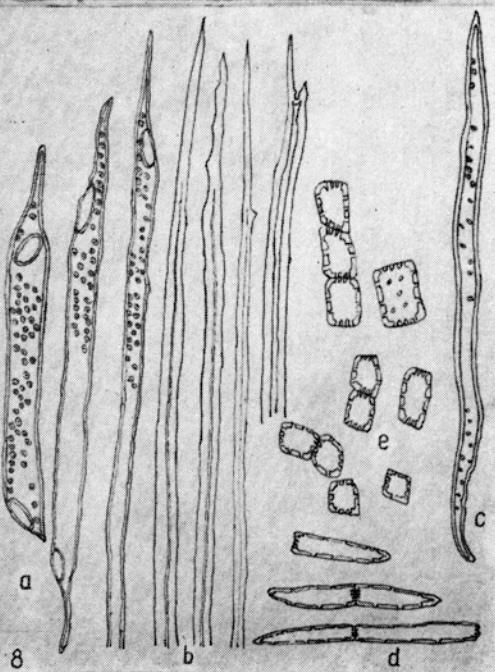
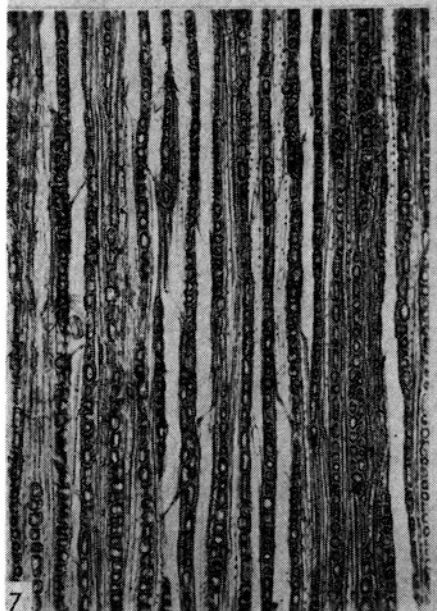
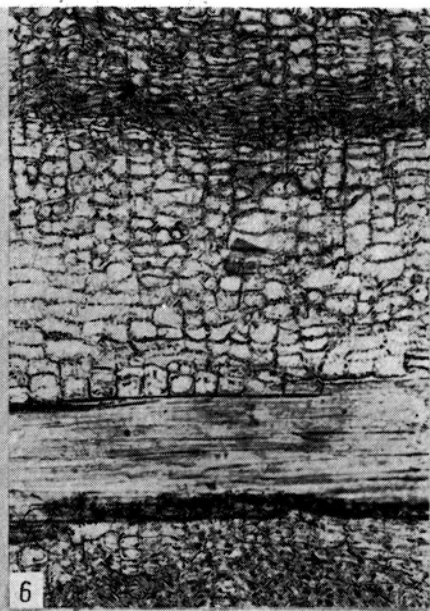
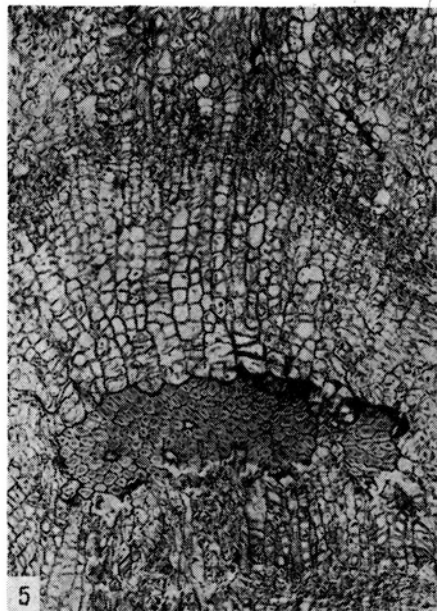


Fig. 5. Transverse section through the pericyclic-fiber bundle (treatment like in fig. 2). Around the bundle a wide zone of newly formed tissues.

Fig. 6. Longitudinal radial section of the pericyclic-fiber bundle shown in fig. 5.

Fig. 7. Longitudinal tangential section through the normal (control) wood.

Figs. 5-7: Magn. $\times 145$

Fig. 8. Macerated components of the normal wood shown in fig. 7.

a — vessels, b — fibres, c — tracheids, d — wood parenchyma, e — ray parenchyma.

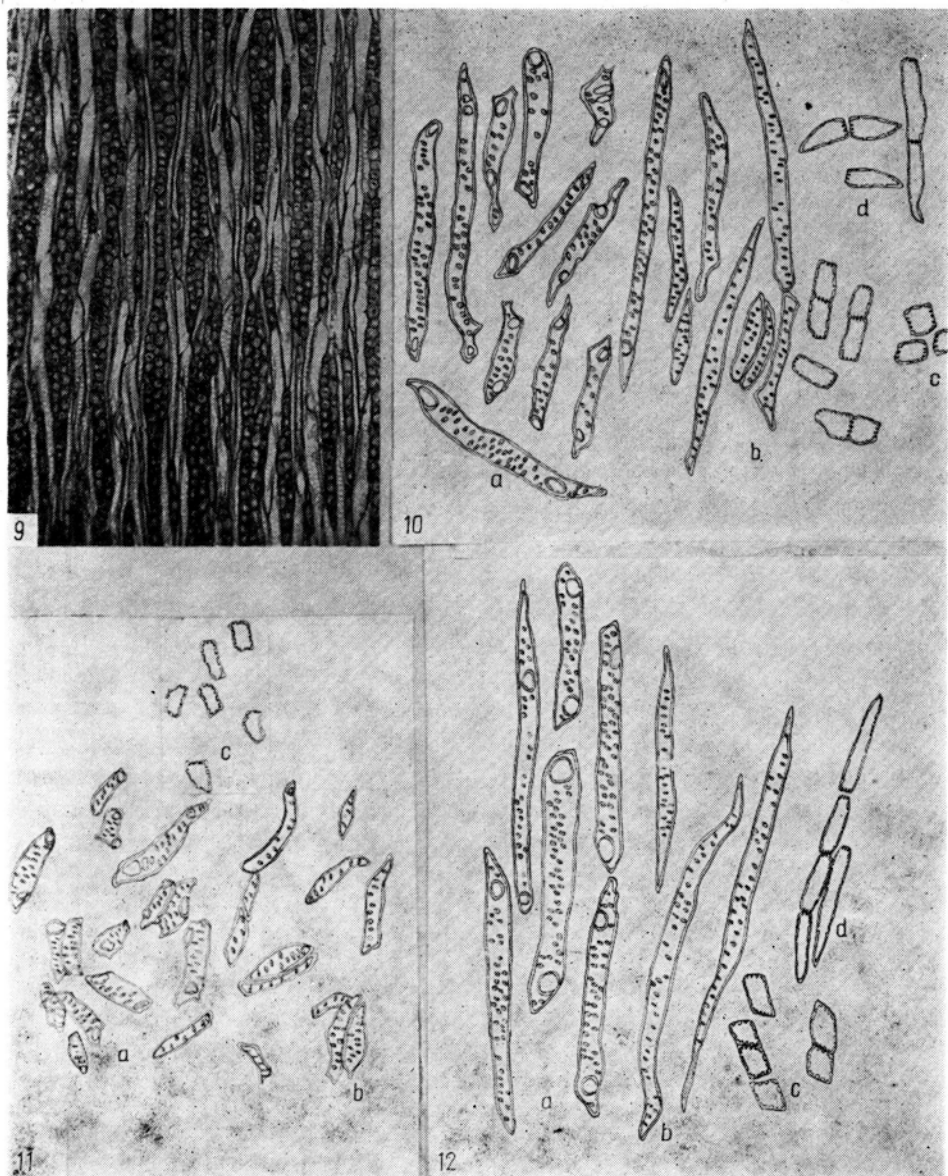


Fig. 9. Longitudinal tangential section through the wood formed after NAA + BA + IT 3456 (Magn. $\times 145$). The transverse section of the same wood shown in fig. 2.

Fig. 10. Macerated components of the wood shown in fig. 9.

a — vessels, b — tracheids, c — ray parenchyma, d — wood parenchyma.

Fig. 11. Macerated components of the wood formed around the pericyclic-fiber bundle as shown on fig. 5 and 6.

a — vessels, b — tracheid-like elements, c — parenchyma.

Fig. 12. Macerated wood components formed after treatment with NAA + BA.

a — vessels, b — tracheids, c — ray parenchyma, d — wood parenchyma.

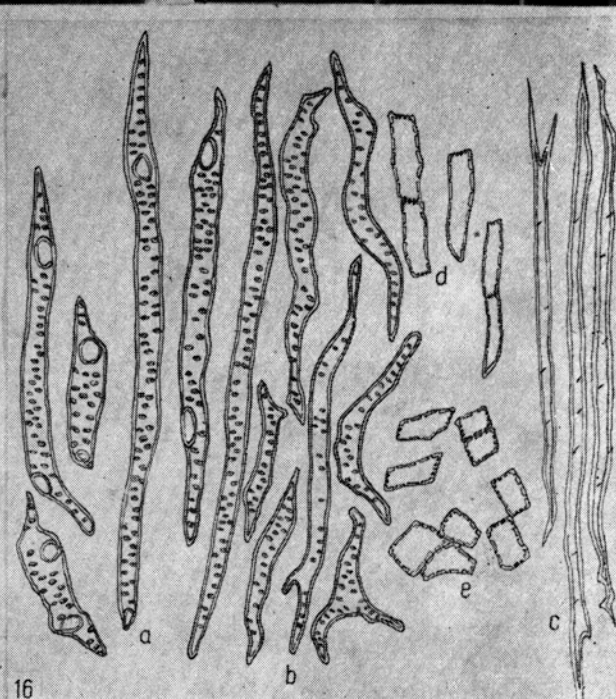
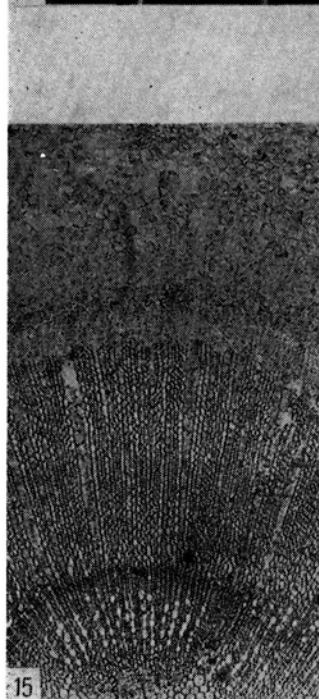


Fig. 13. Experiment B. The appearance of naturally developing apple seedlings after winter chilling (control).

Fig. 14. The appearance of apple seedlings after chilling and treatment with IT 3456 through one root before the buds unfolded.

Fig. 15. Transverse section through the thickened shoot under the terminal bud (the place indicated by arrow on fig. 14). Magn. $\times 56$.

Fig. 16. Macerated wood components from the place indicated on fig. 14. The transverse section of the same shown on fig. 15.

a — vessels, b — tracheids, c — fibers, d — wood parenchyma, e — ray parenchyma.

short and narrow vessels, tracheids and complete absence of fibers (Fig. 12). In normal apple wood there are present long and narrow vessels, tracheids and fiber tracheids (Fig. 7, 8).

Morphactin applied alone to the decapitated and disbudded shoots produced no effect whatsoever. Addition of morphactin to auxin or gibberellin or to the mixture of both did not increase the initial response. Thus, the width of the new xylem was the same as (see Pieniążek and Saniewski 1968b) in the treatment without morphactin. The non-competitive effect of morphactin with respect to gibberellin was evident. This was stated also by several other authors (Tognoni et al. 1967; Krelle and Libbert, 1967; Köhler, 1968).

Very pronounced changes in the anatomy of apple shoots on the distance of about 8 cm. from the treatment were induced when NAA, BA and IT 3456 were applied together (Fig. 2, 3, 4). New, wide growth-ring was produced but the distortions in the xylem were found, and the ratio of components was altered (Fig. 9, 10) as compared with NAA+BA treatment.

The changes due to the addition of IT were also observed in the parenchyma and in the vicinity of pericyclic fibers, where the centers of meristematic activity were found (Fig. 2, 3, 4, 5, 6). In the newly differentiated tissues short and distorted vessel and tracheid-like elements were very numerous (Fig. 11). Therefore, the additional wood was formed outside the phloem. The individual, newly differentiated components showed different stages of lignification assessed on the basis of different intensity of staining of cell walls. There were also the xylem components which remained unstained. The perforations were found in unusual places like in the center of the cells. Some of them were sometimes composed of 2—3 openings.

The changes in the thickness of the phelloderm were also observed after this treatment. Three to five layers of cells were found as compared to one layer present in all the other treatments.

The results obtained in 1967 and 1968 were similar.

In the Experiment B Antonovka seedlings were treated with IT 3456 through one root.

The seedlings were chilled during winter but the buds were not yet active when IT 3456 was applied. It was evident that morphactin was probably transported to the terminal buds because before they started to grow the thickening of the stem under the buds was observed. The treated seedlings started growing later than the water treated controls. When the buds opened the leaves were smaller and malformed and the shoots bent (Fig. 13, 14). Similar thickening and bending of shoots after spraying the trees in May with morphactin was observed by Bubań, Sagi and Porpacz (1968) in apple cv. Jonathan and Starking.

The section through the thickenings showed a wide new increment (Fig. 15). The wood was composed mainly of irregularly shaped tracheids, small tracheid-like vessels and fibers (Fig. 16). In the case when the shoots did not grow out, almost an identical picture was obtained as when treated with NAA, BA and IT 3456 in Exp. A (Fig. 2). Thus a wide, new increment was produced, and the newly formed cells in the parenchyma and the xylem showed similar distortions.

DISCUSSION

The present results showed that morphactin IT 3456 exerted profound influence on cambial activity and xylem differentiation of apple shoots only in the presence of exogenous auxin and cytokinin both in dormant trees or when the leaves and buds were removed. However, when the seedlings were chilled and ready to resume the growth in the spring morphactin alone was necessary to bring about similar structural changes. One was led to believe that in this case the changes were produced by the interaction of morphactin with the endogenous pool of growth regulators.

The distortions in the differentiation of xylem elements were induced by morphactin alone in the intact apple seedlings when it was applied through the root when the buds were still inactive although sufficiently chilled to resume growth. However, this production of distorted elements was of short duration, once the new shoots were produced and elongated the normal differentiation pattern was restored.

Recently Luckwill and Whyte (1968) showed that cytokinins appeared in the xylem sap of apple trees in early spring and reached their maximum concentration about the time of full bloom, after which they decreased in concentration. No hormone activity could be detected in the sap collected during the period from late September to January.

Therefore, as we have earlier suggested (Pieniążek, Saniewski 1968 a) the observed effect of morphactin on apple trees which ended their rest is very likely due to an interaction with endogenous auxins and cytokinins. No competitive action of morphactin with gibberellins in apple shoots could be found.

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SUMMARY

The effect of growth regulators (NAA, GA, BA and morphactin IT 3456 applied singly and in the mixtures on cambial activity and the differentiation of xylem in apple shoots was investigated. Four year old trees cv Antonovka growing in the orchard, and its seedlings after chilling were the object of the experiment.

Morphactin applied singly to the decapitated and disbudded shoots of 4-year old trees did not induce any visible changes as compared with control shoots. However, the treatment with NAA+BA+IT exerted very profound influence on cambial activity and xylem differentiation of apple shoots. The newly differentiated xylem elements were more distorted, shorter and narrower than those obtained after NAA+BA treatment.

Similar changes were induced by morphactin alone when applied to a single root of chilled seedlings. Morphactin was then transported to the terminal buds before their opening and the thickenings under the buds became visible before the growth began. On transverse sections the newly formed increment was composed of distorted elements. The meristematic activity occurred not only in the cambium but also in the parenchyma and around the pericyclic-fibers. These changes were similar to those found in the decapitated and disbudded shoots when morphactin was applied together with synthetic auxin and cytokinin.

The above results lend support to our earlier suggestion that the observed effects of morphactin on many plant species are due to the interaction with endogenous auxins and cytokinins. No support for competitive action of morphactins with gibberellins in apple shoots could be found.

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Anatomiczne zmiany w pędach jabłoni traktowanych morfaktyną IT 3456 i innymi regulatorami wzrostu (NAA, GA, BA)

Streszczenie

Badano wpływ różnych regulatorów wzrostu (NAA, GA, BA i morfaktyny IT 3456) stosowanych pojedynczo i w mieszaninach na aktywność kambium i różnicowanie nowotworzącego się drewna w pędach jabłoni.

Materiałem doświadczalnym były 4-letnie drzewka odmiany Antonówka rosnące w sadzie i jej siewki przyniesione do cieplej szklarni po przejściu okresu spoczynku.

Morfaktyna stosowana pojedynczo nie wywołuje żadnych uchwytynych zmian w pędach drzewek 4-letnich w porównaniu z pędami kontrolnymi.

W pędach traktowanych NAA + IT, BA + IT, GA + IT, BA + GA + IT, oraz NAA + GA + IT otrzymano taki sam efekt, jak w poszczególnych kombinacjach bez dodatku morfaktyny, jeśli chodzi o aktywność kambium (por. Pieniążek i Saniewski 1968b).

Jednakże traktowanie pędów mieszaniną regulatorów NAA + BA + IT powoduje bardzo wyraźne zmiany anatomiczne w porównaniu ze zmianami wywołanymi przez NAA + BA.

W pędach traktowanych NAA + BA wytwarza się dość szeroki przyrost nowego drewna o małych światłach na przekroju poprzecznym, natomiast w pędach traktowanych NAA + BA + IT oprócz wytworzenia szerokiego przyrostu nowego drewna obserwuje się bardzo wyraźne zmiany w strefie włókien perycyklicznych i kory pierwotnej (Fig. 2, 3). Zmiany te wyrażały się w zakładaniu merystemów wokół wysp włókien perycyklicznych i na terenie całej kory. W nowo powstałych tkankach różnicowały się krótkie, zdeformowane elementy naczynio- i cewkopodobne.

Drewno otrzymane po zadziałaniu na pędy NAA + BA + IT wykazywało analogiczny skład jak przy kombinacji NAA + BA, przy czym elementy drewna były jeszcze bardziej skrócone i węższe oraz bardziej zdeformowane.

Podobne zmiany anatomiczne, jak wyżej opisane pod wpływem NAA + BA + IT, otrzymuje się w pędach po potraktowaniu siewek przechłodzonych samą morfaktyną poprzez korzenie. Otrzymane wyniki dyskutuje się na tle współdziałania z endogennymi regulatorami wzrostu.