The influence of IAA content on the growth and development of healthy and virus X, Y and X + Y infected potatoes

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INTRODUCTION

One of the most obvious effects of viral infection in plants is expressed in their dwarfing. This means a continuously proceeding reduction of the height of stems as well as in the number of leaves per plant together with the decrease of the leaf area, what finally results in the decrease of the plants dry weight, and as in the case of potato — in poorer yields.

The development and growth of the plant organism is strictly related, as in all other organisms to the development of new cells, tissues and whole organs. This development is regulated by a group of chemical compounds commonly named growth regulators or growth substances.

Accordingly to different authors [1-3] the accumulation of virus material in young, rapidly developing plant organs is much weaker as in those which are older physiologically. As far as the potato is concerned Ramshorn [4] found, that the virus infected tubers did not show during the first stage of sprouting any acceleration in growth of the physiologically younger top eyes. Nevertheless it was also stated by many authors, that further growth of virus infected potatoes usually proceeds much quicker in the first stage of development, than that of the healthy ones [5—8]. All these observations seem to indicate a possible relationship between the virus pathogen and the plant growth substances.

Although there is already an ample literature on plant hormones little is known about the possible relationships between the pathogenic agents and the accumulation of growth substances in plant tissues.

In the year 1939 two papers were published almost simultaneously by Jahnel [9] and Lucas [5] in which the authors came to quite different conclusions concerning the auxin contents in healthy and leaf-roll infected potato tubers. Jahnel claimed, that he obtained less auxin from the diseased tubers, and especially when they were still comparativelly young. On the contrary, Lucas stated, that during the initial stage of development, the potato stems, containing the leaf-roll virus grow with a much higher speed as the healthy ones. She found in the leaves of the leaf-

-roll infected plants, which were collected in the third week after sprouting, almost 50% more auxin diffused into the agar, as compared with the healthy leaves. However after a month the healthy plants seemed to contain much more auxin, than the diseased ones. These first controversial results stimulated other scientists to investigate the problem in greater detail. Söding [10], Funke [11], Söding *et al.* [12], Pavillard [13], and finally Meyer [14] have determined the contents of auxins in healthy and virus infected organs of the potato plant, and mainly in tubers. Results obtained by those authors clearly showed, that healthy potatoes contain generally more auxin, than those infected with either of the common potato viruses X, Y or leaf-roll. In the subsequently published papers Funke and Söding [15] and Söding [16] have proposed even a method for differentiating between healthy and virus infected potato tubers accordingly to their auxin content. However the application of more exact methods of auxin determination in different plants revealed many discrepancies in comparison with the results previously obtained, concerning the amount of auxins in healthy and virus infected plants [7, 17-20].

A different group of scientists, mainly of the french and british origin [1, 21-25] confirmed in their investigations results obtained by Söding *et al.*, concerning the observed differences in auxin content between healthy and virus infected plants. They tried to explain the reasons of this phenomenon, the more so since it was found, that also other plant pathogens, such as bacteria, fungi and also eelworms usually induce in the infected plant an increase of the auxin content [26-28].

In the year 1944 Best [29] showed an increase of scopoletin formation in tobacco leaves which were infected with the potato virus X. Similar observations were made later on by Limasset *et al.* [21], Andreae and Andreae [30] on tomatoes (*Lycopersicum esculentum*) and potatoes infected with either TMV or potato viruse X, Y or leaf-roll. They have shown, that scopoletin inhibits *in vitro* the degradation of IAA by indolylacetic oxidase. Further investigations carried out in the same line [17] clearly demonstrated, that both scopoletin and also many other coumarin derivatives can initiate the photooxidation of IAA, and that its degradation may be stimulated or inhibited by that compound depending on the environmental conditions. Later on the works of Martin and Morel [31] and Martin [25] showed an increase in the contents of scopoletin glycoside and certain derivatives of caffeic acid in tobacco leaves infected with TMV. These results can certainly explain, at least to some extent, the decrease in the auxin level in virus infected plants. Nevertheless it must be stated, that there is no complete conformity between the accumulation of scopoletin and the decrease of auxins in the plant tissues [26, 32].

In the year 1935 Thimann [33] demonstrated, tryptophan being a precursor in the synthesis of beta-indolylacetic acid which proceeds through 3-indolylpyruvic acid (IPyA). It was also shown in the case of the crown gall bacteria (*Agrobacterium tumefaciens*), which causes tumorous growth on plant roots and stems, that IAA synthesis is here dependent on the presence of tryptophane in the medium [34].

Andreae and Thompson [35] have shown for the first time, that potato tubers, collected from leaf-roll infected plants are lacking tryptophan and contain very

small amount of tyrosine. It is not yet clear whether the increase of tryptophane in plant tissue is due to the synthesis trough certain precursors or results from protein decomposition. The possible connection between tryptophane and IAA content in plant tissue is therefore still subjected to discussion. On the other hand, according to Sequeira and Williams (1961), cited by Sequeira [36], certain kinds of bacteria (*Pseudomonas solanacearum*) can synthesise IAA also from other precursors and not solely from tryptophane, and large amounts of this compound are formed also on media completely deprived of tryptophane. When the medium was supplied by tryptophane marked with C^{14} the radioactive carbon was found in cell proteins but not in beta-indoleacetic acid. More recently Wightman [37] has shown that IAN is the main auxin synthesised in plants belonging to the family *Cruciferae.*

Other investigations pertained to the problem of the effect of auxins on virus multiplication in plant tissues. IAA inhibited the formation of necrotic lesions on tobacco leaves infected with TMV [21, 23, 38—40]. Similarly Kutsky [41, 42] demonstrated an inhibitory effect on TMV multiplication of other plant hormones: IBA (indolebutyric acid) and 2,4 D. A very strong inhibition of TMV lesion formation on leaves of petunia (*Petunia hybrida*) was exerted by kinetin (6-furfuryloadenine). All those results clearly demonstrate a strong influence of auxins on virus multiplication in the infected plant host.

On the other hand however the problem of the reciprocal influence of virus material on the accumulation of auxins in infected plants together with the general problem of growth inhibition and dwarfing remains still obscure in many points and requires more detailed studies.

The aim of the present investigation was the determination of the auxin content in healthy and virus infected potato plants during different stages of development, from the sprouting up to the time of flowering. Most controversial data in the literature pertains to this very problem. In my experiments only the effect of beta-indoleacetic acid was studied as it represents in the potato plant quantitatively the main compound among the natural growth hormones [43]. For comparison also the effect of extra added IAA in the growth and development of healthy and virus infected potato plants, was studied.

Investigations were carried out in years 1964-1966 in the greenhouses of the College of Agriculture in Cracow.

MATERIAL AND METHODS

PLANT MATERIAL

During the years 1964-1965 experiments were carried out with two potato varieties: Epoka and Dar. Both these varieties differ considerably regarding the duration of their vegetation period. The resulting differences in the time of sprouting obscured to certain extent the course of the rate of growth, especially in its initial stage. In the year 1966 experiment was carried out only with the wariety Dar, but with larger number of replications.

Seed material taken for experiment was collected from experimental plots where during several consecutive years potato plants were infected artificially with the potato virus X. The infection with the potato virus Y proceeded in field conditions in a natural way [44]. Healthy tuber material was derived from those plants which did not show any symptoms of virus infection and which also gave negative results in precipitin tests against viruses X, Y and S. Virus infected tubers were derived on the contrary from those plants, which showed distinct symptoms of infection with viruses X or X+Y and gave the same diagnosis in the precipitin test. Plants infected with either viruses S or leaf-roll, as well as with diseases caused by fungi were not used as source material in the experiment.

In order to eliminate possible differences which may result from different contents of storage materials, tubers used in the experiment were carefully selected for their weight, and only those of the average weight of 60-80 g were taken for planting.

OBSERVATIONS OF THE DEVELOPMENT OF PLANTS AND THEIR HEALTH CONDITIONS

The selected tubers were stored untill the next year in a cellar, where the temperature varied between +2 to +8°C. In the spring they were planted into pots 16×16 cm in diameter, as soon as they begun to sprout. The pots were filled up with steamed garden soil and after planting they were placed in the greenhouse. The temperature in the greenhouse was continuously recorded on a termograph. The appearance of first sprouts was noted on all tubers.

The moment when ca. 70% of the planted tubers was already showing sprouts was taken as starting point for detailed observations of the development of stems. Beginning from this moment the whole material was examined, first every 3 day and later on every 6-7 day by measuring the height of each stem (from the soil level up to the growing point), and by counting their number. Two times during the whole period: first after two weeks from the beginning of detailed examinations and second time: 7 days after flowering, when the plants already stopped further growth, the number of leaves on each stem was recorded as well as the length of internodes. At the same time the health conditions of each plants were thoroughly verified. Apart of visual observations serological precipitin tests on viruses X, Y and S were performed together with reinoculations on A_6 (*Solanum demissum hybrid* with the variety *Aquila*). The same procedures were used to test those plants from which samples were taken each time for quantitative IAA analysis.

THE USE OF CORN COCLE (*AGROSTEMMA GITHAGO*) AS A TEST PLANT

The biological activity of chromatograms was determined by means of the hypocothyl test of *Agrostemma githago* L., according to Borriss [45]. For this purpose seeds of the corn cockle were sown out in the boxes 40×30×6.5 cm large, filled out with a 3 cm thick layer of a mixture of sterilised garden soil and sand in the proportion 3:1. The seeds were then covered with a thin layer of soil, after which the boxes were covered and placed in a dark room for 3-4 days at a temperat-

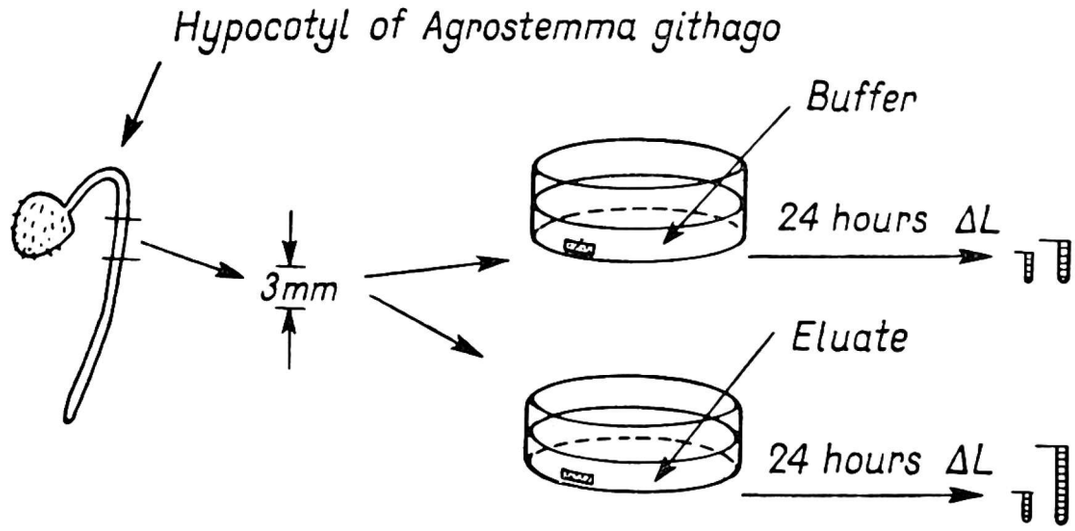


Fig. 1. Scheme of the *Agrostemma* test.

ure of + 22°C. After this period the 4 cm long hypocotyls were cut off from the sprouted seeds and decapitated by removing the cotyledons and the testa together with the bent part of the hypocothyl. The rest, having the length of ca. 3 cm was used for the test (Fig. 1).

TREATMENT OF TUBERS WITH BETA-INDOLYLACETIC ACID

Potato tubers were treated with IAA by implication of this substance inside the tuber tissue according to slightly modified method of Heilinger [46]. A piece of tuber tissue was cut out by means of a corkborer from the bottom part (heel part) of the tuber. In this trough, approximately 3 cm deep, 1 ccm of the appropriate solution of IAA was poured in and the opening was closed by means of a "stopper" cut out from a healthy tuber. The remaining slits were sealed with

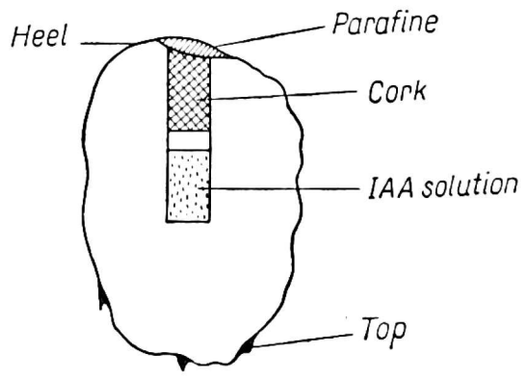


Fig. 2. The methode of supplying IAA to the tubers.

paraffin (Fig. 2). In control series tubers were treated with 1 cc of distilled water instead of IAA. IAA used in this experiment was manufactured by Fisher Sci. Comp. N. Y. U.S.A.

PREPARATION OF EXTRACTS FOR QUANTITATIVE DETERMINATION OF IAA

A slightly modified method of Nitsch [47] and Kefford [48] for determination of growth regulators in green plant material was applied.

30-40 g of fresh tissue (leaves and upper parts of stem) were homogenized with 4 vol. of ethanol for 5 min. The homogenate was left overnight at −7°C after what it was filtered and centrifuged two times for 15 min. at 3000 rpm. The supernatant was condensed under pressure on water bath at 50-60°C and then acidified up to pH below 3.0. The acidified extract was centrifuged again at 3000 rpm for 10 min. The supernatant was mixed with 3 vol. of peroxidase-free ethyl ethier, shaked vigorously for 5 min., and after further 7 min. ether layer was poured out and new portion of ether was added to repeat the extraction. The acidified ether fractions from 3 consecutive extractions were poured together and evaporated in vacuum on a water bath at 40°C.

CHROMATOGRAPHY OF EXTRACTS

The residue remaining after evaporation of ether was taken up in 1 ml of peroxidase-free ether and placed by means of a pipette on a Whatman 1 chromatographic paper. Chromatograms were developed in a solvent consisting of a mixture of isopropanol, ammonia and water in proportions vol. vol. 100:14:6, Chromatographical separation was performed in glass cylinders by ascending method, two samples being run simultaneously: one representing the extract from healthy and the other from virus infected plant. Before the run the paper strips were exposed to the vapours of the developing solvent and subsequently they were dipped with one edge in the solvent for the development to proceed. The separation was continued until the front of the solvent reached the distance of 17-18 cm from the start line. The whole analysis was performed in the dark. After the run the paper strips were dried, up in a stream of warm air and the IAA spots were indentified by their fluorescence in the UV light and by their colour reaction with the Salkowski reagent [48].

BIOLOGICAL ACTIVITY OF THE ELUATES (BIOTEST)

The dried up chromatograms were cut into 2 cm vide transverse strips and each strip was separately eluted after being cut in small pieces, for 5-7 hrs in 10 ml of the *Agrostemma* buffer: 0.544 g $Na_2HPO_4 \cdot 2H_2O$+1.451 g KH_2PO_4 in one liter of distilled water. The final pH of the buffer was 6.3.

The 3 mm sections of the *Agrostemma* hypocotyls were then placed in the eluates and their growth was controlled and measured under the microscope. In the control series other sections were placed in the pure buffer solution. The quantative determination of the IAA content in the eluates was made by comparison of the obtained values with the standard curve of growth designed for *Agrostemma* hypocotyl sections treated with following concentrations of IAA added to the buffer: 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} gramm per liter. Results were expressed in gamma units (10^{-6} g) per 1 kg fresh weight of the tissue, and analysed statistically according to the formula given by Leopold [49].

RESULTS

GROWTH AND DEVELOPMENT OF HEALTHY AND VIRUS X, Y AND X + Y INFECTED POTATO PLANTS

After the tuber material had been selected, the experiments were carried out according to the method described. 30 healthy tubers and 30 tubers from plants infected with the virus X or X+Y were taken for each experiment.

1. Sprouting. In the course of 3 years of experimentations sprouting of tubers was irregular. Differences in the rate of sprouting amounted both with regard to the two varieties and individual years, to 12-16 days. Tubers infected with the potato virus X, Y or X+Y, sprouted ealier, similarly as it had been noted in previous experiments [8]. In 1966 the virus infected tubers startet shooting 15 days following plantation, whereas healthy tubers sprouted 20 days after being planted.

Full data concerning the course of sprouting are collected in two successive Tables: 1 and 2. They show that virus diseased tubers sprouted in all experiments on an average of 3 or even 5 days earlier than healthy ones.

2. Rate of growth in the developmental period, from sprouting to blossom. The rate of growth was expressed by the increment of the main shoot growing out from the top eye.

As it could be expected periodical increments of stems were at the begining not big, within several milimeters. With the advancement of plant development they reached, at examined intervals up to 70 and 90 mm. Increments of shoots were irregular. This phenomenon occured equally in all experiments.

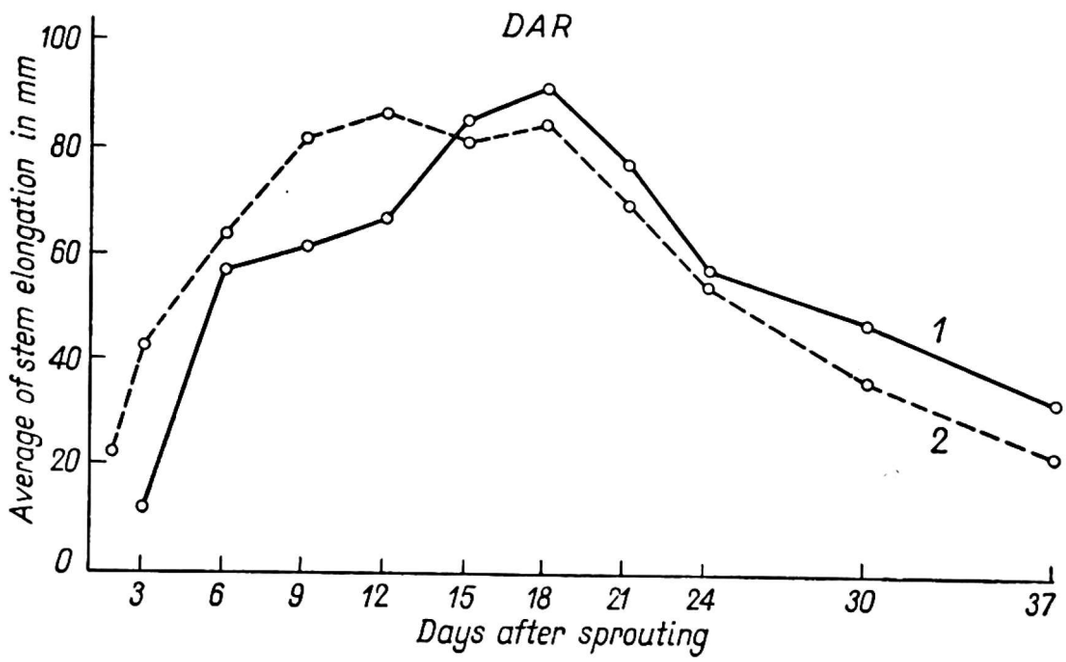


Fig. 3. The rate of growth of healthy and virus infected potatoes variety Dar. *1* — healthy, *2* — virus X, Y and X + Y.

The curves in Figs 3 and 4 show the mean increase of shoots of healthy and virus diseased plants. The highest increase of healthy potato shoots was recorded between the 6th and 24th day after sprouting over 70% of total tubers. An intensive

increment of shoots, which at that time were thin and long, was followed by a period of slower increase marked out by thickenning of stems and especially of shoots growing from the top eyes. At the same time a more intensive growth of leaves could be observed. This period ends with blossoming coming, according to variety, between the 52nd and 59th day after sprouting.

The rate of growth of virus infected plants shown in diagrams by broken lines reveals, in comparison to healthy plants some distinct differences. They are marked out primarily in the first period of potato development. On about the 12th day after measurements had started, plants with first symptoms of mosaic surpassed in height the control plants by an average of 46 mm. During of successive measurements no higher increments in the virus X, Y or X+Y infected plants, as compared with healthy ones, were noted. As it can be seen from Figs 3 and 4 the characteristic

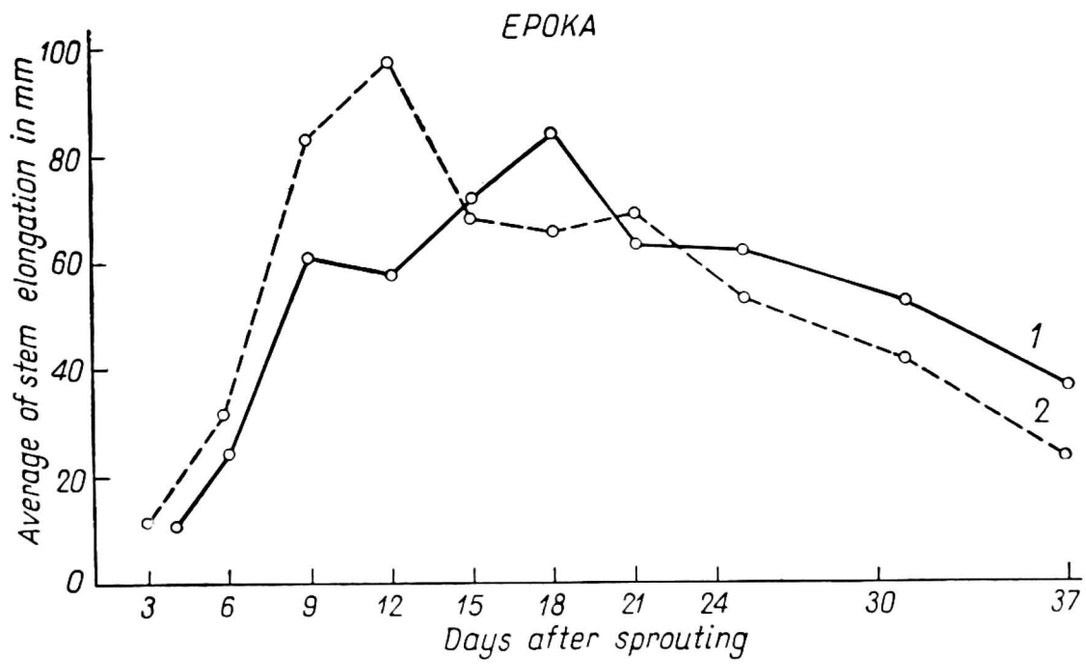


Fig. 4. The rate of growth of healthy and virus infected potatoes variety Epoka. *1* — healthy, *2* — virus X, Y and X + Y.

process of potato dwarfing takes place under the influence of virus disease. In the final effect, in the blossom stage healthy potato shoots exceeded in height the virus infected ones by an average of 6.33 cm. Development of plants after blossoming was examined at 6-7 days intervals. In that stage of development the increments were considerably lower than in the period preceeding the blossom stage.

3. Number of stems. The number of stems is to a great extent one of the characteristic features of each variety although big differences may occur within varieties. Moreover, greenhouse conditions influence those differences between individual plants. In the carried out experiments that difference was revealed in both varieties and in individual years, but in virus infected plants the number of sprouts oscillated within higher numbers. In an extreme case of virus X infected variety Dar these differences varied from 1 to 14 sprouts. Stems growing out in big numbers (11-14) from the virus diseased tubers were in most cases thin and thread-like, as compared to the stems from plants which had only 3 or 4 stems.

With the big number of sprouts one can distinctly observe a dominating growth of 2-3 main sprouts while the other ones most often remain thread-like. Besides having higher individual differences in the number of stems the plants infected with viruses X, Y and X+Y had on an average a bigger number of stems than the healthy ones.

4. Number of leaves on the main stem. In the 3 years-lasting experiments the number of leaves on stems differed quite distinctly with plant growth. The 2-3 weeks old potato sprouts had on an average 4-8 leaves on the main sprout. In the same period the average number of leaves on the main stem of the variety Dar was 2.6 of a leaf bigger than in the variety Epoka. In the blossom stage the number of leaves per stem grown out from the top eyes, fluctuated considerably from 12 to 19.

In the course of all 3 years a distinct tendency to a decrease in the number of leaves, in plants growing from virus infected tubers was observed. A lower number of leaves was recorded in the blossom stage of development.

5. Length of internodes. This feature also varies with individual varieties. There are some varieties with distinctly long internodes, and others with short ones.

Varieties Dar and Epoka, taken for experiments, showed in greenhouse conditions an unequal length of internodes. In the first stage of growth internodes of both varieties were distinctly longer at the bottom of the stem and shortest at the top of it. On the 14th day after sprouting healthy plants of variety. Dar had averagely 6 internodes of following lengths, counting from the bottom of stem: 42 — 31 — — 22 — 19 — 15 — 6 mm. In the period of a most intensive growth (up to the blossom stage) elongation of the middle internodes was observed. In that period the average lengths of internodes of 8 healthy plants of the variety Dar, with 16 leaves on the stem were as follows: 46 — 63 — 55 — 68 — 78 — 124 — 126 — 78 — — 64 — 68 — 35 — 42 — 43 — 28 — 12 mm.

In the case of virus infected potato plants longer internodes in the bottom part of stems were the only recorded difference. This feature was distinctly visible in the initial stage of plant growth.

IAA CONTENTS IN HEALTHY AND VIRUS INFECTED POTATO LEAVES

1. Preliminary experiments. Five weeks before the proper experiment was started a series of potato tubers were planted out. When sprouted plants reached the height of 20-30 cm, the extracts and then the chromatograms and eluates were prepared out of them according to the method previously described. The biological activity of the eluates from separate zones of the chromatogram was examined by taking out from initial volume of 10 ml of the buffer solution for *Agrostemma*, the doses of 2.5 ml of eluate. To such prepared solutions 6 sections of *Agrostemma* hypocotyl were placed for 24 hrs. After that period the length of each section was measured (Fig. 1).

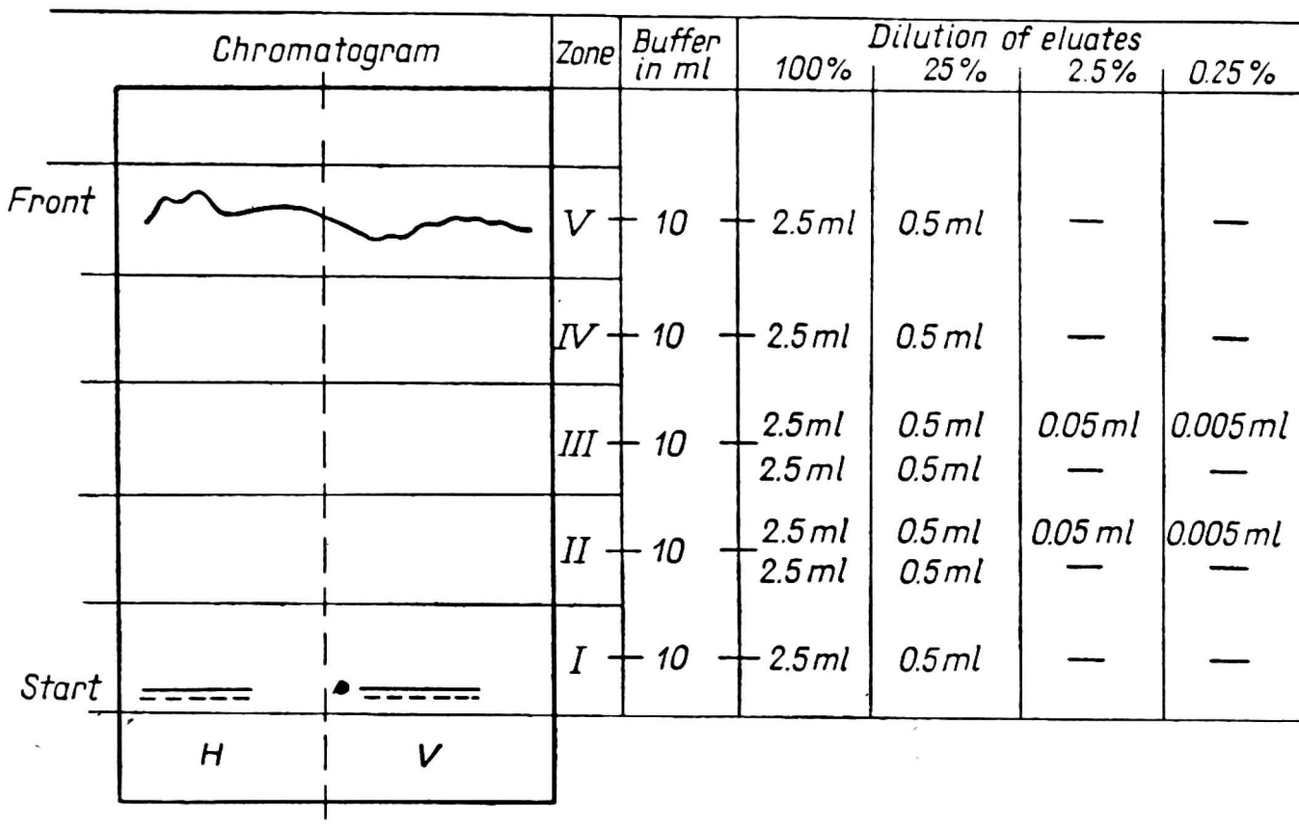


Fig. 5. Dilution of eluates from different zones of chromatogram. *H* — healthy sample, *V* — virus sample.

Since it is known that, depending on the conditions in which chromatography is carried out, most of the biological active substances including IAA are found in Rf from 0.2-0.6 a series of dilutions of the eluate were prepared from those zones. Fig. 5 shows in a schematic form the devision of the chromatogram into zones I-V, taking the dilution of eluates in particular zones into consideration. The preliminary experiments aimed to examine what organs of potato plant — leaves or stems, contain more IAA and in what proportion. The idea was to choose the proper material, richest in IAA, for further extracts.

Figure 6 shows average results of 3 analyses of the increase of *Agrostemma* sections in the eluates from leaves and stems of the zones II and III. It is seen, that the leaves, especially from the II zone of the chromatogram (Rf 0.2-0.4), have the highest contents of biologically active substances.

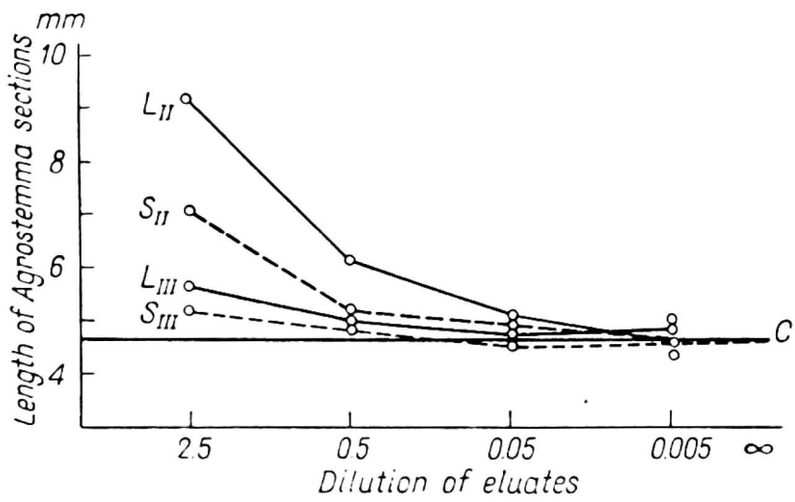


Fig. 6. The growth of *Agrostemma* sections in eluates from leaves and stems of potato.

On the basis of those experiments, the leaves and the top parts of stems only were taken for extractions.

2. IAA contents in plant extracts. Each analysis of the experimental material was controlled in two ways: one of them consisted of sections of *Agrostemma* hypocotyl grown for 24 hrs on the buffer solution; the other control was the growth of *Agrostemma* sections on the known water concentrations of IAA solutions. The first control served only to calculate the average increase of sections in relation to their increase in the buffer itself. The other control in a series of different IAA concentrations gave a standard curve which was then used for estimation of IAA concentration in the investigated extracts. Results were expressed as gamma-equivalents (10^{-6} gram) per kg fresh weight.

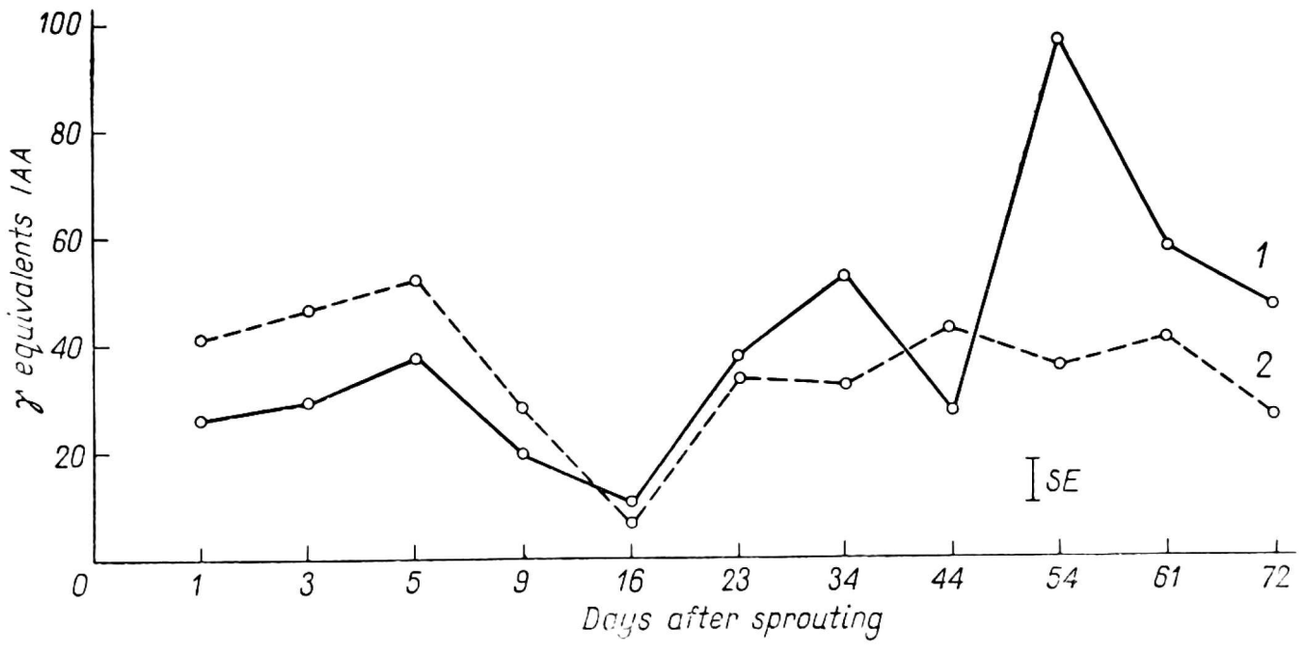


Fig. 7. IAA contents in healthy and virus infected potato leaves expressed in gamma equivalents per kg fresh weight. *1* — healthy leaves, *2* — virus X, Y and X + Y infected leaves.

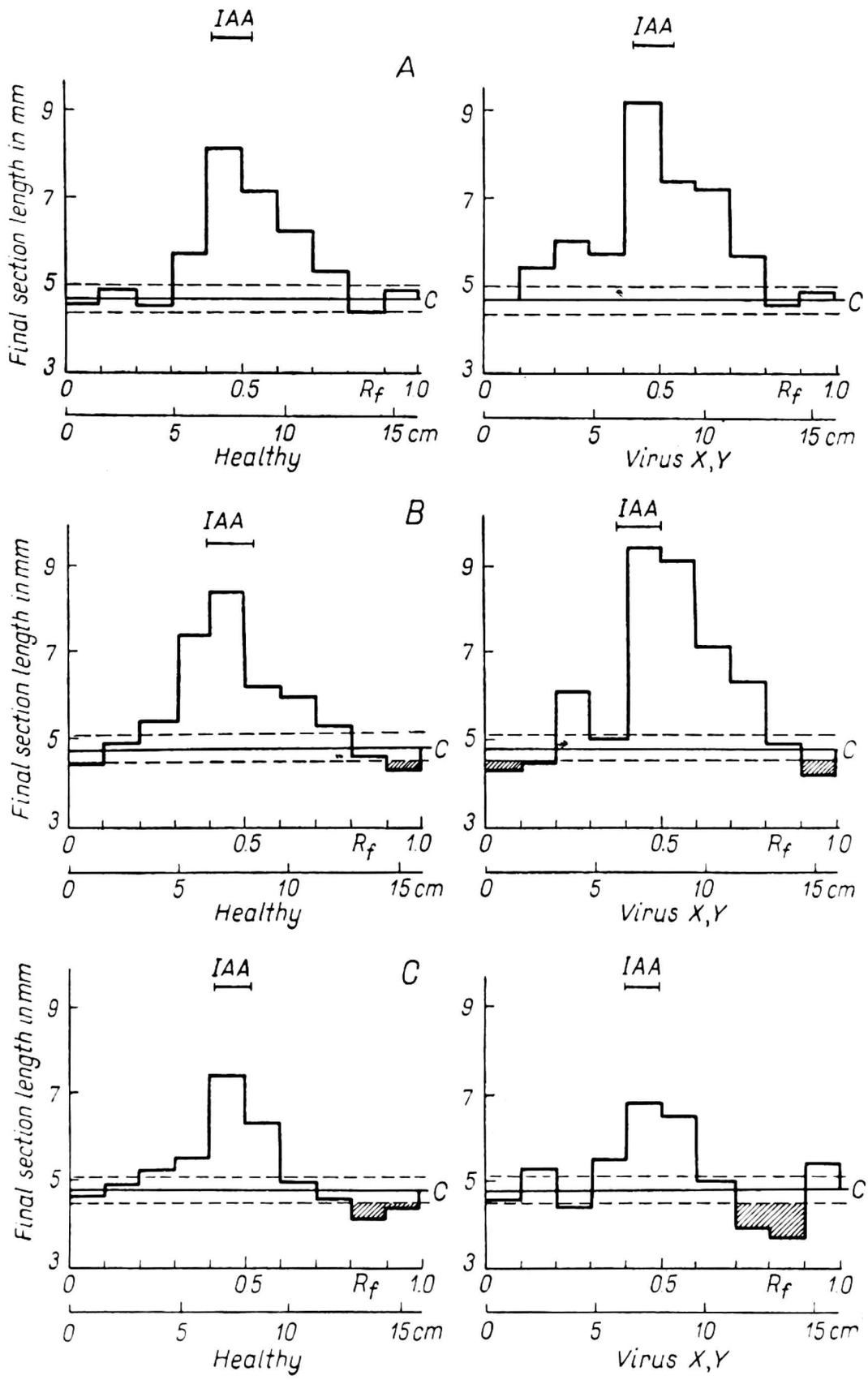
Results of analyses are presented on Fig. 7. Each point on the graph represents a mean value from 12 measurements of *Agrostemma* sections.

As it is seen, the IAA content in the investigated samples variated considerably, reaching the minimum of 9 gamma eq. in the 16th day after sprouting, and a maximum of 96 gamma on the 54th day. Healthy plants exhibited 3 distinct maxima of IAA content on the 5th, 34th and 54th day after sprouting and a decrease at the 16th day. These decrease could be interpreted as caused by the exhaustion of natural IAA resourses from the tuber, the haulms being not yet able to synthesise their own IAA.

Variations of IAA content in virus infected plants showed distinct differences. It is interesting to note that in the first 15 days after sprouting the IAA content in those plants was higher than in healthy ones. Three analyses out of four, made in that period, showed statistically significant differences. On the other hand in the successive period of plants development the IAA contents in healthy plants were

higher than in those infected with viruses. In 7 analyses only one showed higher IAA content in virus infected plants.

Several histograms (Fig. 8 *ABCDE*) are presented showing the growth rate of *Agrostemma* sections, kept in eluates from different zones of the chromatogram. On the right side there are histograms of healthy plants and on the left side, those of infected with viruses X, Y or X+Y. Histograms *AB* come from the first period of potato development ($A = 5, B = 9$ day). Histogram *C* shows a distinct decrease of IAA contents on the 16th day. Histogram *D* shows the results of an analysis



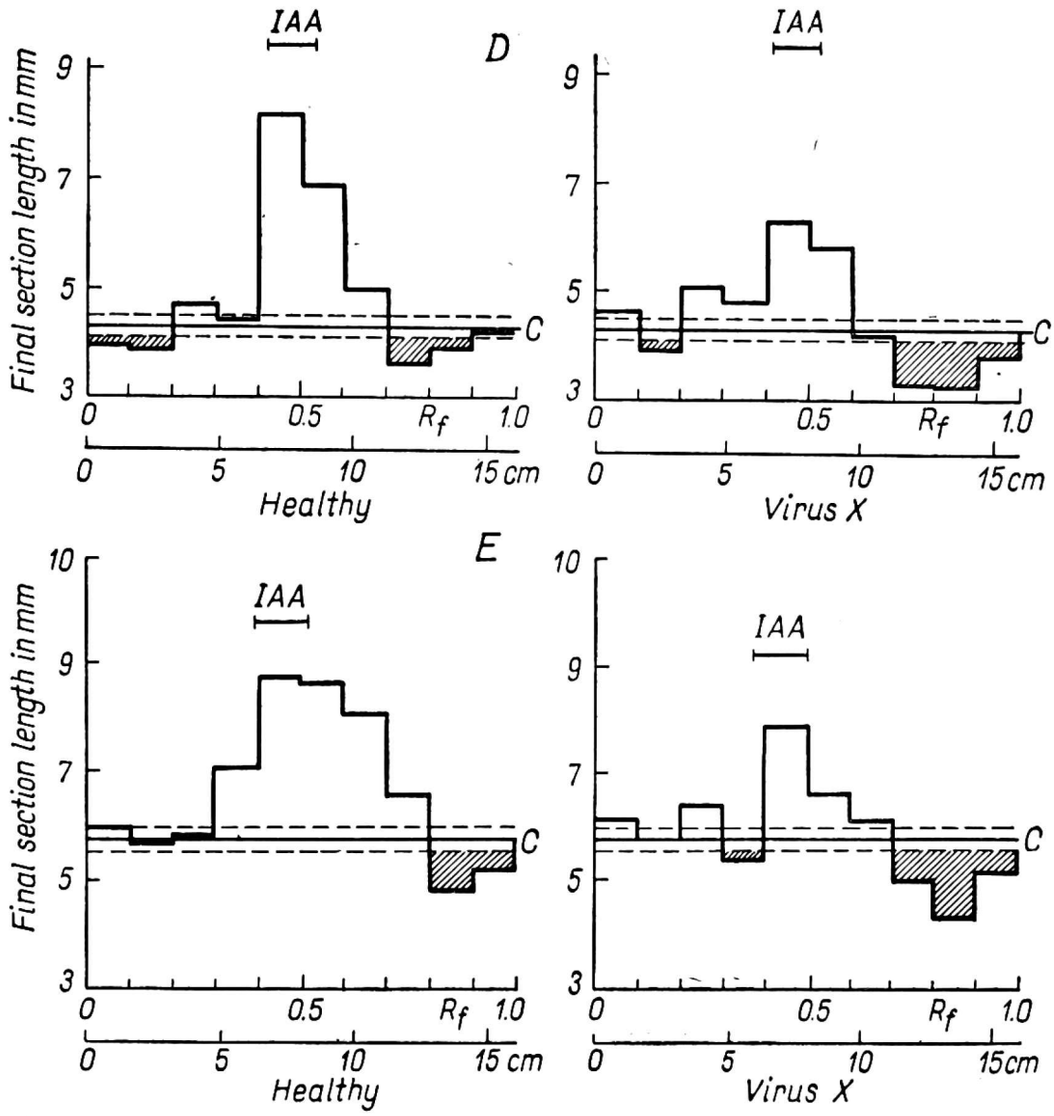


Fig. 8. Growth of *Agrostemma* sections in eluates from chromatograms prepared from the acid fractions of ether extracts of potato leaves. Left side — extracts from healthy leaves; right side — extracts from virus infected leaves. *A* — 5th, *B* — 9th, *C* — 16th, *D* — 54th, *E* — 72nd day after sprouting.

on the 54th day in the initial blossom stage and the histogram *E* dates from the final period of plant development, when the leaves were already senescent.

Histograms *AB* show, that the activity of eluates from virus infected plants was higher than from healthy ones. The level of inhibitors is in that period was low. Histograms *DE* show a decrease of eluates activity (lower IAA content), and accumulation of the inhibitor. The observed decrease of IAA content is much greater in extracts from plants infected with the viruses X, Y or X+Y. The obtained data of IAA contents (Fig. 7) in successive developmental stages of healthy and virus X and Y infected potatoes, seem to confirm the previously described observations of a faster growth of virus infected potatoes in the initial stage of development and next their distinct inhibition in later stages.

EFFECT OF IAA ADDED TO HEALTHY AND VIRUS X, Y AND X+Y INFECTED POTATO TUBERS

The intensive growth and development of virus infected potatoes as compared to the healthy ones in their first stage of growth, and the inhibition of that process in later stages has been many times confirmed [5, 7, 8]. This suggests that the growth-

-stimulating substances play a peculiar role in the development of virus infected potatoes.

In order to examine this phenomenon more closely a cycle of experiments, lasting 3 years was conducted. Five different liquid solutions of IAA were used. Their concentrations were as follows: 50 mg/L; 100 mg/L; 500 mg/L; 1000 mg/L; and 2000 mg/L (50 ppm, 100 ppm, 500 ppm, 1000 ppm and 2000 ppm). Each one of the IAA concentrations was added in the volume of 1 ml to 40 tubers, 20 of them were healthy and the remaining 20 were infected with potato virus X or X+Y.

1. Sprouting. The tubers to which the IAA solutions has been added, before they were planted, did not sprout uniformly. In comparison to the untreated tubers they were characterized by earlier sprouting. This is particulary evident in the healthy potatoes in which the sprouting started between the 10th and 13th day after planting, whereas in the control — untreated tubers the sprouting started on the 20th day. Figure 9 shows average results of 3-year lasting experiments;

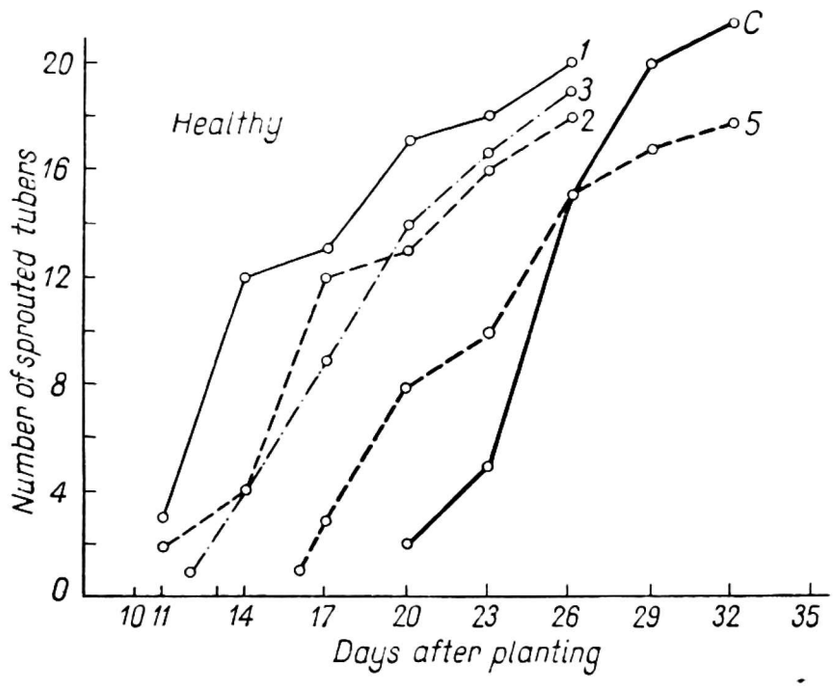


Fig. 9. The influence of different IAA solutions on the rate of sprouting of healthy potato tubers. *1* — 50 ppm IAA, *2* — 100 ppm IAA, *3* — 500 ppm IAA, *5* — 2000 ppm IAA, *C* — control (H_2O).

the rates of sprouting of healthy tubers according to the IAA concentration. Each point in the diagram represents a mean value obtained out of 60 tubers (20 from each year). As it can be seen, the lowest concentration of IAA (50 ppm) had an accelerating influence on the rate of sprouting. 75% of the tubers in this group of concentration sprouted on the 20th day after the had been planted, whereas the tubers of the control group sprouted only in 10%. Figure 10 shows the same relations in potatoes infected with the viruses X, Y and X+Y.

Potatoes growing out from virus infected tubers sprouted 3-4 days earlier than healthy potatoes (Tables 1 and 2 and Figs 9 and 10). It is interesting to note that after the IAA in the first 3 concentrations (50 ppm, 100 ppm, 500 ppm) had been

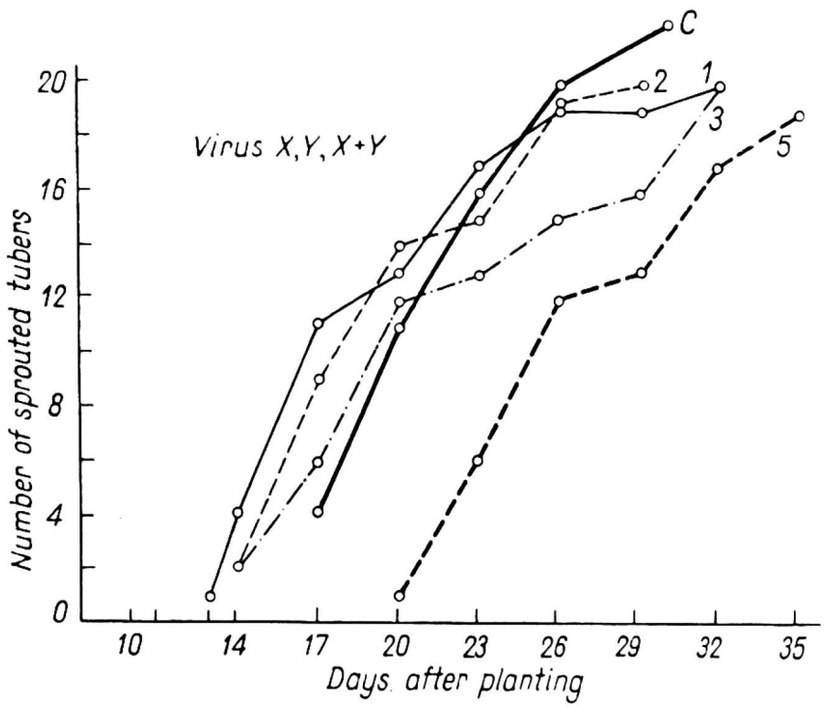


Fig. 10. The influence of different IAA solutions on the rate of sprouting of virus infected potato tubers. *1* — 50 ppm IAA, *2* — 100 ppm IAA, *3* — 500 ppm IAA, *5* — 2000 ppm IAA, *C* — control (H_2O).

added to the virus infected tubers before planting, their sprouting process, although accelerated, showed in comparison to similarly treated healthy tubers an average retardation of 2-3 days. The distinct inhibiting influence of the highest concentration of IAA (1000 and 2000 ppm) exerted in the course of sprouting is quite evident.

Table 1

The rate of tuber sproutings of the varieties Dar and Epoka expressed in per cent of sprouted plants (healthy tubers)

Years	Days after planting													
	Dar							Epoka						
	15	17	20	23	26	29	32	15	17	20	23	26	29	32
1964*	—	—	10.0	20.0	63.3	86.6	93.3	—	—	3.3	16.6	56.6	83.3	96.6
1965*	—	—	6.6	20.0	50.0	90.0	100.0	—	—	13.3	26.6	53.3	86.6	96.6
1966**	—	—	8.3	45.0	71.5	83.3	95.0	—	—	—	—	—	—	—

* 100% = 30 tubers.
** 100% = 60 tubers.

Comparing the controls (tubers with water added, Figs 9 and 10) with the rate of sprouting of the undamaged tubers (Tables 1 and 2) one can distinctly observe that the operation of cutting out some parts of tubers for the purpose of adding the IAA did not play any role in either accelerating or inhibiting the sprouting. The virus infected tubers in the control series sprouted 3 days earlier than the healthy ones.

Table 2

The rate of tuber sprouting of the varieties Dar and Epoka expressed in per cent of sprouted plants (virus diseased tubers)

Years	Days after planting													
	Dar							Epoka						
	15	17	20	23	26	29	32	15	17	20	23	26	29	32
1964*	—	13.3	30.3	56.6	73.3	93.3	93.3	—	16.6	46.6	76.6	93.3	100.0	100.0
1965*	—	10.0	43.3	70.0	83.3	96.6	96.6	—	13.3	50.0	70.0	86.6	86.6	96.6
1966**	5.0	11.6	25.0	43.3	63.3	75.0	93.3	—	—	—	—	—	—	—

* 100% = 30 tubers.
** 100% = 60 tubers.

2. The first stage of development. The fact that healthy potatoes sprouted 8 days earlier and the virus infected potatoes treated with IAA 4 days earlier than those of the control group, influenced their growth in the course of the first 6 days. At the moment when healthy untreated plants sprouted in 75% reaching on an average 14 mm sprout height, potatoes treated with IAA had on an average

Table 3

The average yields per plant in 1964-1965 years (mean of 60 plants)

	Yields in gramms	
Potatoes	IAA	H_2O (control)
Healthy	80.75	65.00
Virus diseased	52.35	37.91

the height of 78 mm. At the same time potatoes grown out from the virus infected tubers treated with IAA had an average height of 124 mm, whereas those that had not been treated were only 26 mm in height. Thus, the growth stimulating substances added to the tubers in the first stage of development evoked in virus infected plants a more intense stimulation than in the healthy ones.

3. The second stage of development. In the next 9 days following the 6-days period, healthy potatoes grown from tubers treated with 50 ppm of IAA showed, contrary to the first period, bigger increments of sprouts than those that had been grown from similarly treated tubers infected with virus X, Y, or X+Y (Fig. 11). As it has been previously proved, in the virus infected potatoes, that had not been treated with IAA, the increments up to the 15th day were higher than in the healthy potatoes (Fig. 12). Comparing the increments of virus infected potatoes treated with 50 ppm IAA — in the period from 6th to 15th day from the moment of sprouting — with the increments of potatoes treated with water, one may note

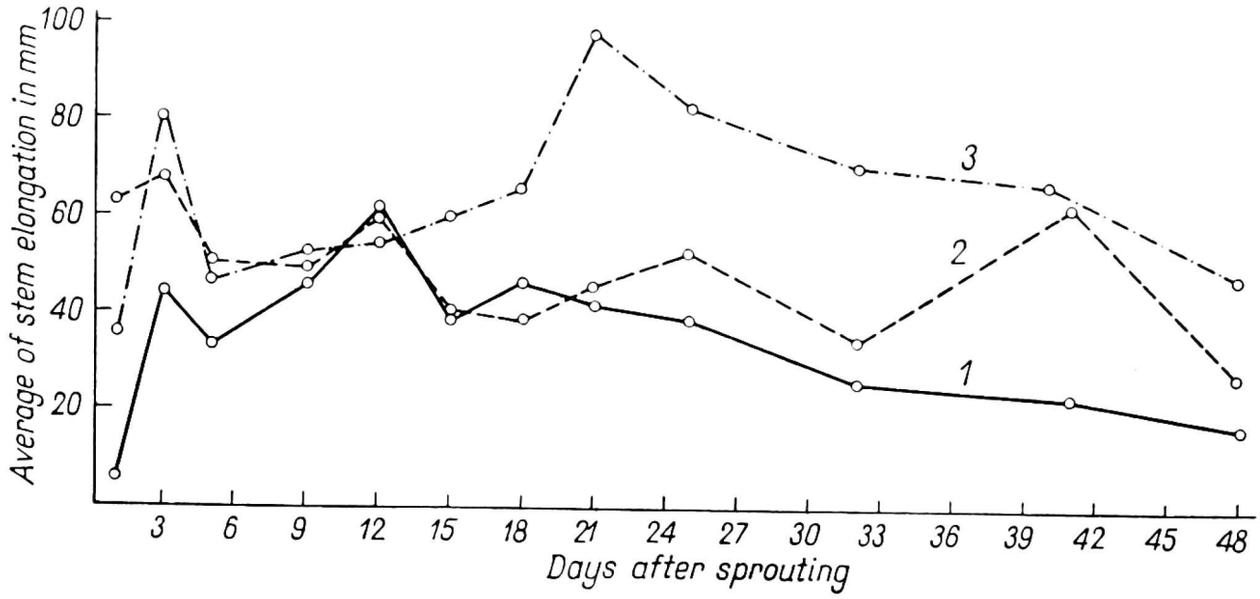


Fig. 11. The rate of growth of healthy and virus X, Y and X + Y infected potatoes treated with 1000 ppm, and 50 ppm dose of IAA. *1* — virus X + 1000 ppm IAA, *2* — virus X + + 50 ppm IAA, *3* — healthy + 50 ppm IAA.

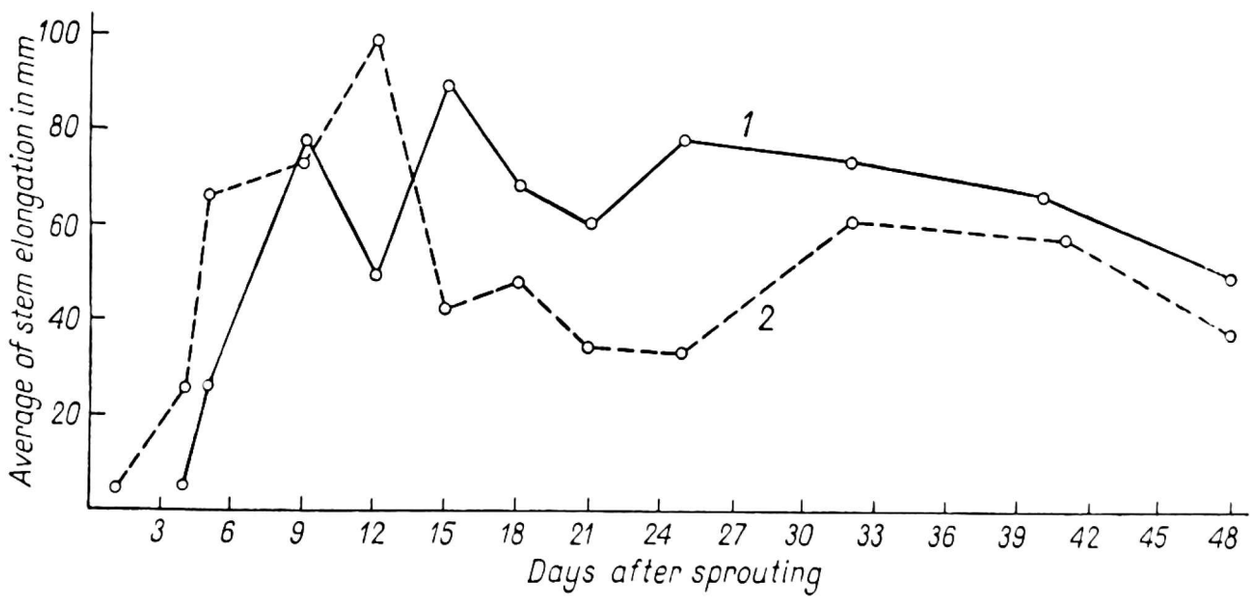


Fig. 12. The rate of growth of healthy and virus X, Y and X + Y infected potato plants (untreated). *1* — healthy + H_2O, *2* — virus X + H_2O.

a distinctly inhibiting effect of externally added growth substance on the increase in growth of virus infected potatoes at that stage of development.

4. Development of plants 25 days after sprouting. In the next 10 days period of development a distinctly stimulating effect of IAA added to the tubers can be cleary noted in the healthy potatoes, especially on the 21 day after sprouting (Fig. 11). On the other hand, in comparison with the control group, the influence of IAA was not marked at all at that stage of development (Fig. 13).

A through analysis of plant development comparising the number of stems, their height, the number of leaves on the central stem, and the lenght of internodes, was carried out each year throughout the 3-years period, prior to the blossom stage i. e. on the 25th day after 70% of the whole material had germinated.

Average values of the 3-years experiment are shown in the Figs 14 and 15 in which each point represents mean value of 60 measurements.

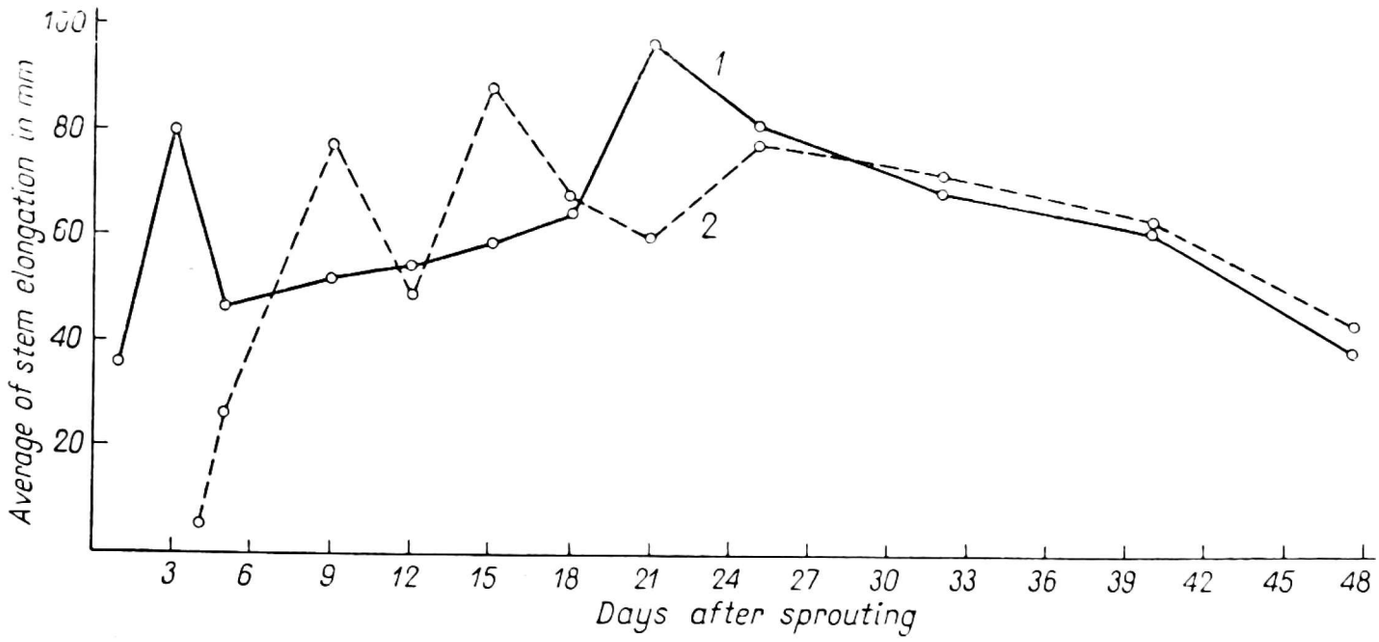


Fig. 13. The rate of growth of healthy potato plants treated and untreated with IAA. *1* — healthy + 50 ppm IAA, *2* — healthy + H_2O.

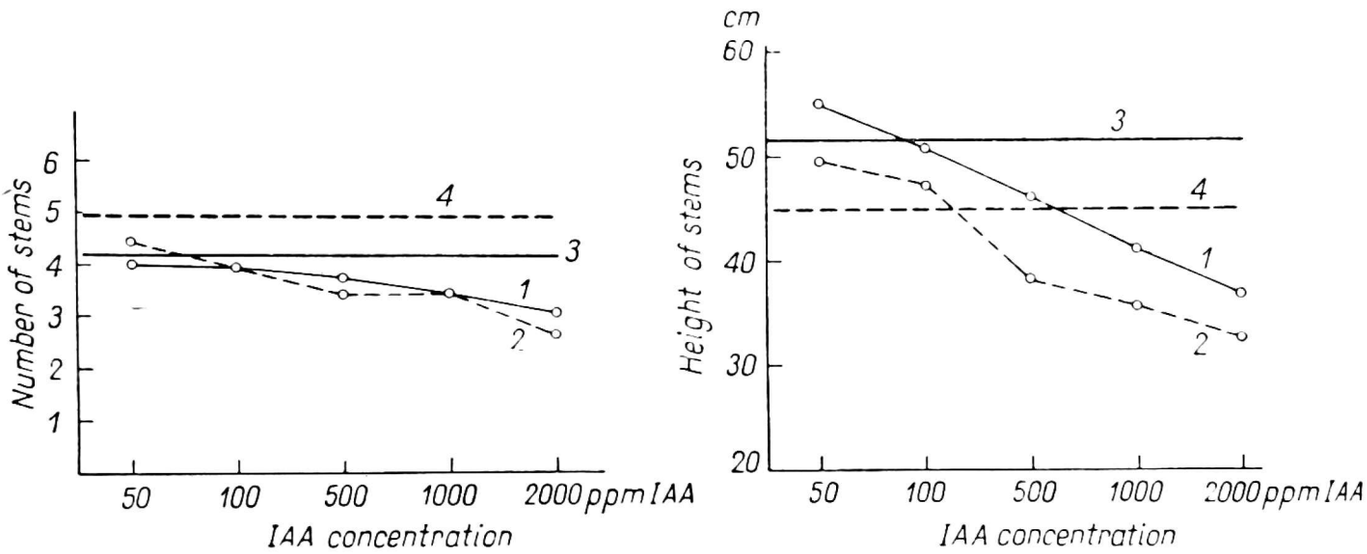


Fig. 14. The influence of different IAA solutions on number and height of stems of healthy and virus infected potato plants. *1* — healthy, *2* — virus, *3* — healthy H_2O, *4* — virus H_2O.

The comparison of control samples (with water) with the experimental ones, in which the IAA of different concentrations was added to the tubers shows, a distinctly stimulating action of the concentration 50 ppm, whereas the highest concentration of 2000 ppm exerted strongest inhibiting effect on the development of potato.

Healthy potatoes to which 50 ppm of the IAA was added had on an average — as compared with the control group — higher sprouts (by 3.6 cm) and longer internodes (by 2.5 mm). With the toxic action of 2000 ppm of IAA the height of sprouts was by 14 cm. shorter and the internodes were shortened by 7 mm.

In the potatoes infected with the viruses X, Y and X+Y, desapite poorer increments resulting from the IAA being added to the tubers, a comparatively higher growth of plants treated with IAA — characterizing the first stage of development — was still maintained. Differences in height of sprouts amounted on an average to 4.6 cm and the differences in the length of internodes — to only 1.7 mm.

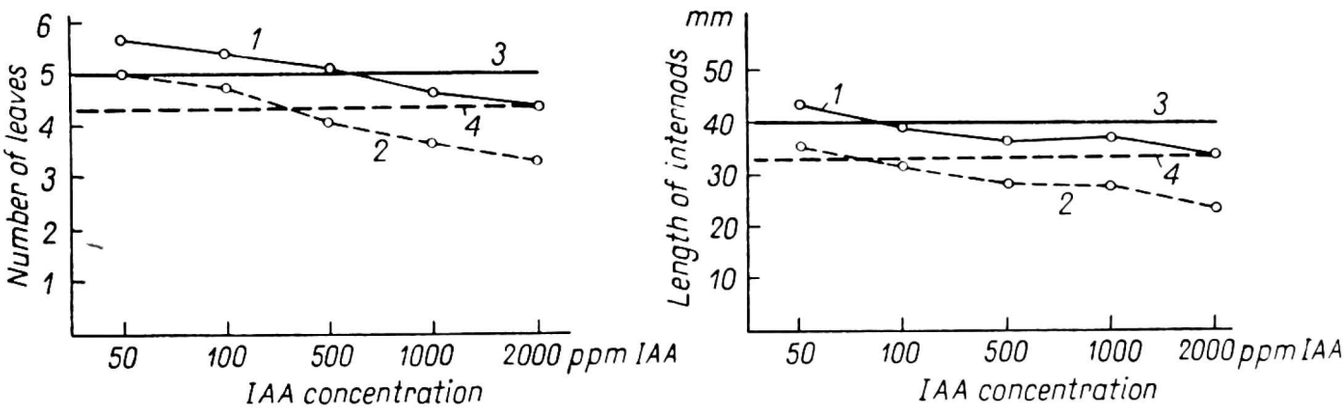


Fig. 15. The influence of different IAA solutions on number of leaves and lenght of internods of healthy and virus infected potato plants. *1* — healthy, *2* — virus, *3* — healthy H_2O, virus H_2O.

The inhibiting action of high concentration of IAA was more distinctly marked in the virus infected potatoes than in the healthy ones (Figs 14, 15).

5. Production of tubers. Yields of tubers collected in the above described pot experiments are shown in Table. 3.

It is interesting that potatoes treated with IAA, both the healthy and the virus infected ones, produced bigger yields of tubers. This is rather suprising because in the period of tuber formation the virus infected plants treated with IAA did not show any signs of a more intensive growth.

IAA CONTENTS IN HEALTHY POTATOES AND IN THOSE TREATED WITH IAA

Results obtained in previous experiments (1964-1965) concerning the influence of different IAA concentrations on the development of healthy potatoes and those infected with the viruses X, Y and X+Y, showed a distinct stimulating effect of the concentration 50 ppm in the first stage of development. Therefore in analysing the IAA contents in the extracts orginating from the leaves of healthy and virus infected potatoes treated with IAA, only those plants were considered which had been externally treated with a 50 ppm dose of this compound.

In Fig. 16 the results of 9 analyses of the IAA contents in potatoes have been shown, which prior to being planted were treated with 50 ppm of the synthetic IAA. The analyses comprised the whole period of the development, begining from the 5th day after germination up to the 71st day when the haulms were still fully green. At the same time potatoes that had not been treated with IAA were already showing some signs of ageing.

Figure 16 distinctly shows, that the level of IAA extracted from the leaves of healthy potatoes was higher during the whole course of the investigated period as compared with plants infected with the viruses X, Y or X+Y. The only higher contents of IAA in virus infected potatoes appeared on the 18th day after sprouting reaching 40.5 gamma of IAA equivalents per 1 kg of fresh weight, whereas in the healthy plants it amounted to 38.4. This difference was found however to be statis-

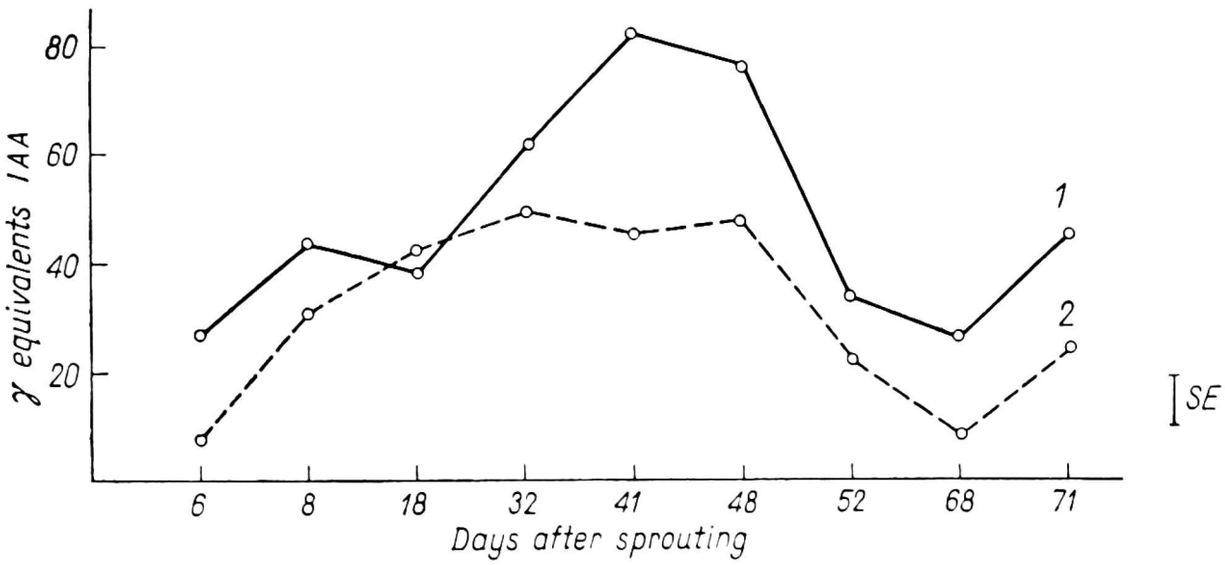


Fig. 16. IAA contents in extracts of plant tissues from the tubers treated with 50 ppm IAA before planting. *1* — healthy leaves, *2* — virus X, Y and X + Y infected leaves.

tically insignificant, since it did not exceed the smallest proved difference (SE) amounting to ± 8.32 gamma of the IAA.

In the 8 remaining analyses higher contents of IAA in the extracts from healthy potatoes were proven statistically significant.

An interesting result obtained in this series of analyses was, that the addition of IAA to both healthy and virus infected tubers caused a distinct "leveling" in the observed on the 16th day decrease of the IAA content in plants, that had not been treated with this compound.

The IAA added to the plants „increased the supplies" which held out till the 48th day after germination; the following extractions showed a decrease of IAA below the level of control plants to which no IAA had been added.

Another remarkable result is the fact that the addition of IAA to virus infected tubers prior to planting, exterted in comparison with virus infected but not IAA treated potatoes — an inhibiting influence on the contents of this growth-stimulating substance (Figs 7 and 16).

In connection with the prolonged period of vegetation in the IAA treated plants an insignificant increase of IAA contents in both examined series of potatoes appeared, on the 71st day after sprouting.

DISCUSSION

The vegetative development of healthy potatoes and potatoes infected with the viruses X, Y and X+Y (without addition of IAA) which showed a more rapid growth of diseased plants in the first stage of development, has confirmed in general — the results that had been previously noted by Lucas [5], Jones [7], and Jaros [8].

On the other hand Bald and Hutton [6] found that healthy potatoes exceeded in height, in all stages of development, potatoes infected with the leaf-roll virus. This was most likely the effect of this virus which influences to a very high degree

the shortening of stems [50, 51]. The conducted experiments also showed that potatoes with viruses X, Y or X+Y sprouted 3 days earlier than healthy ones. The first observation of a more rapid sprouting of virus infected tubers (leaf-roll) was made by Ramshorn [4].

The analysis of IAA contents in the first stage of development of the virus infected potatoes (15 days after germination) distinctly showed, that this earlier germination and a more intensive growth can be explained by a higher accumulation of growth-stimulating substances in the virus infected plants. The fact, that they may be more rapidly activated by the virus may also play here a certain role.

Sequira and Kelman [26] as well as Fehrmann and Dimond [28] showed in regard to the bacterial and fungal pathogenes, that IAA contents in the cells of the host increased as well. In further stages of development of healthy and virus infected plants some essential changes in IAA contents occur. In healthy plants that content increases throughout the whole course of vegetation, whereas, in virus infected plants it decreases below the level of the first stage of development.

In order to make sure whether the deficit of auxins in the second and third stage of development of the virus infected potatoes can be compensated from outside a method of supplying IAA to the tubers, prior to planting, was applied.

The influence of growth-stimulating substances supplied to the plants externally (from the outside) has been thus far examined exclusively on cut-off organs (leaves, leaf-disks, tuber-disks etc.) immersed in a solution containing a given biologically active substance. Many authors ([39, 40, 42] and the others) noted a high activity of supplied auxins through a very short time followed later on by a quick inactivation. Bennet-Clark and Wheeler [52] found that absorbtion of IAA by disks of potato tubers (from the solution in which they were immersed) ceased after 24rs time almost entirely.

The applied method of implicating IAA in a natural way, proved to be successful. A distinct decrease of IAA contents in the control-group plants, appearing on the 16th day after sprouting, was completely abolished in plants which had been treated with this substances was marked up to the 46th day after sprouting this might indicate that the period of IAA activity applied to the plant in that way, was prolonged.

As it follows from the above described experiments, addition of synthetic IAA to virus infected tubers did **not** result in a more rapid growth of examined plants nor in a higher level of the contents of growth-stimulating substances.

According to the investigations of Still *et al.* [53] the increase of auxin contents above the normal level in a plant may lead to its accumulation in leaves in a non-toxic but already inactive form. Under the influence of the enzyme triphosphopyridine IAA — amassed in excess may be also oxidised to 3-methyleneoxindole, a strong inhibitor of growth.

There is a possibility that in potatoes infected with viruses X, Y or X+Y, accumulation of large quantities of IAA during the first stage of development, and subsequently their comparatively rapid decrease leading to smaller increments of sprouts, may be connected with a biochemical transformation of this growth-

-stimulating substance into compounds exerting an inhibiting action on the plant growth.

The yields of potatoes (healthy ones and those infected with the viruses X, Y or X+Y) obtained in the course of experiments run in 1964-1965 were, as regards potatoes treated with IAA, higher than those of plants with no addition of IAA. This might be explained by their prolonged vegetation period and also by a specific action of the IAA itself, strengthening the potato roots [54] and formation of stolons and tubers (Harmey *et al.* 1966).

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