

**EXAMINATION OF GROWTH-INHIBITING SUBSTANCES SEPARATED BY PAPER CHROMATOGRAPHY IN FLESHY FRUITS  
IV. PAPER CHROMATOGRAPHIC ANALYSIS OF LEMON JUICE CONTAINING GERMINATED SEEDS**

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**Introduction**

In the course of paper chromatographic examinations of growth-inhibiting substances of various fleshy fruits (6, 7, 8) it could be established that the derivatives of cinnamic acid and benzoic acid summarized under the name of  $\beta$ -inhibitor-complex are mainly, and the essential oils partly, responsible for the commonly known inhibitory activity of fruit juices. From these results it can be concluded that in the overripe, rotting fruits already containing germinated seeds these inhibiting agents are missing or only present in insignificant amounts. The aim of this work is to prove experimentally this conclusion.

**Material and method**

The lemons used for the experiments were kept from spring to autumn for a period of about 6 months in a closed glass container. During this time the seeds contained in the fruits, but for a few exceptions, germinated (*Photo 3*) and the sprouts and roots of some larger seedlings even penetrated through the exocarpium becoming thus also visible from the exterior. (*Photo 1 and 2*).

100 ml. fruit juice (pH 2,75 and 2,4 respectively) was pressed out of the lemons containing germinated seeds as well as out of normal ones used as controls. The growth-inhibiting substances were extracted in the manner described in a previous paper (7) with peroxide-free ether at 0° C., then five 20 cm. chromatograms, each having 3 start points, were prepared with isopropanol: ammonia: water 10:1:1 solvent on Sch & Sch No. 2043b paper. Two of these chromatograms were bio-assayed with wheat coleoptile sections and two others with Papaver seeds (the procedure was similar to that reported previously 7), the fifth was sprayed with FeCl<sub>3</sub> reagent after it had been analysed in UV light. The average results were calculated as percentage of the controls.

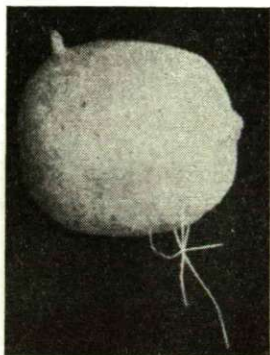


Photo 1:  
Lemon fruit containing seedlings

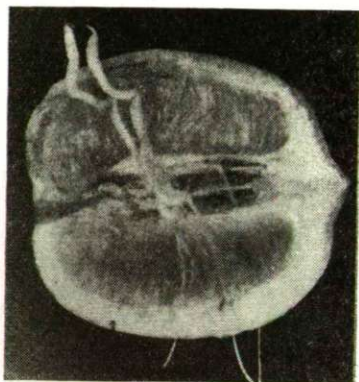


Photo 2: Length-section of the  
fruit illustrated in Fig. 1.

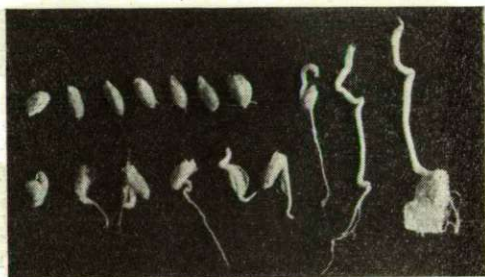


Photo 3: Germinated seeds and seedlings prepared from the fruit

### Experimental results

The results of the bio-assay of the chromatograms are illustrated on *Figs. 1. and 2.*

Part A) shows the growth and germination responses observed in the eluata of the chromatograms made from the fruit juice of normal lemons. Both the coleoptile- and the seed test indicate a pronounced inhibition in the range of  $R_f$  0,0—0,2 where the tannic acids and the short chain carbonic acids — in this case mainly citric- and malic acid — are located (8). A total inhibition prevails in the area between  $R_f$  0,65—0,85 in the so-called  $\beta$ -inhibitor zone (3, 6, 7, 8, 9) and at  $R_f$  1,0 where the essential oils are situated (8).

Part B) of *Figs. 1 and 2* on the other hand, exhibits the results regarding the identical amount of fruit juice of lemons containing germinating seeds and seedlings, respectively. The lower sector of these chromatograms ( $R_f$  0,0—0,2)

the coleoptile sections do not indicate any inhibition, on the contrary a rather mild promotion may be observed. In the *Papaver* seed test the germination inhibition, which may be attributed to short chain organic acids localized here, is also quite insignificant. According to earlier results (6, 7, 8, 9) the promoting spot obtained with the coleoptile test at  $R_f$  0,35, which however, the seed test does not indicate, is due indole-3-acetic acid.

Both types of biological assay prove unequivocally that the group of aromatic acids summarized under the name of  $\beta$ -inhibitor complex ( $R_f$  0,65—0,85), which exert in the fresh lemon a very strong inhibiting activity, occurs in the juice of fruits containing germinated seeds only in quite insignificant quantities. The same holds good for the essential oils ( $R_f$  0,95—1,0).

The results of the analysis of the chromatograms in UV light and the spraying with  $FeCl_3$  are in good agreement with those of the biological experiments. On the chromatograms of normal lemons the substances of the in-

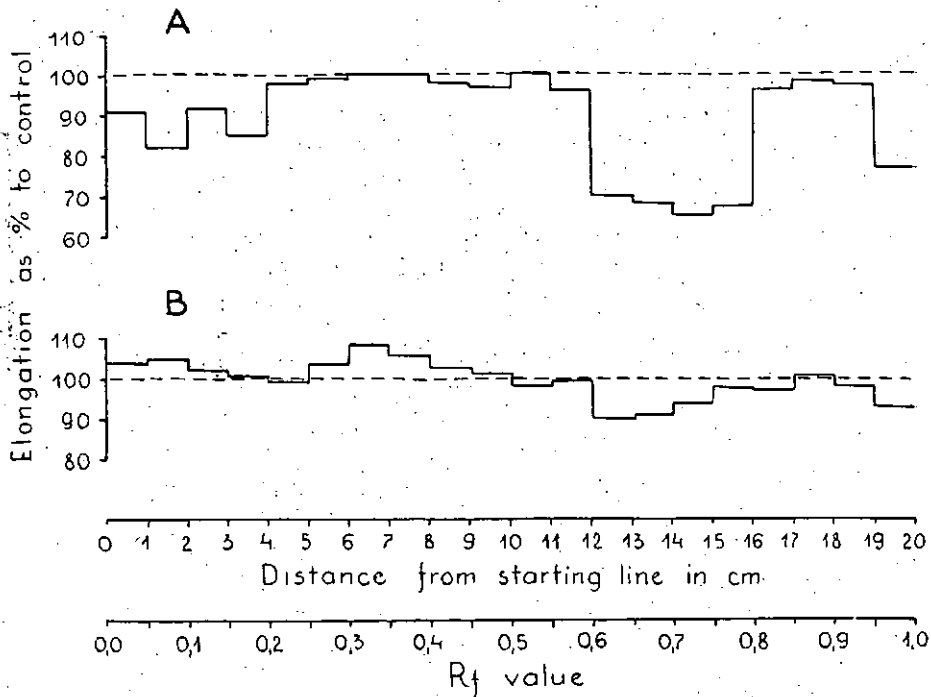


Fig. 1: Elongation of wheat coleoptile sections in the eluate of 1 cm. paper segments of the chromatograms made with the ether extract of normal lemon (A); and of that containing germinated seeds (B). Standard error  $\pm$  2%.

hibiting zones show a bright fluorescence (described in detail in 8) and after treatment with  $FeCl_3$  the spots of the tannic acids ( $R_f$  0,05—0,25) and the salicylic acid ( $R_f$  0,65) are well visible. On the other hand, on the chromato-

grams of the fruits containing germinated seeds the corresponding spot are much fainter and smaller in UV light and do not give any colour reaction after treatment with  $\text{FeCl}_3$ .

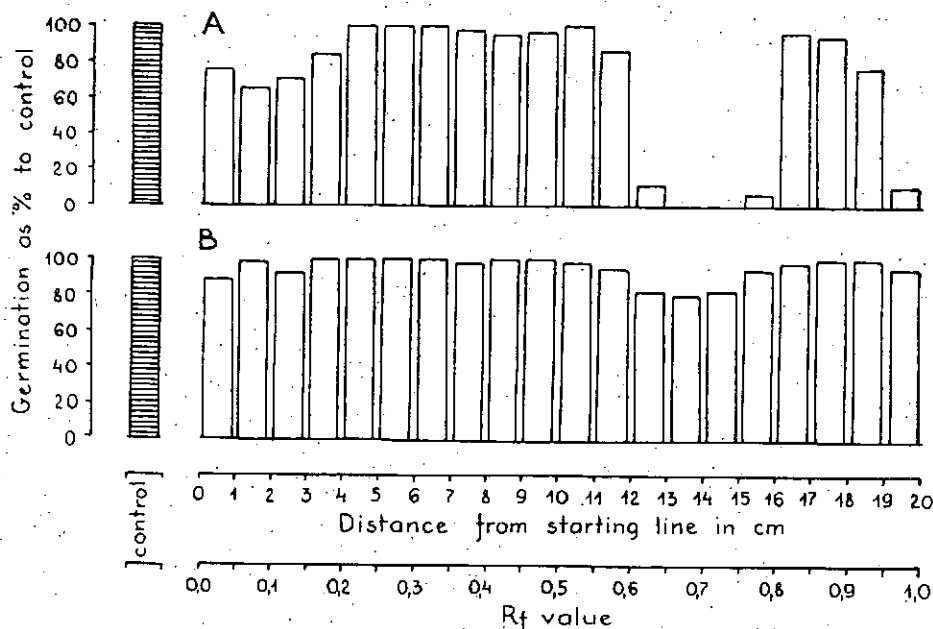


Fig. 2: Germination of *Papaver* seeds on 1 cm. paper segments of the chromatograms made with the ether extract of normal lemon (A); and of that containing germinated seeds (B). Standard error:  $\pm 2\%$ .

### Discussion

In a previous paper (6, 7) the conclusion was drawn that the inhibiting activity of fruit juice must be mainly attributed to aromatic acids (denoted as  $\beta$ -inhibitor complex) and essential oils and that in this respect the action of aliphatic carbonic acids is only secondary. The results reported in the present paper are entirely in accordance with this establishment and confirm it. Indeed, in the juice of lemon containing germinated seeds the  $\beta$ -inhibitor complex — which in the course of the identification (8) proved to be a mixture of salicylic acid, *o*- and *p*-coumaric acid and ferulic acid — is no more present in biologically significant quantities and similarly the amount of essential oils and aliphatic carbonic acids is also reduced.

For the correct interpretation of the data one must take the circumstance into account that the inhibitory responses observed at the biological test and demonstrated on the Figs. were obtained with highly condensed substances and that the same quantity of inhibitors is actually distributed in 100 ml. juice, i. e. in about 1 1/2 lemons of medium size. Consequently, it may be stated that the content of inhibitors in the juice of lemons containing ger-

minated seeds is so insignificant that in spite of the advantageous conditions it cannot retard the growth of the seedlings.

The reduction of the amount of aliphatic acids is shown on the one hand, by the fact that their inhibitory effect turns into a promoting one which is a characteristic feature of the low acid concentrations, and on the other, by the rise of the pH of the fruit juice.

On the chromatograms of the lemons containing seedlings the presence of IAA — which cannot be detected on those of freshly picked fruits — is marked. Most probably the formation of IAA is a result of the decomposition taking place in the fruit tissue.

The fact that seeds germinate in fleshy fruits is described in numerous earlier papers (1, 2, 4, 5) as a rare phenomenon. It is very likely that also on these cases this is due to the absence of the inhibitors mentioned above.

### Summary

Ether-extractable inhibiting substances of the juice of lemons containing germinated seeds and seedlings respectively, were chromatographed and bio-assayed. According to the results the derivatives of cinnamic- and benzoic acid summarized under the name of  $\beta$ -inhibitor complex — which is fresh lemons primarily responsible for the strong inhibitory action of the fruit juice — can only be found in quite insignificant quantities in these fruits. The amount of the other inhibiting agents in fruit juice, the essential oils and aliphatic organic acids decreases to a great extent too.

### References

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