Kinetin induced breaking of dormancy in 8-month old apple seedlings of 'Antonovka' variety

Wpływ kinetyny na przełamanie spoczynku zimowego 8-miesięcznych siewek jabłoni odmiany 'Antonówka'

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INTRODUCTION

Winter dormancy is an obligatory state for apple trees, and only a prolonged effect of low temperatures between 0 and 10°C brings them out of rest period. The cultivation of this species is restricted then to the temperate zone, and only recently the breeders were fortunate to produce two apple varieties for warm regions — Tropical Beauty in Australia and Golden Dorsett in Bahamas.

Many different substances were used to break the dormancy in apple and other species but without much success. Recently Chvojka (1961, 1962, 1963) has shown that kinetin applied to petioles stimulates the growth of the resting apple buds. His experiments were conducted on Boskoop and Ontario varieties with various concentrations of kinetin and 6-benzylaminopurine at different periods of the year. Thus it was established for the first time that dormancy of deciduous trees can be broken with the help of a purine compound.

The evidence is also very limited concerning the transport of kinetin in the intact plants (Miller, 1961; Wickson, Thimann, 1958, 1960; Witwer, Dedolph, 1963). It indicates that when kinetin is applied to the surface of a leaf its effects are restricted to the treated tissues. Recently Osborne (1964) studying the movement of isotopically labelled benzyladenine in the petiole segments of *Phaseolus vulgaris* has found that this substance is not only transported readily through the tissues but that the movement is also basipetally polar.

Chvojka's experiments with apple trees treated with kinins with the purpose of regulating their growth and metabolism are of special interest since they were performed not only on herbaceous plants or their parts but for the first time on trees. Radioactive

6-benzylaminopurine, as revealed by autoradiography, is localized in the vicinity of the point of application, mainly in the bark and in the phloem. It is little transported toward the apical end, somewhat more toward the base of the shoot (X-ray film darkens several cm away from the point of application). After an extended period of time another radioactive substance was isolated from the experimental material differing from 6-benzylaminopurine in its R_f value. This substance is being further investigated.

The information on histological changes induced by kinetin is also scanty. Sorokin et al. (1962) investigating the effects of auxin and kinetin in the pea epicotyl have found a considerable increase of mitotic activity, and the newly formed secondary xylem of one to several layers apparently regular and functional.

In the present study an attempt was made to investigate the effect of kinetin on unbranched 8-month old apple seedlings at all stages of rest period, the transport of this compound in the main stem, the development and the rate of growth of buds and shoots, and the induced histological changes.

MATERIAL AND METHODS

Six month old 'Antonovka' seedlings growing in pots were brought from the open on September 1, 1963 into the heated greenhouse where the temperature was maintained in winter at 12 to 13°C and supplied with additional light.

The terminal buds were already formed and no new extension growth was produced by the plants upon their transfer to the greenhouse. The leaves were not shed and even at the end of January some of the control seedlings kept their old leaves.

Every week beginning with Oct. 25, 1963, three seedlings were taken for treatment with kinetin solution applied to their petioles, as described by Chvojka (1961) and shown on Fig. 1. Two plants serving as controls were treated in the same way with distilled water. Kinetin was applied only once to each plant during the rest period.

Kinetin synthesized by dr T. Bany of Lublin Medical Academy was used throughout the experiment in two concentrations — 100 µg/ml and 200 µg/ml. It was dissolved in a small amount of hot distilled water acidified to pH 2.0, and after making it up to the desired volume, it was brought to pH 5.5. This procedure makes the kinetin more soluble in water (Chvojka, Veren, Kozel, 1961).

Four leaves below the apical bud were cut off and the stubs of their petioles were inserted in the thin rubber tubing whose other end was immersed in small glass tubes containing 2 ml of kinetin solu-

Fig. 1. Illustrates the method used for treating the seedlings with kinetin through petioles

Fig. 2. Kinetin soaked pads applied to the stem

tion. The solution drawn into the tube before connecting it with the petiole was fed automatically for few hours to several days.

The leafless seedlings were treated in two different ways: 1) the apical bud was removed and the tube applied directly to the stem, and 2) the leaf scars were slightly uncovered with a sharp razor blade and the stem wrapped up in a piece of surgical gauze soaked in kinetin solution. The gauze was covered with polyethylene to keep it moist for several days. One ml of solution was added from the top every third day and the gauze removed on the ninth day from the start of the treatment (Fig. 2).

The observations of growth were made regularly, the length of new shoots measured, and some plants fixed in 70 % alcohol for histological study. The cross sections were examined fresh and after staining them with acid fuchsin and malachite green after previous bleaching in a mordant (Eau de Javel).

RESULTS

Dormant seedlings with some of the leaves retained

Eight month old unbranched apple seedlings broke their dormancy after single application of kinetin solution to four petioles. Thus a full confirmation of Chvojka's results was obtained. The concen-

Fig. 3. Seedling treated with kinetin on Oct. 29, 1963. The development of dormant buds on Nov. 12, 1963. Notice the growth of buds far down from the point of application of kinetin

Fig. 4. Seedling treated with kinetin on Oct. 30, 1963. Close up of bud in the leaf axil far down from the apical bud and further advanced in development than the upper buds

Fig. 5. Seedling treated with kinetin on Oct. 25, 1963. The new laterals on Dec. 3, 1963.

Fig. 6. Control seedling kept in a heated greenhouse since Sept. 1, 1963 retaining its leaves but not extending in length. Picture taken on Jan. 25, 1964

tration of 200 $\mu\text{g/ml}$ used by Chvojka on 5-year old trees, was found too high for the seedlings, and the plants were injured. That is why later only the 100 $\mu\text{g/ml}$ concentration was used.

The kinetin stimulated the buds into growth in two weeks from the treatment at all stages of rest period. The dormant seedlings resumed their growth not only in October but also in November, December and January.

The swelling of the buds was very pronounced and rapid but their subsequent growth into shoots very slow. After 40 to 50 days the longest shoots did not exceed 8 cm in length (Fig. 5). In most cases the apical bud formed a rosette of numerous, small leaves.

The kinetin supplied to the petioles directly under the apical bud stimulated in many cases first the buds far down from the point of application in the petiole (Fig. 4), and then or at the same time in the buds close to the treated petioles. The presence of leaves on a plant enhanced the basipetal transport of kinetin and the swelling of the buds in their axils. Thus the transmission of kinetin effects was observed on a distance of about 20 cm.

In the case of higher concentration of kinetin used — 200 $\mu\text{g/ml}$ the shoots were severely damaged in their top parts, subsequently the buds developed only in the lowers parts. In several plants, two months after the injury became visible, a slow growth of buds started on apparently regenerated axis.

Not much can be said about acropetal transport of kinetin because no seedlings were treated so far in the middle of the stem. In most cases though, the apical bud developed when the petioles directly under it were treated.

Dormant seedlings with one remaining leaf

In January several plants in the greenhouse have lost all their leaves but one close to the apical bud, so the kinetin could be applied to the leafless plant through one remaining petiole. In this case only the apical bud and three or four directly below it began swelling. As yet (February) no information is available as to their further development, but it is obvious that in the absence of the leaves the transport of kinetin is restricted to the few cm (about 5) below the point of application. No control plants treated in an identical manner with distilled water resumed their growth.

Control plants

The seedlings were brought into greenhouse on September 1, 1963 when their extension growth was terminated and the apical bud formed. Until February 1964 some of the seedlings have lost all their

leaves while others had kept several of them (Fig. 6) that were of dark green color.

On two plants out of 150 in the experiment, three buds in the leaf axils swelled at the beginning of February, and an apical bud developed on one, while the rest remained dormant. The cross section on Fig. 7 shows a dormant cambial zone from a control seedling kept in the heated greenhouse over winter. No ring of new growth was detected on plants retaining their leaves.

Samish reports (1960) that in warm countries in spring, towards the end of rest, deciduous trees will normally have no leaves. Under particularly favorable conditions part of their leaves may, however, be retained. He had occasion to observe that, in these cases, the buds in the axil of such leaves will start to grow earlier than buds the subtending leaves of which have dropped. On the basis of the above observation, one may say that the conditions in the greenhouse were especially favorable for some seedlings and less for others.

Histological Changes

The only shoot developed on a plant treated with 200 $\mu\text{g/ml}$ of kinetin was 1.5 cm long. On a cross section some vessels looked normal but their formation was soon arrested and they were replaced by tra-

Fig. 7. Control seedling from the greenhouse. Inactive cambial zone and the last summer growth ring (Magn. 370 $\times$)

cheids. On a fresh section all tissues were browned and the vessels occluded. A different picture was seen on cross sections from a lateral shoot grown on a plant treated with 100 $\mu\text{g/ml}$ kinetin. The shoot was 7.3 cm long, and in this case large parenchyma cells could not be distinguished from the vessel elements.

Fig. 8. Kinetin treated seedling. Spring-like ring of new growth (Magn. 185 $\times$)

Fig. 9. Cross section of a control seedling grown in the orchard. Spring and summer wood (Magn. 185 $\times$)

Spring wood formation was stimulated by kinetin treatment along the entire length of the stem. Treating the buds with 200 $\mu\text{g/ml}$ resulted in haphazard growth rings, very irregular in thickness, several layers wide close to the swollen buds, and very narrow along the internodes. The cross sections were made at several heights of the stem, and the irregular, spring like layers were found even close to the base. In the case of lower concentration — 100 $\mu\text{g/ml}$ — the new growth rings were in width (Fig. 8) and structure not differing from a spring wood at a control plant grown in the orchard (Fig. 9). The thickening of the axis was considerable. At some nodes where several buds were close together the main axis had doubled its girth by February.

Treating leafless seedlings with kinetin through the decapitated stem

Leafless dormant seedling grown in the open until November 26, 1963 and then brought into the greenhouse, were after removing the apical bud treated with kinetin solution. After two weeks three buds below the cut developed and produced three long shoots (Fig. 10-

-right). The control plant (Fig. 10-left) treated in the same manner with water opened just one bud that did not grow into a shoot. On a later date (plant decapitated on Dec. 12, 1963) the control seedling developed also a shoot. At the end of December the same treatment resulted in a comparable growth of treated and control plants.

The basipetal transport of kinetin in leafless plants was slower in the absence of leaves and affected only three to six buds below the cut

Fig. 10. Left: control seedling decapitated on Nov. 26, 1963. Right: decapitated seedling treated on the same date with kinetin. Three vigorous shoots on Jan. 25, 1964

(about 5 cm), but the growth of shoots was very vigorous on plants treated at the end of November whereas the controls failed to extend at all. The internodes of treated plants were of normal length and the leaves large.

Leafless seedlings treated with kinetin soaked pads

The seedlings brought from the open and treated with kinetin solution in the above described manner, produced the leader and lateral shoots (Fig. 11). The development of buds in this treatment was good along the entire length of the stem but very irregular, depending probably on the degree of injury inflicted with the razor blade, and on the concentration of kinetin used. Sometimes the shoots were formed close to the top, sometimes on a lower part of the stem.

The buds started growing 14 to 20 days after treatment. Better results, as in previous treatments, were obtained with a lower concentration of kinetin (100 $\mu\text{g/ml}$), although the length of shoots was not affected.

Fig. 11. Left: control seedling treated with water soaked pad on Dec. 21, 1963. Right: treated with kinetin soaked pad. Picture taken on Jan. 25, 1964

Lanoline paste with kinetin or kinetin and IAA

Dormant seedlings kept in the open until November, when all the leaves were shed off, were treated on December 3, 1963 with a small amount of lanoline alone (control), lanoline plus kinetin, and lanoline plus kinetin and IAA smeared on slightly wounded leaf scars. The treatment gave no result whatsoever in the case of lanoline, and lanoli-

Fig. 12. Tumors on the buds after smearing them with kinetin plus IAA in lanoline paste on Dec. 3, 1963. Apical bud produced a vigorous shoot. Seedling was kept in the greenhouse since Sept. 1, 1963 and lost its leaves

ne with kinetin. After two months small tumors appeared only on the buds smeared with the paste containing besides kinetin IAA (Fig. 12). Those tumors were still enlarging in size in February. No tumor engulfed the apical bud and a vigorous shoot developed. Unfortunately no plants were treated with IAA alone, so it cannot be said whether the growth of the terminal shoot was due to IAA alone or to an interaction of kinetin and IAA.

The experiment with a solution containing kinetin and IAA applied to the petioles gave nonconclusive results, probably due to a too high concentration of those compounds.

DISCUSSION

Experiments conducted with unbranched apple seedlings during their dormancy permitted to study the transmission of kinetin effect from the treated buds. Contrary to most reports that kinetin is transported only with difficulty up and down a long stem, a basipetally polar transport on 20 cm or more was observed on plants with the lower leaves intact.

In the absence of the leaves the transport was limited to about 5 cm affecting three to six buds on the decapitated stem, which produced vigorous lateral shoots. Only recently Osborne gave an evidence of a rapid polar transport of kinetin but her experiment was made only with detached petiole segments of *Phaseolus vulgaris* 5.4 cm long.

Kinetin without doubt breaks the dormancy of apple seedlings at all stages of dormancy, and its effect manifests itself two weeks after the treatment, regardless of the technique used. The stunted growth of laterals on the seedlings treated with 200 $\mu\text{g/ml}$, and to a lesser degree of those treated with 100 $\mu\text{g/ml}$ supplied to four petioles at the same time, could be due to the amount of solution used (8 ml). Two ml applied to the decapitated stem, and the same amount to one petiole only, produced more vigorous growth. In the future lower concentrations and smaller amount of solution will be tested supplied several times instead of one.

Can kinetin replace in apples the cold treatment necessary for breaking of dormancy? No definite answer can be given as yet on the basis of the above described limited experiments. Australian workers Bottomley, 1963; Goldavre, Bottomley, 1959; Zwar, Bottomley, Kefford, 1963; Zwar, Kefford, Bottomley, Bruce, 1963 report that kinins found in young apple fruitlets and other apple tissues differ from kinetin, and the work on their identification is proceeding. Thus it could be that supplying kinetin — cell

mitotic activity is increased and a basipetal resumption of cambial growth correlated with bud growth. The renewed production of phloem and xylem tissue along the entire length of the kinetin treated seedlings, and the growth of laterals shows that transpiration and translocation of water and nutrients is taking place. Supplying kinetin just once, too much of it or at too high a concentration cannot substitute fully for the cold treatment.

The seedlings partially exposed to cold (four to six weeks of temperature below $+10^{\circ}\text{C}$) and then treated with kinetin either through the decapitated stem or through the leaf scars, develop vigorously growing shoots. The control plants treated with water alone because of insufficient chilling do not resume their growth at all or they grow very poorly. Thus it appears reasonable to suppose that after partial chilling only the initial stimulation for growth is supplied by kinetin, and later on endogenous growth regulators produced during the chilling in the plant are sufficient for normal growth.

SUMMARY AND CONCLUSIONS

The effect of two concentrations of kinetin (200 and 100 $\mu\text{g/ml}$) supplied through the petioles in a single treatment, on growth of the resting unbranched 8-month old apple seedlings of 'Antonovka' variety was studied. The plants were grown in pots in a heated greenhouse since September 1, 1963 and during winter supplied with additional light of low intensity. All treatments with kinetin led to the breaking of buds and growth of new shoots during all the stages of rest period of seedlings with their leaves retained or without them. Chvojka's findings were fully confirmed, and the growth began two weeks from the treatment. The subsequent growth was slow and tortuous in the unchilled seedlings, the leaves small, numerous and in rosettes, the extension growth haphazard and stunted. The longest lateral shoots of about 7 cm were obtained. Control plants treated with water remained dormant.

The basipetal transport of kinetin was good, and in the seedlings retaining their lower leaves it was observed on the distance of 20 cm or more.

Cross sections of kinetin treated plants showed irregular cylinder of spring like wood formed along the entire length of the plant, whereas no new growth ring was detected in the control plants. Young shoots produced by kinetin treated plants showed a distortion of primary xylem differentiation.

The best response to kinetin was obtained in the partially chilled dormant seedlings that have shed all their leaves in the field. Decapitated and with the apical bud intact seedlings treated with a single dose of kinetin produced vigorous, normal shoots. The transport of kinetin in the absence of the leaves was limited to three or six buds below the point of decapitation.

A tentative conclusion is reached that kinetin does not replace fully the cold treatment but as a strong stimulator of mitotic activity provides a sufficient stimulus for the breaking of rest of dormant buds.

STRESZCZENIE

Badano wpływ dwóch stężeń kinetyny (200 i 100 $\mu\text{g/ml}$) na rozwój pąków w czasie spoczynku zimowego 8-miesięcznych nierozgałęzionych siewek jabłoni odmiany 'Antonówka'. Kinetynę wprowadzano w roztworze wodnym do uciętych ogonków liściowych, oraz do pędów po usunięciu pąków szczytowych i za pomocą kompresów na zranione żyłką ślady liściowe. Siewki traktowano kinetyną jeden raz w różnych okresach spoczynku. Siewki zostały przeniesione do szklarni dnia 1-go września 1963 r. po utworzeniu pąka szczytowego i zakończeniu wzrostu na długość. W ogrzewanej i doświetlanej w czasie zimy szklarni siewki pozostawały w spoczynku, ale traciły bardzo wolno liście i niektóre z nich zachowały po kilka liści do końca stycznia 1964 r.

Siewki traktowane kinetyną rozwijały pąki w dwa tygodnie po zabiegu, zarówno przy podaniu im tej substancji w listopadzie, grudniu, jak i w styczniu. Wzrost pędów na długość siewek nie poddanych działaniu chłodu był bardzo powolny i najdłuższe przyrosty osiągnęły długość około 7 cm. Rośliny kontrolne traktowane wodą pozostały w spoczynku.

Wpływ kinetyny podanej do pąków w górnej części siewki przenosił się na pąki położone w dolnej części rośliny na odległość około 20 cm. Transport kinetyny zachodził najdalej u siewek, które zachowały liście w dolnej części pędu. Pąki w pachwinach liści w dolnej części rośliny rozwijały się wcześniej niż pąki położone wyżej.

Siewki traktowane kinetyną wytworzyły pierścień nowego drewna wiosennego na całej długości pędu, podczas gdy w siewkach kontrolnych przekroje poprzeczne nie ujawniły żadnej działalności miazgi. Młode pędy, które wyrosły na siewkach traktowanych kinetyną, wykazywały nienormalności w różnicowaniu się naczyń pierwotnych.

Siewki traktowane kinetyną po częściowym działaniu obniżoną temperaturą w polu rozwijały się najlepiej. Siewki te straciły najpierw wszystkie liście i dopiero wtedy zostały przeniesione do szklarni, gdzie działano na nie kinetyną po ucięciu pąka szczytowego lub też w postaci kompresów.

Transport kinetyny siewek nieulistnionych był słabszy i dochodził od trzech do sześciu pąków poniżej miejsca traktowania (około 5 cm), jednak wytworzone przez nie pędy były długie i silne.

Autorka uważa, że kinetyna nie zastępuje wprawdzie całkowicie działania chłodu w procesie spoczynku zimowego u jabłoni, ale stymulując podziały mitotyczne w pąkach powoduje ich rozwój, a nawet w niektórych przypadkach, wzrost pędów.

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