Carotenogenesis in carrot roots

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Contents

Summary

A. In a carrot root two activities may proceed side by side. One is the primary vegetative growth of the root, resulting in enlargement of the root. The other is ripening, which is seen in the form of a thickening along the whole root and of rounding at the tip, and, in the carotene carrots, the orange coloration due to carotenoid production. Different conditions may favour one activity more than the other.

B. As the root grows its carotenoid concentration gradually increases up to a certain (fluctuating) maximum. This is caused by the increase of carotenoid in the individual cells of the ripening tissue, but also by the increase of the ripe upper part of the root in proportion to the whole root. The carotenoid contents of carrot roots should therefore be determined before the maximum has been reached and be related to the size of the roots. The safest way is to assess them in a series of successive harvests.

C. The carotenoid content that can be reached in carrot roots primarily depends on adequate photosynthesis.

If the roots are grown in a well-drained soil of good texture, there are two groups of growth conditions which may further modify the carotenoid content in relation to root weight. These are:

1. Such conditions as plant density, soil-moisture content, and plant-food supply, which control the growth rate and growth limits of the roots. A higher growth rate will result in a more rapid enlargement of the proportion of the ripe upper part of the root and give roots large enough to have a relatively high carotenoid content in a shorter time. Narrower growth limits will reduce or stop primary vegetative growth at an earlier period and favour earlier ripening (including increasing carotenoid content).
2. Temperature. When high, this factor favours ripening, and when low it favours primary vegetative growth. The favourable effect of relatively high temperatures is lost when the oxygen concentration in the soil atmosphere is 6 % or less.

D. A good theory on which to base further work would seem to be that carotenogenesis in the carrot root takes approximately the following form:

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Photosynthesis produces certain carbohydrates which are transported to the root, and at some phase of conversion are necessary for protein synthesis (primary vegetative growth) as well as for carotenoid synthesis (colouring).
The priority pattern for the two consumption processes is controlled by temperature. At a relatively low temperature (*e.g.* 8° C) protein synthesis has a high degree of priority; the same is true of carotenoid synthesis at a relatively high temperature (*e.g.* 20° C).
The priority pattern does not work when one of the consumption processes ceases for any reason. If primary vegetative growth is stopped at a low temperature through lack of space, water or food, protein synthesis will stop as well and the available quantity of carbohydrate compound can be used for carotenoid synthesis.

E. The consequences for the evaluation of field trials and the breeding of carrot varieties of a good colour are discussed.

1. Chemical classification of carotenoids

The carotenoids are mainly tetraterpenes. These compounds belong to the isoprenoids which can be thought of as polymerization products of the five-carbon compound isoprene, C_5H_8, although isoprene itself has not been found in plant material.
The lower isoprenoids with 10, 15, 20 or 30 C atoms form the bulk of the essential oils and resins; the higher ones, the polyterpenes, $(C_5H_8)n$, are rubber and gutta percha. The tetraterpenes, $C_{40}H_{64}$, which form the carotenes, occupy an intermediate stage.
The colourless phytofluene is, however, the only carotene which actually has the empirical formula $C_{40}H_{64}$. All other carotenes have the empirical formula $C_{40}H_{56}$.
Xanthophylls are oxygenated carotenes and together with the carotenes belong to the carotenoid group.
The carotenes occurring in orange carrot roots are β- and α-carotene and traces of γ-, δ-, ζ -carotene and lycopene. The total carotenoids in the roots usually consist of > 90 % of carotenes and < 10 % of xanthophylls (BOOTH, 1945).

2. Carotenoids synthesized in the root

According to BONNER (1950), all carotenoids appear to be synthesized *in situ* in the organ in which they are found. That this may be true of carrot roots is suggested by the following two viewpoints:

1. Both the roots and leaves of the carrot may contain carotene, but there is no correlation between the carotene concentrations in the two organs. White carrots, which have no root carotene, have about the same leaf-carotene concentration as varieties with an exceptionally high root-carotene concentration. Moreover, the seasonal variation in the root-carotene concentration has no positive or negative counterpart in the leaves (BOOTH, 1957).
2. Cut-off slices of carrot root, when floated on a synthetic nutrient medium containing a solution of mevalonic acid, $HOOC \cdot CH_2 \cdot \underset{\displaystyle CH_3}{\underset{|}{C(OH)}} \cdot CH_2 \cdot CH_2OH$, are able

to synthesize β-carotene and to incorporate the mevalonic acid in it, so that before the mevalonic acid is incorporated it loses its carboxyl group (BRAITHWAITE and GOODWIN, 1957). The carrot slices will also incorporate acetate into β-carotene, but mevalonic acid was found to be a much better precursor than acetate (GOODWIN, 1958).

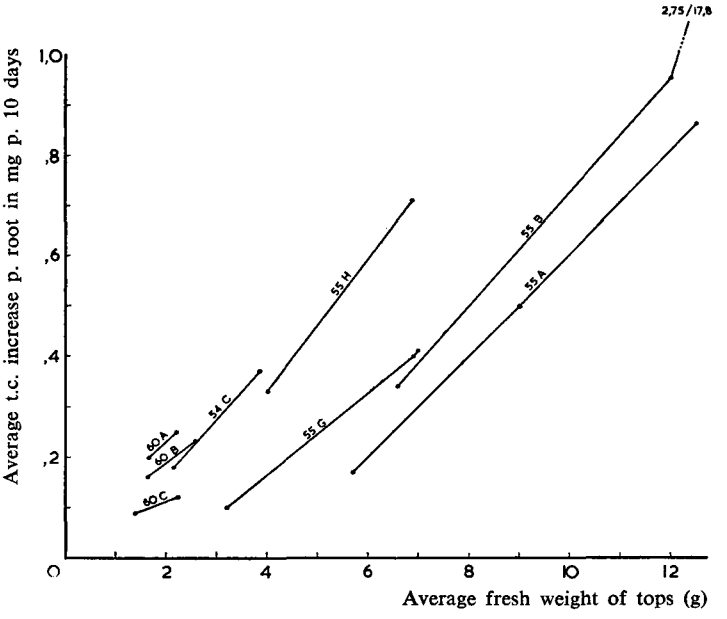
It is interesting that MODI and PATWA (1961) report the occurrence of mevalonic acid in carrot roots in quantities of 0,3—0,6 mg per 100 g of fresh material.

These two points indicate that root carotene can be synthesized in the root itself, and suggest that it probably always is.

3. Rôle of photosynthesis

The carbon of the root carotenoids, however, must have been initially supplied by the photosynthesis of the leaves, there being no other source.
From the data of our experiments of 1954—1960 we were, in fact, able to deduce two interesting correlations illustrating this aspect. One is the correlation between the size of the top as expressed by its fresh weight and the rate of the total-carotenoids (t.c.) increase per root (FIG. 1). The other is the correlation between the percentage of dry matter of the roots and their t.c. content (FIG. 2).
FIG. 1 shows the average values for the top-weight/t.c.-increase ratio under the contrasting treatments of eight experiments. Each of the experiments 60A, 60B, 60C contains carrots grown in pots with a low soil-moisture content (s.m.c.), as compared with other carrots grown in pots with a high s.m.c. In experiment 54C, carrots grown

FIG. 1. Correlation of average total-carotenoids (t.c.) increase per root and average fresh weight of tops in experiments from 1954 to 1960



The averages were calculated from data collected at the successive harvests per treatment per experiment.
Correlation coefficients:
For all experiments r = 0,874***
For 60A, 60B, 60C, 54C, 55H r = 0,957***
For 55A, 55B, 55G r = 0,926***

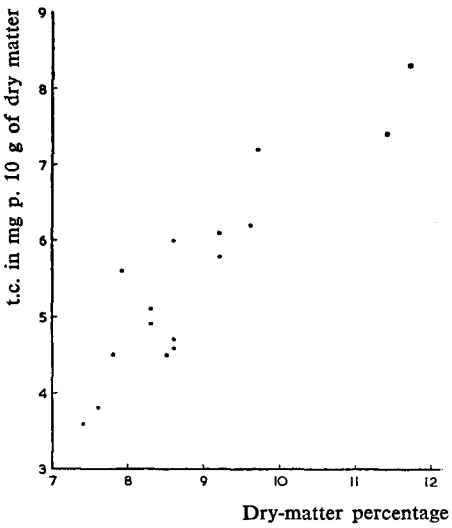


FIG. 2
Correlation of average total-carotenoids (t.c.) concentration and average dry-matter percentage of the roots in experiments from 1954 to 1960

The averages were calculated from data collected at the successive harvests per treatment per experiment.
Correlation coefficient: r = 0,907***

at 8° and 18° C are compared, and in the experiments 55H, 55G, 55B and 55A carrots grown at different plant densities. The tops are heaviest at the higher s.m.c.'s, at 18° C, and at the widest plant distances.

Taking all experiments together, the correlation coefficient r = 0,874, which is highly significant (P = 0,1). Taking separately a group I of 60A, 60B, 60C, 54C and 55H, and a group II of 55G, 55B, and 55A, the correlation coefficients are r = 0,957 and r = 0,926 respectively (both P = 0,1).

In this connection it is interesting to note that in the experiments of group I the carrots were grown under conditions which were not optimal for leaf growth (conditioned rooms or glasshouses, or a high, dry, sandy soil), and that in the experiments of group II they were grown under conditions which were more conducive to leaf growth (frame, or in the open, both on a low, sandy soil). It can be concluded, therefore, that the rate of increase of the t.c. in the root of a carrot is very closely correlated to the size of its top (here expressed by fresh weight). This is especially true when the general conditions for leaf growth are more or less comparable for the pooled experiments.

FIG. 2 shows the relationship between the level of the dry-matter percentage and that of the t.c. content of the roots. In most cases a single dot represents the average of the data from one treatment in one experiment, but sometimes that of the data from the same treatment in two or three experiments which had given similar results. The values were calculated from the results of our 1954—1960 experiments, but the data from our low-temperature and low soil-oxygen experiments were omitted for reasons to be explained later.

The dots in FIG. 2 lay in one trajectory, indicating a close correlation between the percentage of dry matter of the roots and their t.c. content. The correlation coefficient r = 0,907 is, in fact, highly significant.

The percentage of dry matter can be seen as an indicator of the intensity of photosynthesis. It appears, therefore, that the t.c. content of the roots depends very much on the intensity of photosynthesis, as well as on the size of the apparatus used for photosynthesis, *viz.* the size of the top (see FIG. 1).

4. Increase of total carotenoids with root growth

The carotenoid content in a carrot root increases with root growth until a maximum is reached. Any further increases in root-size do not result in any further increases in carotenoid content (BARNESS, 1936; WERNER, 1941; BROWN, 1947; BOOTH and DARK, 1949; LAMPRECHT and SVENSSEN, 1950). We were able to confirm this phenomenon in our own experiments (see under REFERENCES). The maximum carotenoid content reached is not the same in all seasons. Over a seven-year period BOOTH and DARK (1949) found a fluctuation of up to 20 % above or below the average. It is not unlikely that differences in photosynthesis in different seasons will be at least one cause of this fluctuation.

In comparing different varieties or differently treated groups of carrots for their carotenoid content, one should take into account the *carotenoid/root-size* ratio. We therefore made a practice of estimating in any group of carrots, the total-carotenoids (t.c.) content, the average root weight as a measure of root-size, and the dry-matter content. Occasionally the crude-fibre content was assessed as well. In most cases the experiments were so designed that a series of successive harvests could be made for each item. By plotting the successive carotenoid contents on the ordinate and the successive root weights on the abscissa the development of the relative carotenoid content can be visualized in a curve. Different curves can then be graphically compared. As the dry weight was usually found to be more reliable than the fresh weight, the data was normally calculated on a dry-weight basis. In some cases it was calculated on a crude-fibre basis.

5. Degree of ripening of the root coincides with degree of carotenoid increase

What is it that the carotenoid content increases with root growth? It cannot be a function of the photosynthetic activity of the leaves, for it happens under poor or good light conditions. Nor is it top-/root-size ratio, for this decreases during the growth of the plant, mainly owing to the increase in root-size. In fact, if we take the average fresh weight of the top divided by the average fresh weight of the root per treatment per experiment of all our relevant experiments and show this in a graph together with the t.c. content (see FIG. 3), we can see that there is a tendency for the higher t.c. contents to go with the lower values of the *top/root* ratio.

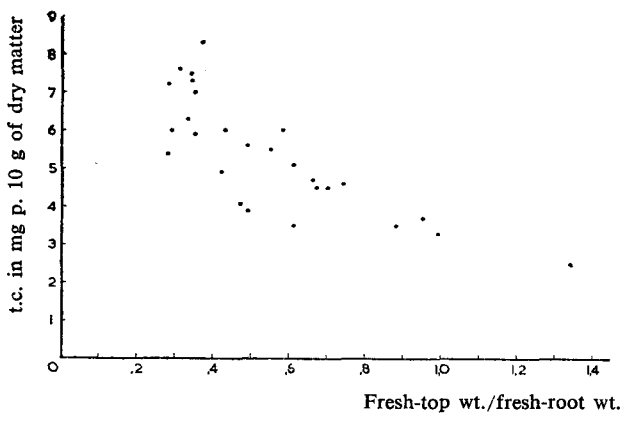


FIG. 3
Negative correlation between the values of the top/root ratio and the total-carotenoids (t.c.) content of the root in experiments from 1954 to 1960

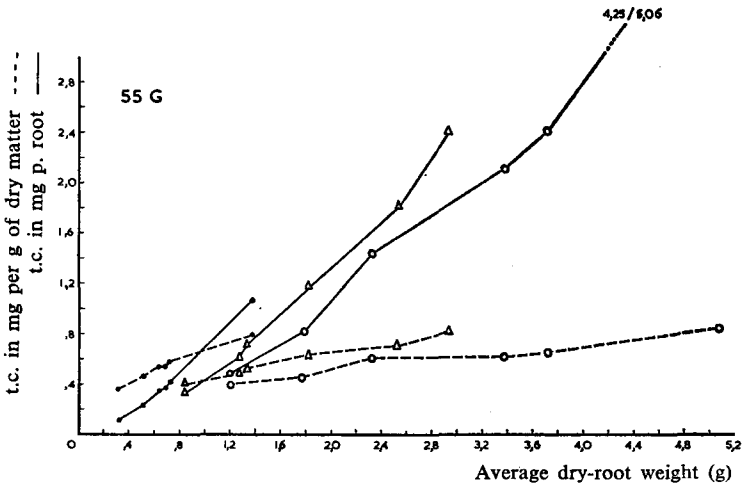
The averages were calculated from data collected at the successive harvests per treatment per experiment.
Correlation coefficient:
$r = -0{,}796^{***}$

The coefficient for the negative correlation between the values of the *top/root* ratio and the t.c. content of the root (r = 0,796) is highly significant. This indicates that the increase of the t.c. content of the root during growth is not caused by an over-capacity of the photosynthesis apparatus.
Hence the reason why the carotenoid content increases with root growth probably resides in the root itself. In our experiments we obtained the impression that the increase in the t.c. content of the root is connected with the degree of ripening reached by a root.
Ripening is normally characterized by a tendency for the root to become smoothly thickened over the whole length and well-rounded at the tip. There is a simultaneous tendency to become more coloured (= increase in the carotenoid content). Ripening does not occur simultaneously in all parts of the root. So long as the root continues to grow at the tip, the latter is not so ripe as the upper part of the root. In due course the upper part will have stopped growing and have ripened. While the root is growing the upper, stationary ripe part normally increases in proportion to the whole root. In addition to the increase in the carotenoid content of the individual root cells, the proportional increase of the ripened part of the root is another factor that increases the carotenoid content of the whole root.

6. Factors influencing the ripening equilibrim of the root

In order to learn more on this matter we studied the influence of plant density, temperature, soil-moisture content and oxygen content of the soil atmosphere. We studied the influence of these factors on the t.c. content of the root with reference to the root weight and root shape.
A carrot root does not develop in different, strictly successive phases of maturation. If we distinguish two elements in its development, *viz.* primary vegetative growth and ripening (= thickening and colouring), experience shows that these two elements usually operate simultaneously in any developing carrot root. Under different growth conditions, however, the degree of activity of one element may differ in proportion to that of the other. Thus a shift in the growth conditions may give rise to a shift in the equilibrium between these two elements, *viz.* in what we have termed the ripening equilibrium.
We will now turn to the results of our experiments on the influence of certain factors on the ripening equilibrium of the carrot root.
FIG. 4 and 5 show the influence of plant density in a summer trial. In FIG. 4 the dashed lines show the trend of the t.c. in mg per g of dry matter, while the solid lines show the trend of the t.c. in mg per root. In both cases it can be seen that at similar dry-root weights the t.c. content of the more narrowly grown roots is higher than that of the roots which had more space. In FIG. 5 roots of about the same dry-root weight, grown at narrow, normal, or wide plant distances, show differences in shape.
Combining the two results, we see that narrowing the plant distances promotes the ripening of the smaller-size roots. This is shown by the degree of thickening of the roots over the whole length, the degree of rounding of their tips, and their carotenoid content. It seems that in this case earlier ripening is caused by the suppression of the primary vegetative growth through lack of space.
The influence of temperature is shown in FIG. 6 and 7. The roots grow more rapidly at 18° C than at 8° C. But ripening is also more rapid at 18° C. At a given dry-root

FIG. 4. The influence of plant density on the ratio of total-carotenoids (t.c.) content and dry-root weight in a summer trial



The dashed lines show the course of the ratio in the successive harvests for the t.c. concentration in mg per g of dry matter; the solid lines show the same course for the t.c. content in mg per root.
• narrow spacing (5 × 1,5 cm)
△ normal spacing (13 × 1,5 cm)
o wide spacing (20 × 10 cm)

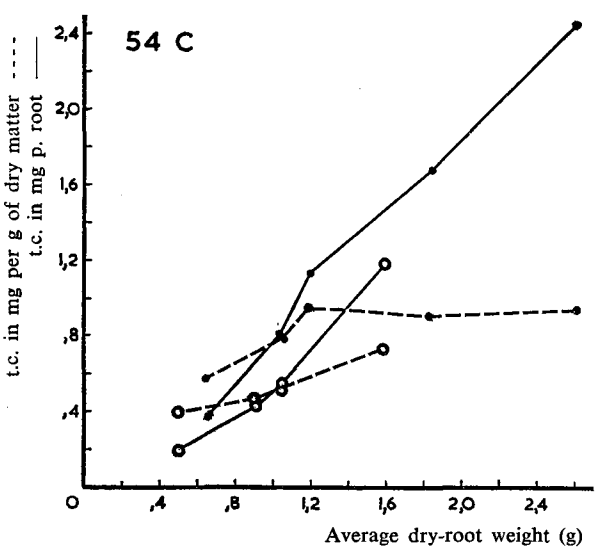


FIG. 6
The influence of temperature on the ratio in total-carotenoids (t.c.) content and dry-root weight in a phytotron trial

The curve for 18° C is based on five successive harvests, the curve for 8° C on the 1st, 3rd, 4th, and 5th harvests only, because the figure assessed for the t.c. content of the 2nd harvest was probably wrong

The dashed and solid lines have the same significance as in FIG. 4.
• 18° C. o 8° C.

weight there is much greater thickening of the roots and rounding of their tips at 18° C than at 8° C, and the t.c. content is also higher at 18° C than at 8° C. In this

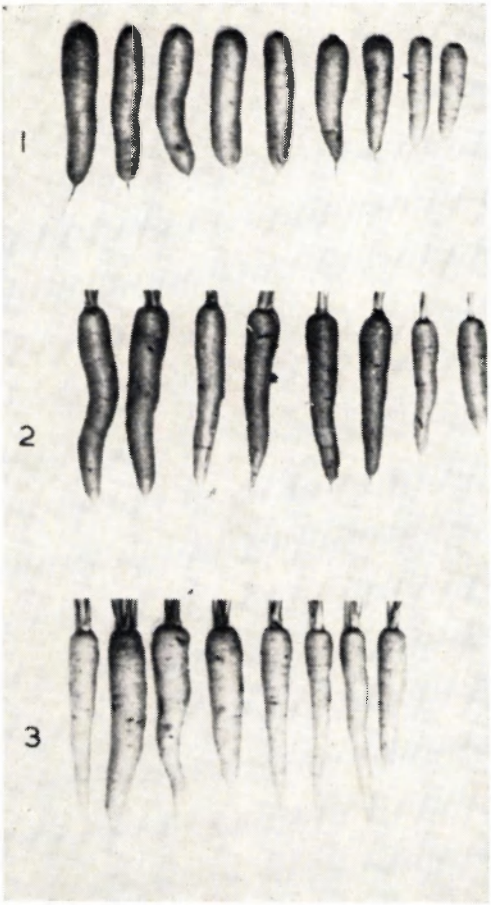


FIG. 5
Samples of roots of about the same average dry-root weight from narrow (1), normal (2), and wide spacing (3) in the same experiment as FIG. 4

Roots from the narrow spacing (1) which have about the same dry-root weight as those from the normal spacing (2), are more thickened over their whole length, more rounded at the tips, and shorter.
A similar difference is visible when roots from the normal (2) and wide (3) spacings are compared.

FIG. 7
Samples of roots of about the same average dry-root weight grown at 18° C and at 8° C

Roots grown at 18° C which have about the same dry-root weight as those grown at 8° C, are more thickened over their whole length, more rounded at the tip and shorter.

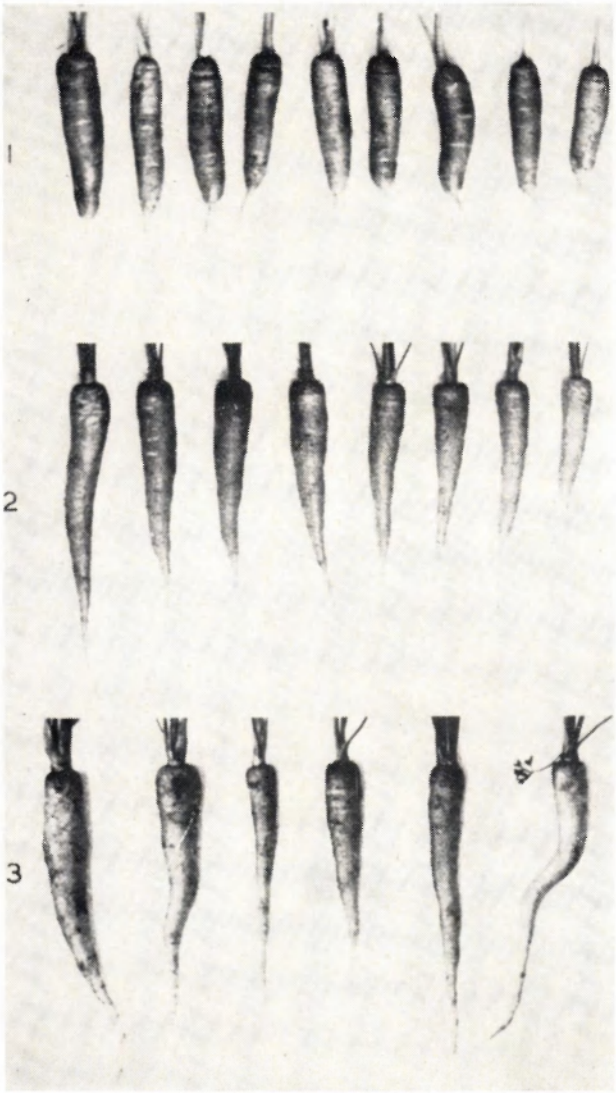
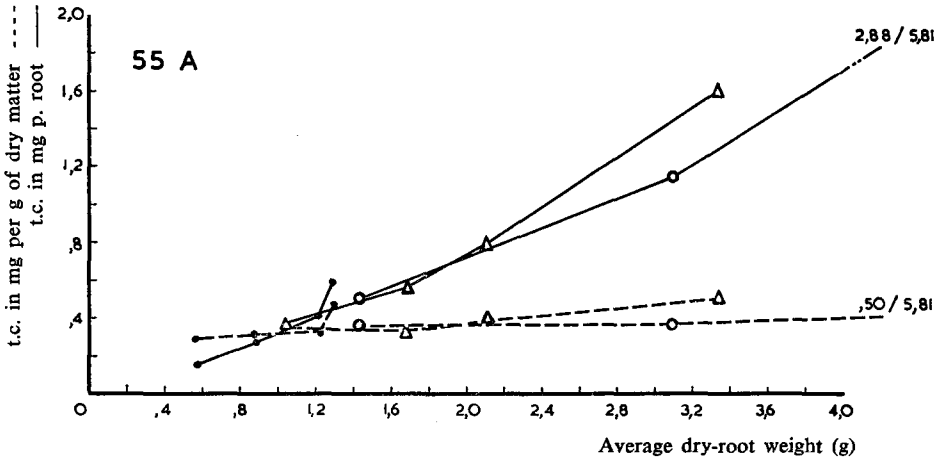


FIG. 9
Samples of roots of about the same average dry-root weight from narrow (1), normal (2), and wide spacing (3) in a cold frame during the winter (same experiment as FIG. 8)

According as the spacings were narrower, roots of about the same dry-root weight became more thickened over their whole length, more rounded at the tip and shorter.

FIG. 8. The influence of plant density on the ratio of total-carotenoids (t.c.) content and dry-root weight in a cold frame during winter (low temperature)



The dashed and solid lines have the same significance as in FIG. 4.

• narrow spacing (5 × 1,5 cm)
△ normal spacing (13 × 1,5 cm)
o wide spacing (20 × 10 cm)

case no external factor can have caused the roots to ripen earlier. It must have been an internal change induced by the higher temperature.

FIG. 8 and 9 give the results of a plant-density trial in a cold frame during the winter, *i.e.* at a fairly low temperature. Growth is slower and the general level of the t.c. content remains lower than in the summer trial of FIG. 4 and 5, this being no doubt due to the low temperature. But we can see that there is substantially the same response to plant density. The t.c. content at the three plant densities remains about the same for a long period. But when the plants become overcrowded the t.c. content rises, probably because further primary vegetative growth is suppressed by lack of space.

FIG. 10 and 11 show the influence of the soil-moisture content (s.m.c.). In the soil with a high s.m.c. root growth is more rapid than at the low s.m.c. At a certain dry-root weight the t.c. content at the lower s.m.c. is higher than at the high s.m.c. Evidently primary vegetative root growth is more or less suppressed at the low s.m.c., causing a ripening of the root at a smaller root-size. This is hardly if at all reflected in the root shape, probably because in dry soil root thickening is somewhat suppressed by the dryness, and in wet soil the available moisture is conducive to thickening.

FIG. 12 shows the results of varying the oxygen content of the soil atmosphere in large pots. Two open control pots were harvested just before the different treatments started; one open control pot was harvested over a period of two weeks with eight pots with reduced oxygen contents, and one open control pot two weeks later.

A dashed line was drawn through the dots of the four control pots for the t.c. in mg per g of dry matter, and a solid line through the dots of the four control pots for the t.c. in mg per root. The dots for the eight pots with reduced oxygen contents were then inserted. The dots for the pots with 9,5 % or more oxygen are all on or very near the control lines. An oxygen content $\geqslant$ 9,5 % evidently does not shift the

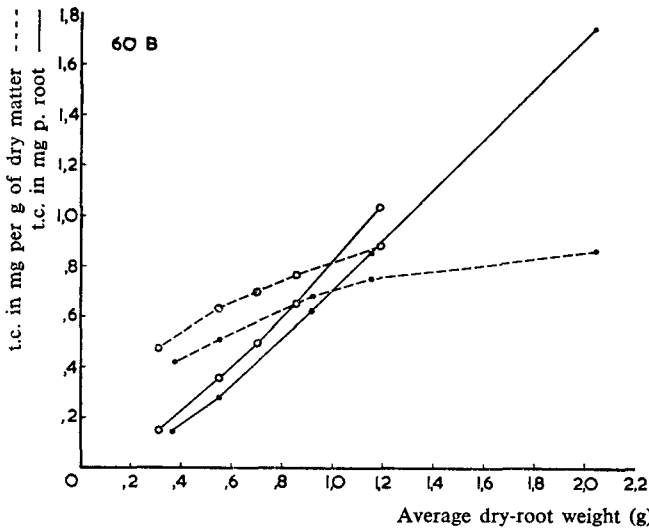
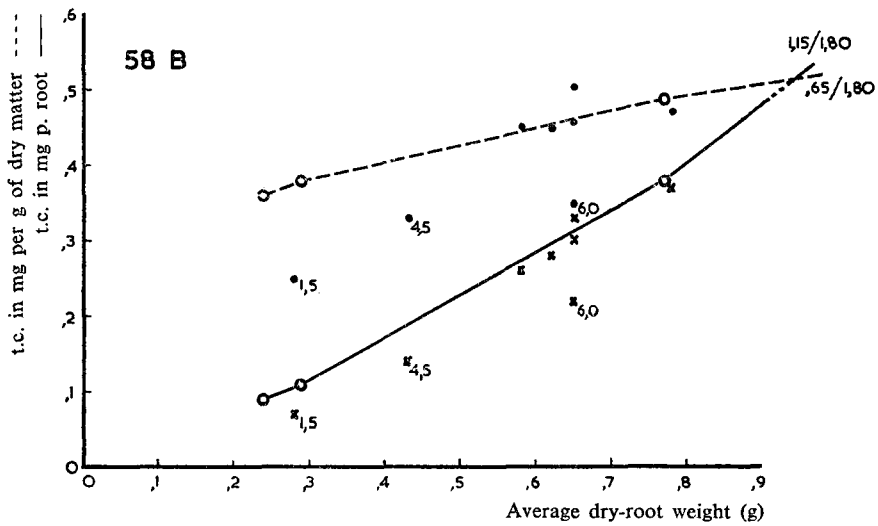


FIG. 10
The influence of the soil-moisture content on the ratio of total-carotenoids (t.c.) content and dry-root weight in a clay soil in containers in a conditioned glasshouse at 14° C

The temperature in the wet and dry containers was registered and automatically adjusted to the same level, if necessary.
The dashed and solid lines have the same significance as in FIG. 4.

o relatively dry
• relatively wet

FIG. 12. The influence of the oxygen concentration in the soil atmosphere in containers placed in a conditioned glasshouse at 17° C



The broken and solid lines have the same significance as in FIG. 4.

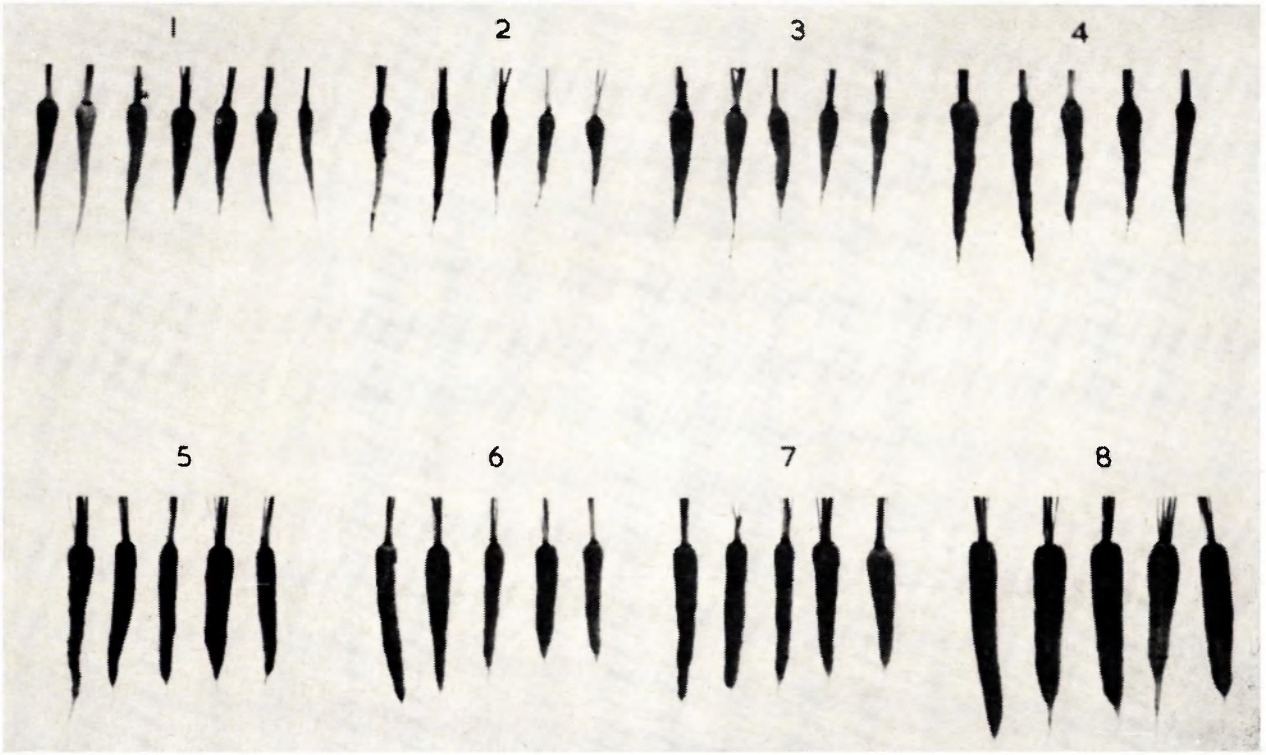
o open control pots.
• x pots with regulated flow of oxygen-nitrogen mixture through the soil; the numerals 1,5; 4,5; 6,0 denote that 1,5; 4,5; 6,0 % oxygen was measured at the outlet of the container. Dots and crosses without numerals represent oxygen percentages of 9,5 up to 18.

ripening equilibrium. But the dots for 1,5; 4,5; and 6,0 % of oxygen are significantly below the control line, which indicates that in this case the ripening equilibrium was shifted.

FIG. 11. Samples of roots of about the same average dry-root weight from relatively dry and relatively wet containers

According as the t.c. content of the roots from the relatively dry containers is higher than that of the roots from the relatively wet containers, more thickened roots might be expected from the relatively dry containers. There is, however, hardly any difference in shape, probably because root thickening was more or less suppressed in the dry soil and favoured in the wet soil.

FIG. 13. Samples of roots from pots with different oxygen contents of the soil atmosphere



1. 22nd April, open pot, just before the differentiation in the oxygen content started.
2. 7th May, after two weeks 1,5 % O_2
3. 7th May, „ „ „ 4,5 % O_2
4. 7th May, „ „ „ 6,0 % O_2
5. 7th May, „ „ „ 9,5 % O_2
6. 7th May, after two weeks 12,0 % O_2
7. 7th May, „ „ „ 18,0 % O_2
8. 19th May, open pot

In comparison with the normally grown roots on 22nd April, those grown at 9,5—18 % O_2 have (on 7th May) become longer and thickened over their whole length. The roots grown at 1,5 % O_2 have the same shape as the control roots on 22nd April; those grown at 4,5 % O_2 have become larger, but the shape has hardly changed. On 7th May the roots grown at 6,0 % O_2 reached the normal size (as compared with those at 9,5—18 % O_2), but their tips are slightly thinner.

FIG. 13 (No. 1) shows roots just before the treatments started. In the atmosphere with 1,5 or 4,5 % oxygen (Nos. 2 and 3) the roots have grown longer, but below the initial thickening no further thickening along the length of the roots has taken place. In the atmosphere with 9,5 % or $> 9{,}5$ % O_2 the roots have grown longer and have also thickened along the whole length. In the atmosphere with 6,0 % O_2 thickening has taken place as well as lengthening, but not quite to the same extent as in the atmosphere with a higher O_2-content.

7. Discussion

K. PAECH (1957) has an attractive theory on the competition of carotenoid and protein synthesis in plants. This theory may be formulated as follows: *Both carotenoid and protein synthesis are primarily dependent on the intermediate compounds of general carbohydrate metabolism. When there is competition protein synthesis takes priority.*

PAECH (1957) adduced evidence in favour of this theory in experiments with young wheat seedlings. He grew these seedlings on water and 0,0; 0,05; 0,1 or 0,2 % urea and analysed them after they had grown for 6, 8 or 10 days. After six and eight days synthesis of soluble nitrogen compounds or protein had increased more with higher doses of urea; at the same time the carotenoid increase was lower with the higher urea doses. After 10 days carbohydrate reserves seemed to be exhausted and disintegration started. The conclusion was that less carotenoid was produced so long as protein synthesis removed the carbohydrate compounds.

Similar results have been observed with carotene-producing fungi and algae. They start real carotene production after a sharp decline or complete stoppage in the growth of the mycelial mat or cell-division. For fungi *cf.* GARTON *et al.* (1951), GOODWIN *et al.* (1952), MASE *et al.* (1957), and for algae DROOP (1955).

The results reported in the foregoing paragraphs do not seem to contradict the PAECH theory.

In the cases of ripening at a smaller root-size owing to a higher plant density or a lower s.m.c., primary vegetative growth is reduced. This means that protein synthesis is also reduced and that it is consuming less carbohydrate compounds. Consequently, most of the carbohydrate compounds available can be used for other consuming processes such as carotenoid synthesis.

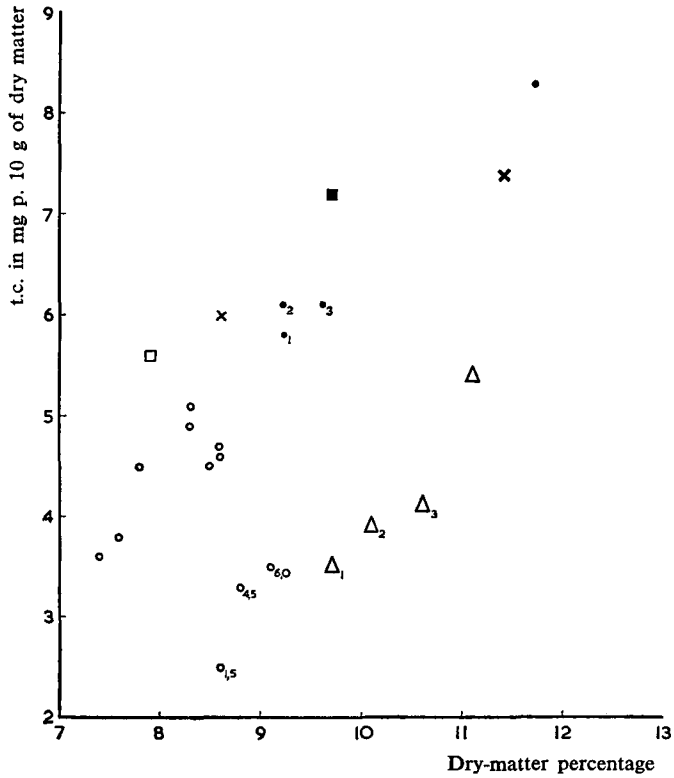
In cases of ripening at a smaller root-size because of a relatively high temperature, the relatively high temperature evidently changes the priority pattern of the consumption of carbohydrate compounds. At a lower temperature less of the available carbohydrate compound is used for carotenoid synthesis; at a higher temperature the greater part is used.

An oxygen content $\leqslant 6$ % in the soil atmosphere also reduces carotenoid synthesis in some way.

We find a confirmation of this in FIG. 14, which contains the same data as FIG. 2 plus the low temperature and the low oxygen data which were omitted there.

The main group in FIG. 14 contains the points for roots grown at different plant distances or s.m.c.'s at temperatures of 14°, 17°, 18° C or at a summer temperature, in a soil with an atmosphere containing at least 9,5 % oxygen. The coefficient of correlation between the t.c. content of the roots and their dry-matter percentage, $r = 0{,}907$, is highly significant.

FIG. 14. Correlation of average total-carotenoids (t.c.) concentration and average dry-matter percentage of the roots



The same data as used in FIG. 2, but now completed with those for plants grown at a low temperature or at a low oxygen concentration in the soil atmosphere.

- • warm (18° C) } Exp. 54C
- △ cold (8° C) }
- • 1, 2, 3 warm (summer). Average of experiments 55B, 55G,55H
- △ 1, 2, 3 cold (winter). Exp. 55A
- × soil dry } Average of experiments 60B, 60C (14° C)
- ■ soil wet }
- × soil dry } Exp. 60A (14° C)
- □ soil wet }
- o soil atmospheres containing 9,5—20,8 % O_2 (17° C) } Exp. 58B
- o 1,5; 4,5; 6,0 soil atmospheres containing 1,5; 4,5 or 6,0 % O_2 (17° C) }

The group below the main group contains the points for roots grown at 8° C or at wintertemperature in a cold frame at Wageningen and for those grown at 17° C in a soil with an atmosphere containing 1,5; 4,5 or 6,0 % of O_2. In this group there is a highly significant correlation between t.c. content and percentage of dry weight ($r = 0{,}919$), but in proportion to the same percentages of dry weight the level of the t.c. contents is much lower than in the main group.

This suggests that at a low temperature, or at a low oxygen concentration in the soil atmosphere, the distribution pattern of carbohydrate compounds for carotenoid synthesis and other consuming processes is actually less favourable for carotenoid synthesis than at higher temperatures and higher oxygen concentrations.
It is interesting to note that a difference in plant distances does not alter the relationship between the t.c. content and the dry-matter percentage, all points being in the same group.
As for the influence of different soil-moisture contents, there is a suggestion that there may be a small difference for plants grown at a low or high s.m.c. Calculation of the regression line of the main group minus the open and blocked squares (= pots with a high s.m.c.) ($\bar{y} = -3{,}4 + 0{,}998\ \bar{x}$) shows that the open and blocked squares differ significantly (P = 1 %) from the regression line. This means that the plants from the wet pots have a higher t.c. content than might be expected from their dry-matter percentage. But this does not necessarily imply a change in the distribution of the carbohydrate compounds between carotenoid and protein synthesis, but only that less carbohydrate compounds were used on cell-wall material owing to the formation of larger cells with thinner walls. In this case more carbohydrate material could be used on carotenoid and protein synthesis than was expected from the dry-matter percentage, without changing the distribution ratio between the two.
We must conclude from the available data that root ripening is not the cause of the change in the distribution of carbohydrate material, but is identical with this change. The change in the priority for protein synthesis may arise from such external causes as lack of space for further growth or a low s.m.c., or by an internal cause induced by relatively high temperature. If the oxygen content of the soil atmosphere $\leqslant 6\ \%$ at the relatively high temperature the priority of the protein synthesis is maintained. It seems probable that the thickening over the whole length of the root and rounding of the tip has the same cause as the colour increase, *viz.* the availability of more carbohydrate compounds.

8. Consequences for the evaluation of field trials

It is obvious from the phenomena described above that carotene contents assessed for carrot roots can only be used as evidence provided they are related to the size and the ripening equilibrium of the roots. This can be further demonstrated by the discussion of some experiments reported in the literature.
In manurial trials on carrots JANES (1946) and GUÉRILLOT-VINET *et al.* (1961) did not find any significant effect of different nitrogen levels on the carotene content of the roots, but in carrots harvested from high nitrogen plots POLLARD (1941) found 125—131 mg carotene per 100 g of dry material, against 100—109 mg in those taken from low nitrogen plots. FREEMAN and HARRIS (1951) and WOLF (1955) studied the effect of five nitrogen levels on carrots grown in nitrogen-deficient soils. In the FREEMAN and HARRIS experiment, a highly significant correlation was found between the manurial nitrogen level and the carotene content of the harvested roots. In the WOLF experiment there was an increase of the carotene content in the roots along the line 0 × N, 1 × N, 2 × N, 4 × N, 3 × N.
The evident conclusion for the latter three experiments is that the carotene production in carrot roots depends upon an adequate supply of nitrogen. But since the average root weights were not published we cannot check the relationship between carotene content and root development in this case. Any conclusion about a direct

relationship between carotene content and nitrogen supply is therefore premature as it is quite possible that:

a. in the roots of the experiments cited the maximal carotene contents had not been reached at the time of harvesting;
b. the level of the carotene content was therefore primarily correlated to the size of the roots, and not to the nitrogen supply;
c. it was only the size of the roots that directly responded to the nitrogen level. When growth rate is high the upper, non-growing, ripe part of the root also rapidly increases; thus there is a rapid increase on the carotene content of the whole root.

If the supposition is correct, the differently fertilized roots might have shown the same carotene content if they had been harvested at a later period when the carotene content in every group had reached its maximum. On the other hand, any factor that under a certain set of conditions favours a more rapid root development may show a more rapid increase in the carotene content; in this respect nitrogen has no specific effect. This may possibly explain the contradictory results of different authors.

Another example is found in an article by KELLY, SOMERS and ELLIS (1952), who studied the effect of boron supply on the growth and carotene content of the roots of *Red Cored Chantenay*. They found in roots grown under conditions of boron deficiency a significantly lower carotene content than in roots with an adequate boron supply. They thought that this might be due to a disruption of the carbohydrate supply to the roots rather than to a direct effect of boron on the synthesis of carotene, there being no apparent relationship between the carotene content and the boron content of the roots. They evidently considered the carotene content of the boron-deficient roots to be sub-normal.

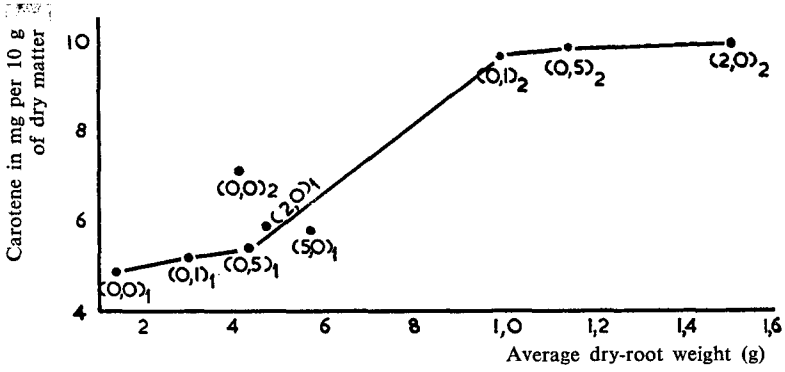
If, however, we compare the carotene contents, paying due attention to the average root weights, we can see that the reverse is true.

The authors grew the roots in pots; in the largest experiment they harvested at two periods and assessed the total carotenoid contents as well as the average dry-root weights. When the data are presented graphically, we obtain the curve shown in FIG. 15. In this figure the carotene contents are plotted on the ordinate and the dry-root weights on the abscissa.

We see the usual curve for the rising carotene content in relation to the increasing dry-root weight. The points for the carotene contents at the different levels of boron supply at the 1st and 2nd harvests are on or very near the curve. Only point $(0,0)_2$, *viz.* the t.c. content of the boron-deficient roots at the second harvest, is above the curve. This means that the t.c. content is higher than would be expected in relation to the dry-root weight reached. This would disprove the theory of a disrupted carbohydrate supply. It seems more probable, therefore, that growth is reduced by boron deficiency, thus preventing protein synthesis from using its normal part of the carbohydrate supply, and consequently, more is available for the carotene synthesis.

A further example is provided by SCHUPHAN (1962) in his study of the effect of the seed-size on the carotene content of the roots of *Nantes*. Screening the seeds produced three groups with diameters $> 1{,}00$ mm; 1,00—0,75 mm and $< 0{,}75$ mm. Over 1959, 1960, and 1961 the average emergence after sowing was 63 % for the group with diameter $> 1{,}00$ mm, 53 % for the group with diameter $= 1{,}00$—0,75 mm, and 27 % for the third group. The two extreme groups were compared for carotene

FIG. 15. The effect of various levels of boron supply on growth and carotene content of roots of *Red Cored Chantenay*, according to data produced by KELLY, SOMERS, and ELLIS (1952)



Sowing date: 20th June 1946, dates of harvesting: 26th September and 8th November. In brackets. boron supply; 1 and 2 denote 1st and 2nd harvest respectively.

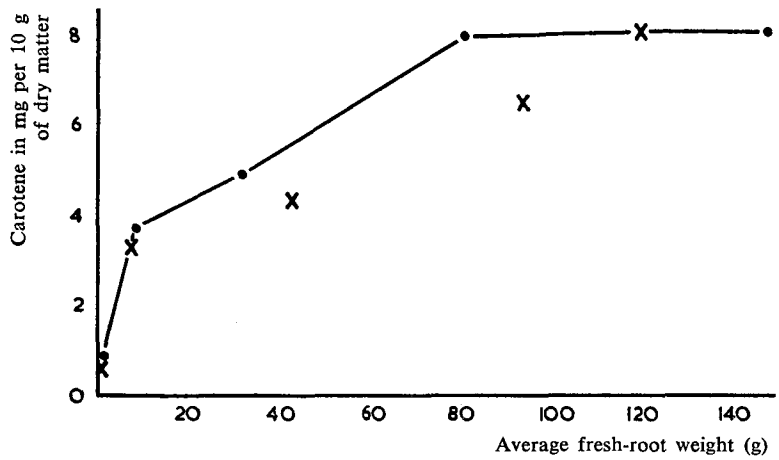
contents. At the start the roots from the smallest seeds grew more slowly than those from the largest seeds. But owing to the greater plant density of the roots from the largest seeds, their primary vegetative growth was soon slowed down through lack of space, whereas the roots from the smaller seeds, owing to the smaller plant density, had more space to grow and soon grew larger than those from the largest seeds. The carotene content of the roots from the largest seeds was higher for much of the season. Ultimately the carotene contents of the roots grown from the two seed groups became about the same. SCHUPMAN (1962) put forward the view that the carotene in carrot roots is an excreted substance of which the concentration depends on the intensity of metabolism, the slower germination of the smaller seeds thus causing a slower metabolism and consequently a lower carotene content.

In order to check the carotene contents presented by SCHUPHAN against the average root weights published, we plotted the two values shown in FIG. 16. If we consider the curve for the carotene contents of the roots grown from the larger seeds as the normal curve, we can compare with this curve the carotene contents of the roots grown from the smaller seeds. We see that:

a. during the period of slow growth of the roots from the smaller seeds their carotene-content/root-weight ratio is normal (first two harvests);
b. during the period of accelerated growth of the roots from the smaller seeds their carotene-content/root-weight ratio is sub-normal (3rd and 4th harvests);
c. ultimately the carotene contents of the roots from both groups of seeds seem to reach the same maximum.

The phenomena mentioned under a and b do not support the idea that a low carotene content is specifically correlated to a low metabolism. The opposite suggestion would have more weight if growth rate and intensity of metabolism could be regarded as parallel phenomena. But, as our experiments and deductions have shown, it is not primarily or solely the intensity of metabolism but the distribution pattern of products of metabolism that controls the carotene synthesis in carrot roots.

FIG. 16. The effect of seed-size on carotene content of roots of *Nantes* in 1960, according to SCHUPHAN (1962)



Sowing April 1961, harvested periodically during June—September.
• Carotene contents for roots grown from large seeds (diameter > 1,00 mm)
X Carotene contents for roots grown from small seeds (diameter < 0,75 mm)

As for the effect of seed-size on carotene content as reported by SCHUPHAN (1962), this does not seem to be a specific effect of the seed-size on the carotene content, but merely a case of the effect of plant density (plant distances) on the carotene content.

Thus we see that it is impossible to interpret carotene contents of carrots in field trials if the average root weights are unknown. And even if they are known, the interpretation still requires very careful consideration.

9. Consequences for the breeding of carrot varieties of a good colour

Assuming the roots to be grown for selection in a well-drained soil of a good texture, there are two groups of growth conditions that may modify the carotene content, *viz.*:

a. conditions such as plant density, soil-moisture content, and plant-food supply, that control the growth rate and the growth limits of the roots;
b. temperature, affecting the ripening equilibrium in the roots, and therefore, we assume, the priority system between protein synthesis (growth) and carotene synthesis (colouring).

It is very difficult to homogenize in a selection plot the conditions mentioned under a. Even if it were possible to homogenize the local atmosphere and soil, differences in the plants would be apt to spoil the homogeneity. Differences in the germination capacity of the seeds or the viability of the plants would be sufficient to cause differences in plant density, thus affecting the carotene-content/root-weight ratio. Of course the aim would be to obtain the maximum possible homogeneity of growth conditions in a selection plot, but one cannot hope to exclude fluctuation in this way. In many cases one must be content to understand afterwards what has happened.

On the other hand the temperature effect may be worth attention as a factor that can be manipulated in breeding work. In a variety suitable for producing roots of a good colour under cool growth conditions the priority system should change at a lower temperature than in a variety suitable for producing a good yield of roots of a good colour under relatively warm conditions. When grown at a fairly high temperature a variety adapted to cool conditions will remain small and very soon ripen, whereas a variety adapted to relatively high temperatures will usually develop poorly coloured and shaped roots when grown at too low a temperature.

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