EXAMINATION OF GROWTH-INHIBITING SUBSTANCES SEPARATED BY PAPER CHROMATOGRAPHY IN FLESHY FRUITS III. CHANGE IN CONCENTRATION OF GROWTH-INHIBITING SUBSTANCES AS A FUNCTION OF THE RIPENING

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Introduction

In previous papers (5, 6) the detection by means of bio-assay of the inhibiting zones on paper chromatograms obtained from the ether extracts of different fruit juices, furthermore the results of the examinations concerning the identification of these inhibiting substances — particularly regarding the β-inhibitor-complex — were published.

The present paper deals with paper chromatographic studies of the change in concentration of the growth-inhibiting substances in fleshy fruits as a function of the ripening. There are namely very few literary data available as to how the amount of the ether-extractable inhibiting substances of the fleshy fruits changes in the course of the ripening, and the few ones also contradict one another (1, 2, 3). This was one the aims which prompted us to carry out these investigations; the other was a pratical one to obtain data concerning the isolation of the β-inhibitor-complex: to establish which is the most suitable stage of maturity for the extraction of these substances and which is the most profitable as regards the yield.

Material and method

The experiments were carried out in 1956/57 with the following three fruits the morphological structure of which varied: *Prunus avium* L. »Early Boppard«, *Ribes uva-crispa* ssp. *reclinatum* (L). Schwarz, and *Fragaria ananassa* Duch. »Eszterházy«.

The period between the deflorescence and the anticipated ripening was divided into five equal parts and the extraction of the growth regulating substances was carried out on samples from the same individuals at the five periods mentioned. The ovules were removed from the freshly picked fruits (in the case of straw-

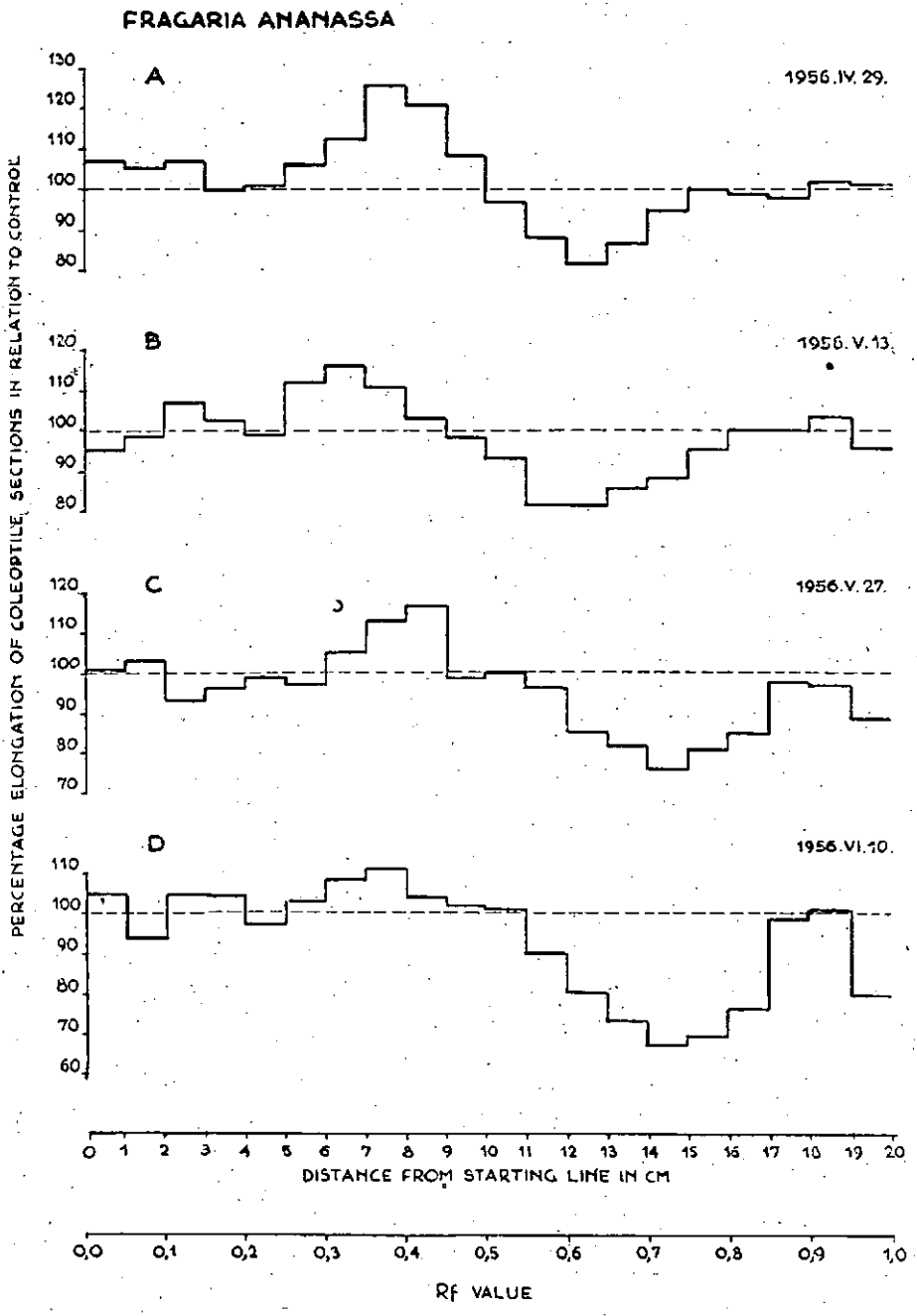

Fig. 1. Elongation of wheat coleoptile sections in the eluate of 1 cm paper segments of the chromatograms made with the ether extract of strawberries the stage of maturity of which differed. (Standard error ± 2%).

berries the achenes) as well as all tissues with the exception of those which turn into flesh. This manipulation was an extreamly complicated procedure and in the case of the youngest fruits of the gooseberries and strawberries the yield was so low that in the first planned period the experiments could not be carried out satisfactorily.

The content of the growth regulating substances of the prepared tissues were related — for the sake of realistic comparison — to the same dry material. The growth substances of the fruit tissues were extracted with peroxide-free ether at 0° C and a quantity corresponding exactly to 3,0 g. of the dry weight of the extract was used for the preparation of the chromatograms. From this quantity five 20 cm ascending chromatograms were made with isopropanol: ammonia (sp. gr. 0,88): water 10:1:1 solvent, on *Sch & Sch No. 2043b* paper, in the dark at 23° C. Three of the developed chromatograms were submitted to three parallel bio-assays and two of them were sprayed with bromcresolgreen and $FeCl_3$ solution after having been analysed in UV light.

The bio-assay of the chromatograms was performed by the wheat coleoptile test in the manner described previously (5, 6). All the data reported represent the average of the growth responses of 30 coleoptile sections.

Experimental results

1. The results obtained with strawberries are shown on histograms illustrating four successive periods in *Fig. 1.*

The growth responses observed between R_f 0,0—0,3 vary to a great extent. In view of the fact that the short chain organic acids and tannic acids localized here exert two kind of activity and their quantity varies considerably, furthermore that the precursors of IAA are also located here, this part of the chromatograms is difficult to evaluate.

At the period of intensive growth the IAA (R_f 0,3—0,4) is present in relatively large amounts in the unripe fruit tissues *(A)* into which it diffuses from the developing ovules. However, in the ripe fruit the size of which does not change any more, the content of IAA decreases significantly *(D)*.

Already the quite young fruits contain the β-inhibitor-complex (R_f 0,55—0,85) in quantities producing definite inhibitions *(A)*. The increase of the inhibitory effect exerted on the coleoptile sections proves that the concentration of the β-inhibitors increases progressively during the ripening *(B, C, D)*.

The essential oils of the strawberries localized in the inhibiting zone under the front line only begin to accumulate in concentrations exerting an inhibitory effect towards the middle of the ripening period, the largest amounts may be found in the completely ripe fruits.

2. The data concerning gooseberries are shown on the histograms in *Fig. 2.*

The results are quite similar to those obtained with strawberries. It seems remarkable that the β-inhibitor-complex is present an approximately identical concentration in the half ripe *(B, C)* and the quite ripe *(D)* berries. The same holds good for the essential oils.

3. The changes in concentration of the growth inhibitors concerning the fleshy fruits of the cherry can be seen on the five histograms of *Fig. 3.*

In the part of the chromatograms in range of the R_f values 0,0—0,25 a mild inhibitory effect appeared everywhere showing that the short chain carbonic acids and tannic acids are present in comparatively larger amounts, i. e. in

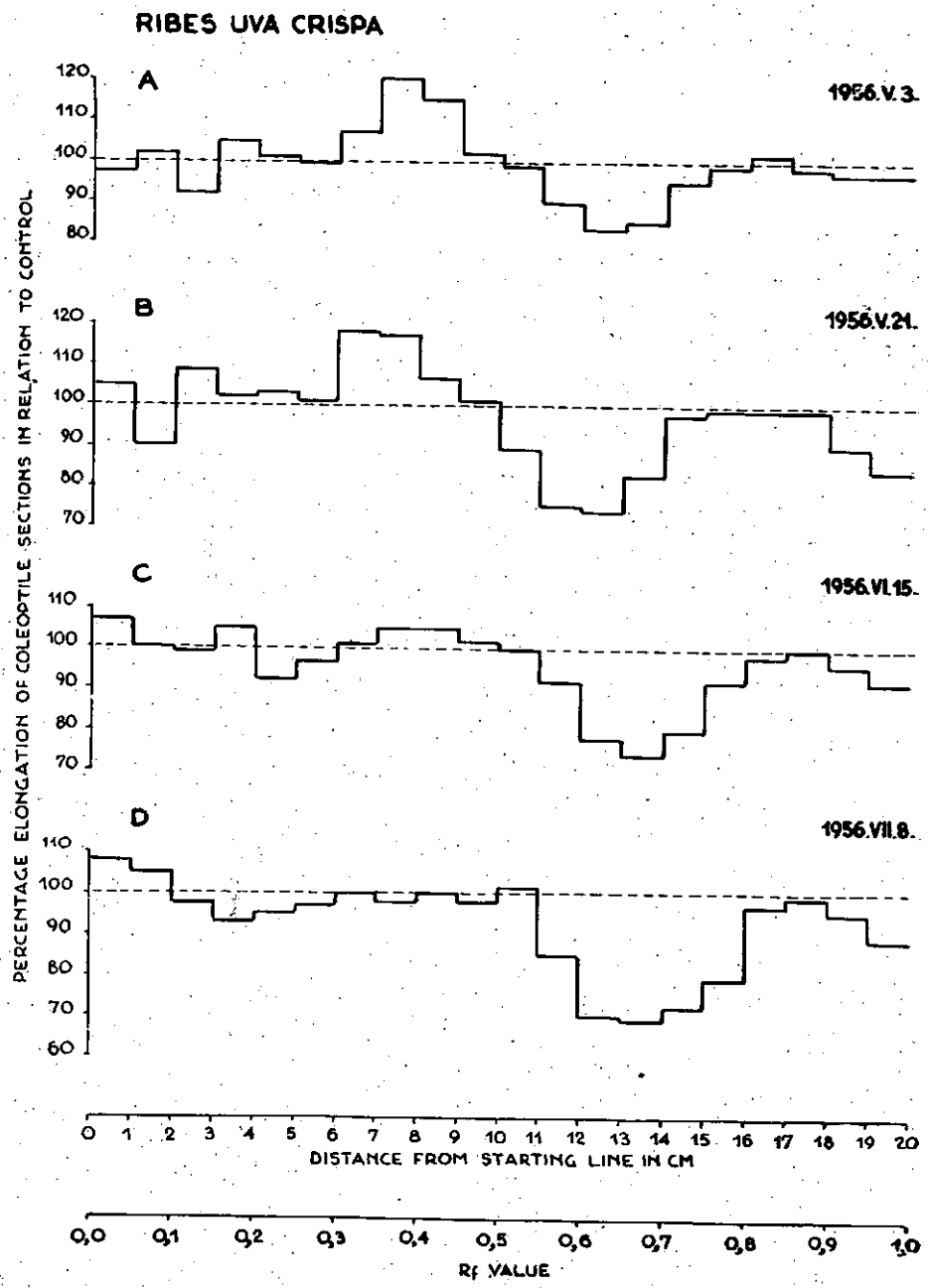

Fig. 2. Elongation of wheat coleoptile sections in the eluate of 1 cm paper segments of the chromatograms made with the ether extract of gooseberries the stage of maturity of which differed. (Standard error ± 2%).

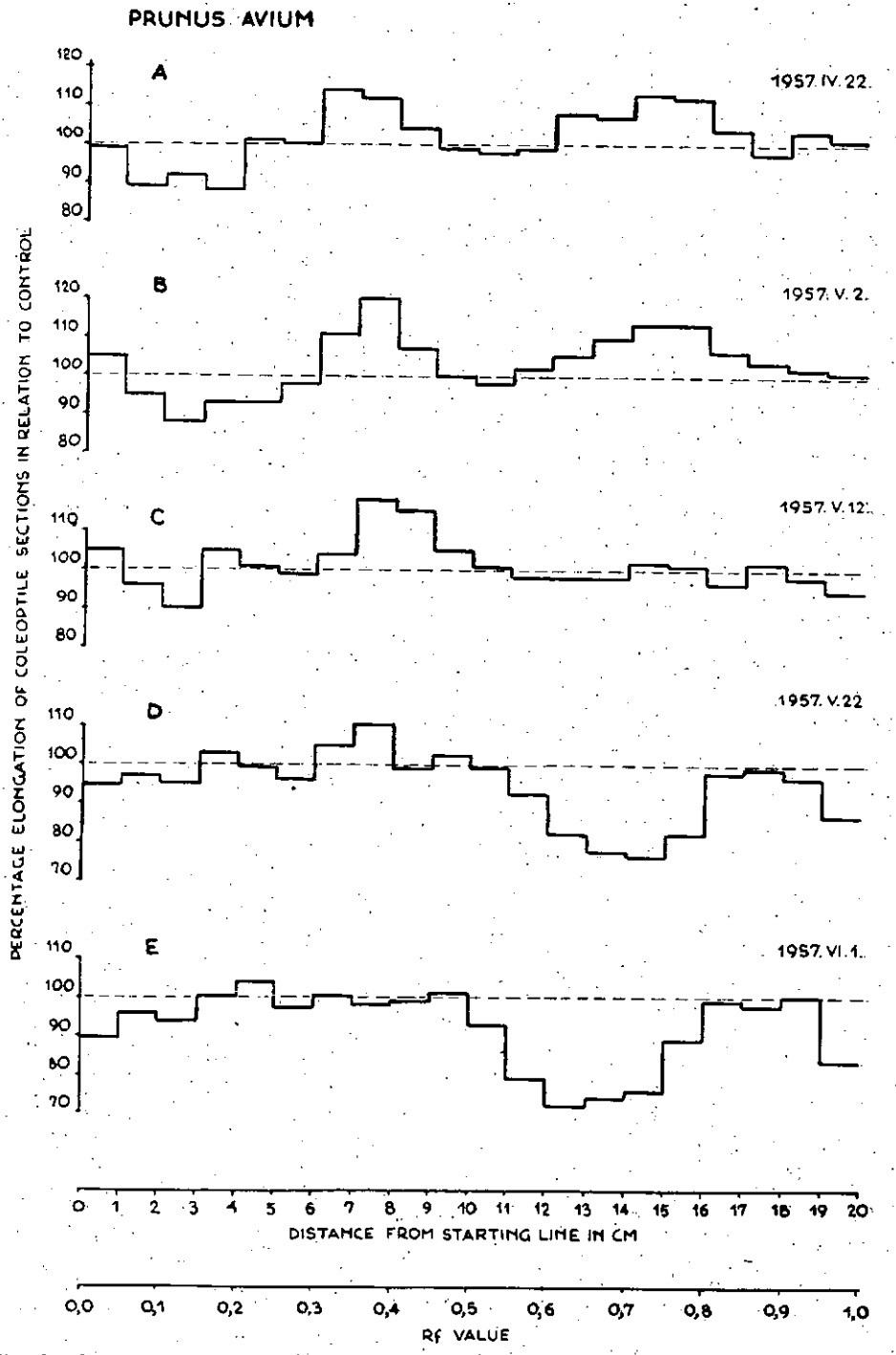

Fig. 3. Elongation of wheat coleoptile sections in the eluate of 1 cm paper segments of the chromatograms made with the ether extract of cherries the stage of maturity of which differed. (Standard error ± 2%).

inhibitory concentrations. This inhibitory effect, however, diminishes during the ripening *(D, E)*.

Concerning the amount of the β-inhibitors, it was surprising that they produced in the young fruits instead of an inhibition a pronounced promotig effect (*A* and *B*) and even towards the middle of the ripening period no inhibitory effect could be detected *(C)*. When the fruits began to get red *(D)* and in the case of the ripe ones *(E)*, however, the inhibiting zone was already marked and their activity was about the same. Hence it could be assumed that the concentration of the β-inhibitor-complex of the cherries examined was initially so low that it elicited a promoting effect in the course of the bioassay. To prove this assumption numerous chromatograms were prepared with the ether extract of 250 g. ripe cherry juice and the corresponding zones (R_f 0,55—0,80) were eluated with ethanol. The eluate was evaporated dry at 50° C at low pressure and the residue was dissolved in 5 ml distilled water. In 3 ml of different dilutions of the standard solution 10 coleoptile sections were incubated. According to *Fig. 4* exhibiting the experimental results, low

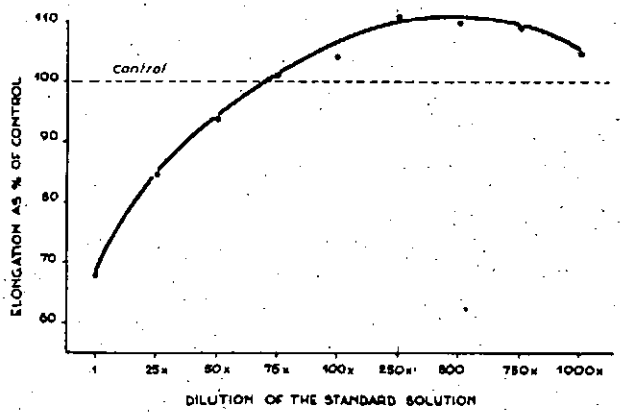

Fig. 4. Growth responses of wheat coleoptile sections in dilutions of the β-inhibitors of cherries. (The averege of 3 parallel examinations).

concentrations of the β-inhibitor-complex of the cherry actually promotes growth.

The amount of the essential oils changes in cherries like in the two fruits dealt with above.

4. Beside the three fruits analysed in detail above, the inhibiting substances of other half ripe fruits (apple, apricot, plum and cucumber) were also examined to establish whether or not the β-inhibitors are already present in the fruit tissue? For comparison an extract corresponding to 0,5 g. dry material was dropped onto each chromatogram.

In most of the fruits mentioned (apple, apricot, plum) a large inhibiting zone between R_f 0,55—0,80 could be observed when they were only half ripe. However, the chromatograms of the half ripe cucumber did not show any inhibition on this area *(Fig. 5)*, although the β-inhibitor zone of ripe cucumbers is very pronounced (6).

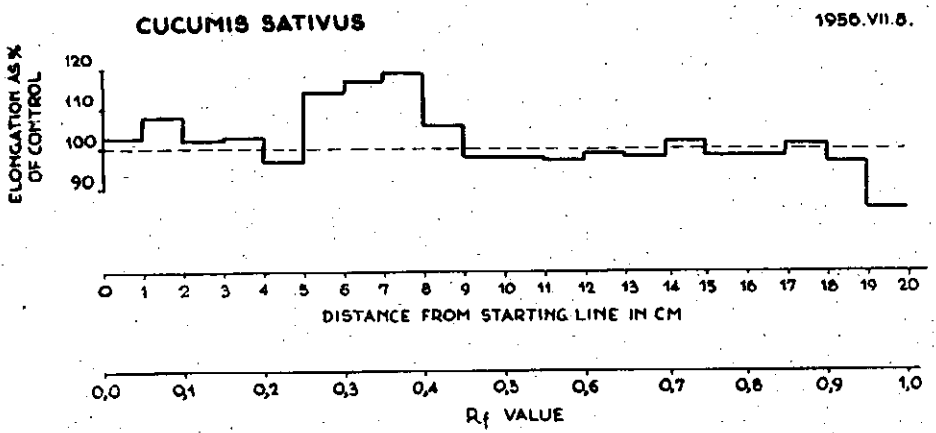

Fig. 5. Growth responses of wheat coleoptile sections in the eluate of 1 cm paper segments of the chromatograms of half ripened cucumber. (The average of 3 parallel examinations).

Discussion

From results obtained at the bio-assay connected with paper chromatography of ether extracts of fruits the maturity of which differed, the following conclusions may be drawn.

It may be established that the high IAA content of the tissues of intensively growing fruits continually decreases in the course of the ripening and when the fruits are completely ripe it cannot be observed at all, or only in insignificant quantities. LUCKWILL (4) also reported the absence of IAA in ripe apples.

The change of the amount of the β-inhibitor-complex during the ripening is not the same in the different fruits, inasmuch as it accumulates in concentration effecting a significant inhibition at the bio-assay in some fruits relatively earlier and in others later. In this respect there are not only differences due to the species, but also some due to varieties, what more probably also to individual deviations caused by external factors. WALGER et al. (7) also mention differences in the concentration of the inhibitors in the individual fruits of squash.

The results point to the fact that the extraction of the β-inhibitor-complex can already be carried out properly in the case of half ripe fruits, however, the isolation is the most profitable if the fruits are quite ripe.

The essential oils of the fruits also begin to accumulate in inhibiting concentration towards the middle of the ripening period, they reach the maximal quantity in full ripe fruits.

These results do not agree with those of MOEWUS (2, 3) according to which cherries and other fleshy fruits contain inhibiting substances in large amounts particularly in the unripe stage, but are rather in accordance with those of KAUFMANN (1), wo states that the riper the cucumber fruits the larger the inhibitory effect they exert on germination of seeds.

Summary

Ether extracts of strawberries, gooseberries and cherries the stage of maturity of which differed were chromatographed and bio-assayed to study the change in concentration of their growth-inhibiting substances, particularly that of the β-inhibitor-complex, in the course of the ripening.

It could be established that during the ripening the high IAA content of the developing young fruits continually diminishes and that it can usually no more be detected when the fruits are quite ripe.

In some of the young fruits the β-inhibitor-complex accumulates in inhibitory amounts relatively earlier and in others later, and reaches its maximum in the entirely ripe fruits. Hence, the extraction of the inhibitors is most profitable when the fruits are in the state of full maturity.

The essential oils of the fruits also begin to accumulate in inhibitory concentrations towards the middle of the ripening period and the largest amount may be found in the quite ripe fruits.

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