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TRANSLOCATION OF C^{14}-PHOTOSYNTHATES IN THE GRAFT MUSKMELON/CUCURBITA FICIFOLIA

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INTRODUCTION

The graft combination of muskmelon on *Cucurbita ficifolia* was shown by WELLENSIEK (1949) to be incompatible unless some foliage is maintained on the rootstock. Removing this stock foliage results in a rapid death of the whole combination; dying significantly starts in the stock. Because of its interesting nature, DE STIGTER (1956) studied this graft in more detail, including items like the relationships between number of stock leaves and scion growth, distribution patterns of starch, root formation in graft-cuttings, behaviour of double graft combinations and histological observations. On the basis of the results obtained, the conclusion was reached that the incompatibility in the absence of stock leaves is not due to a lack of union between the partners, but rather to the lack of a factor, a "specific substance", being produced by the leaves of the cucurbit stock only, but not by the melon scion, and necessary for the proper functioning of the stock's phloem.

From the above *résumé* it is evident that in one way or another, translocation of organic compounds must be involved. In order to check the above hypothesis, and to obtain a more detailed picture of the distribution of photosynthates in this graft, it was decided to study these processes by means of the radioactive carbon isotope C^{14}.

As our problem turned out to be more complex than it had at first appeared, it was felt necessary to reconsider the whole subject, opposing once again the two major points of view of our above-mentioned paper, *i.e.*, the purely structural, anatomical approach, and the more physiological one. In our opinion, the considerations to be developed apply to the physiology of grafted plants in general, and thus are not limited to our special case only.

MATERIALS AND METHODS

As experimental material, muskmelon, *Cucumis melo* L., var. Suikermeloen, and *Cucurbita ficifolia* Bouch. were used. A detailed account of raising the plants, and of the grafting techniques has been given in our previous paper.

As before, the various graft combinations will be indicated by symbols using the following abbreviations and signs:

M and F : muskmelon and *C. ficifolia*, respectively;
\+ and —: with and without leaves on the stock, resp.;
*: place of application of C^{14}.

The young grafts were grown with two or three leaves on the stock. In experiments without stock leaves, these were removed one day before, or just prior to the administration of C^{14}.

Unless otherwise specified, the lowermost leaf of the melon scion was always treated, by exposing it to $C^{14}O_2$. The $C^{14}O_2$ was released from $BaC^{14}O_3$ by lactic acid, and circulated in a closed circuit containing up to six leaf chambers in a parallel arrangement. Thus, a varying number of plants, to a maximum of six, could be treated at the same time. As a rule, 15 μc of C^{14} were given per plant. The exposed leaves did not stay in the photosynthesis chambers for periods longer than one hour; the total duration of the translocating periods varied from a few hours to one day, or in some cases even two days.

At the end of the experimental period, the treated leaves were detached, and the plants cut into several pieces, to facilitate handling after drying. The treated leaves were loosely wrapped in tissue paper to prevent contamination of other parts. The plants were arranged in wire trays, quick-frozen with pulverized dry ice, and vacuum-dried at a temperature of about —15° C; *cf.* YAMAGUCHI and CRAFTS (1958).

The dried plants were carefully flattened, mounted on 14″ × 17″ sheets of paper, and pressed. For autoradiographing, as for the freeze-drying, the procedures described by Yamaguchi and Crafts were followed. The film material used was Kodak No-Screen X-ray film, and the time of exposure 14 days.

For histological details a few microautoradiographs were made, employing a modified stripping-film technique. The material (short pieces of rootstock stem) was dehydrated by freeze-substitution in methyl-cellosolve, embedded in paraffin and sectioned at 15 microns thickness.

For the histological examination of phloem, the tannic acid — ferric chloride — lacmoid staining combination of CHEADLE, GIFFORD and ESAU (1953) was employed, using rootstock material preserved in 70 % ethyl alcohol.

In order to be able to observe and record root growth, and to make autoradiographs of undisturbed root systems, a special method of water culture was devised and successfully used. The unit for each individual plant of this set-up consisted of a shallow tray, sized as to accommodate a 14″ × 17″ piece of black nylon fabric on the bottom, and covered with a removable lid of a lath frame lined with thin polyethylene film. To exclude the light, and excessive heat, this lid was again covered with a piece of black cotton and a piece of cheese cloth, respectively. The nylon fabric on the bottom of the tray was soaked by Hoagland's culture solution continuously dripping down from two pieces of glass tubing introduced through small holes in the plant holder at one end of the tray. Between these two capillaries a plant was fitted in the plant holder, and its roots spread out on the nylon. Twelve trays were arranged in line, with a common supply; they were placed in a slightly slanting position, so that the excess of culture solution could flow off, to be collected and recirculated by means of a pump with float switch.

Apart from the periodic replenishing and renewal of the culture solution, the only maintenance of this system consisted of checking the glass capillaries from time to time. Root growth could be followed and recorded by making white-pencil marks on the black nylon. At the end of an experiment the plants were cut close to the root collar, and the root systems vacuum-dried while still attached to the nylon.

Results and preliminary discussion

The first item to be studied was the translocation and distribution of C^{14}-photosynthates in the absence and in the presence of stock leaves, or symbolically in M*/F— and in M*/F+, respectively. As can be seen from plates 1 and 2, the distribution patterns were remarkably alike: in neither case could any appreciable amount of radioactivity be observed in the root system, 24 hours after exposing one melon leaf to $C^{14}O_2$. Countings performed on thin transverse sections of rootstock stem were hardly, if at all, above background: only about 6 and $2\frac{1}{2}$ counts per minute for M*/F+ and M*/F—, resp., on a basis of two hours counting with a background of 27.2 c.p.m. Even after 48 hours the results were virtually the same. The bulk of downward translocation stopped right at the node above the graft union, only negligeable amounts travelling further downwards. Only in a very few cases, visible and measurable quantities of radioactivity did occur in the stock tissues and roots, both in M*/F— and in M*/F+.

The only difference between the two treatments, though not to be seen in the pictures, was that in M*/F— root growth had stopped completely, while in M*/F+ root growth was continuing normally. Apparently, in M*/F— a condition of incompatibility develops right after the stock leaves have been cut. As was shown before, De Stigter (1956), the sieve tubes of the stock show visible symptoms of collapse as early as one day after defoliation.

That in M*/F+ no, or no appreciable radioactivity is found in the roots, might be taken to mean that the roots are provided with all they need by their own leaves, and thus do not act as a "sink" with regard to the scion as a potential "source" of photosynthates (for the terms sink and source, see Crafts and Yamaguchi (1958)). Control experiments in which both scion and stock were *C. ficifolia*, revealed that in F*/F+ the roots were practically free from radioactivity, while in F*/F— great amounts of radioactivity were found in the roots very soon after the $C^{14}O_2$ was given. These results seem to confirm the view that in these grafts the roots are served only by their own leaves, if present. However, quite different results were obtained in other control experiments with grafts of muskmelon only; here the presence of stock leaves did not prevent the labelled photosynthates from entering the rootstock. Yet, here also, the presence of stock leaves had a suppressing effect, the stock tissues of M*/M+ containing twice as little radioactivity than did those of M*/M—; transverse sections produced 127 and 249 net counts per minute, and per section,

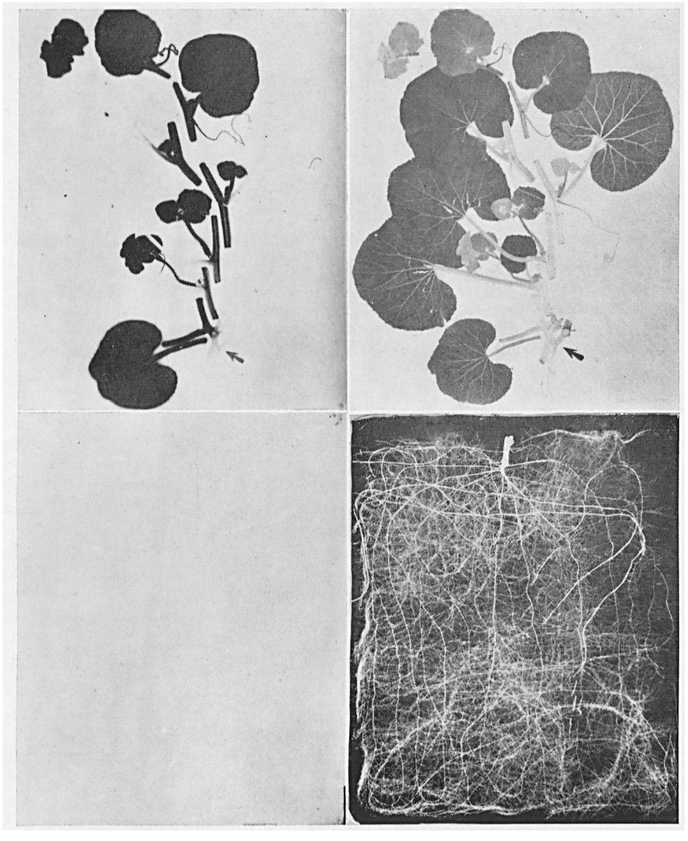


Plate 1. Melon/*Cucurbita ficifolia*, without stock leaves: M*/F–, April 2–3, 1959. *Right:* plant and root system; *left:* autoradiographs of same. Graft union indicated by arrow. Stump of detached stock shoot extends to the upper right of arrow. Melon leaf at lower left was exposed to $C^{14}O_2$, ± 15 μc. Duration of translocation and distribution period: 24 hours. Note that downward translocation practically stops at the node above the graft union. Within the scion, C^{14}-compounds accumulate in parts which are still growing, and hence importing ("sinks"): top parts, axillary shoots, tendrils. As a contrast, full-grown leaves (exporting organs) hardly show up in the autoradiograph.

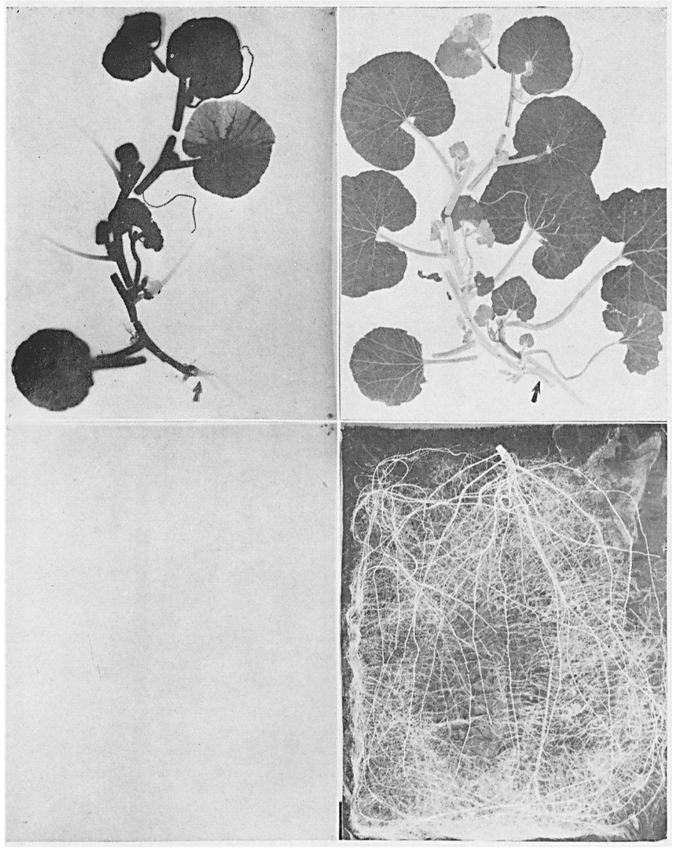


Plate 2. Melon/*Cucurbita ficifolia*, with stock leaves: M*/F+. April 2–3, 1959. Same details as plate 1, except for the stock shoot which occupies the lower right corner. Note that here, too, the roots are free from radioactivity (see text).

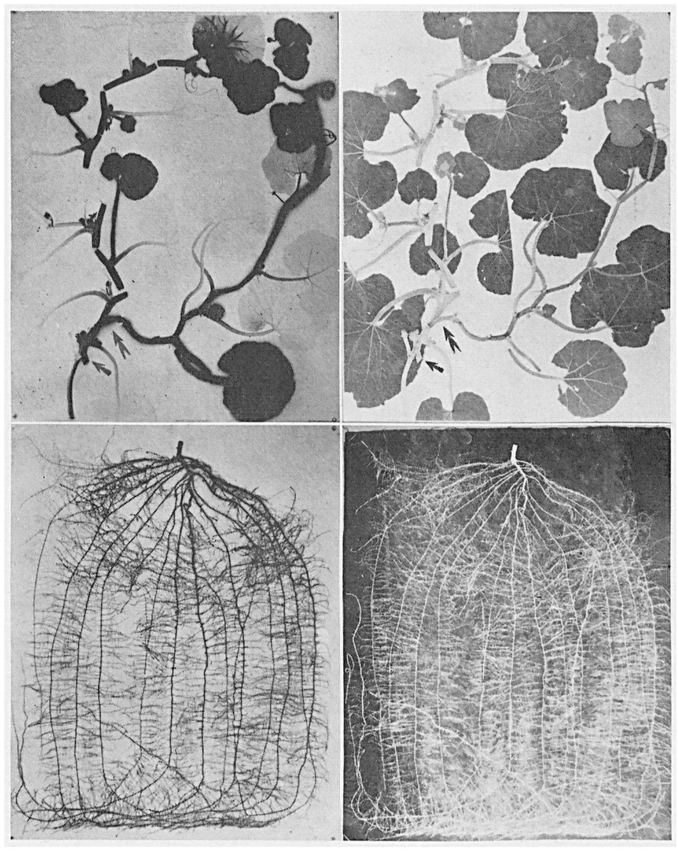


Plate 3. Melon/*Cucurbita ficifolia*, without stock leaves, but with another, laterally inserted *Cucurbita* shoot: F*/M/F—. April 6, 1959. Arrow: original graft union; stump of detached stock shoot extends to upper left. Winged arrow: second graft union, inserted *Cucurbita* shoot fills lower right part. Lowermost leaf of inserted shoot was exposed to $C^{14}O_2$, ± 30 μc. Duration of translocation and distribution period: 6 hours. In contrast with plates 1 and 2, radioactive compounds have travelled to the roots, now even passing two graft unions.

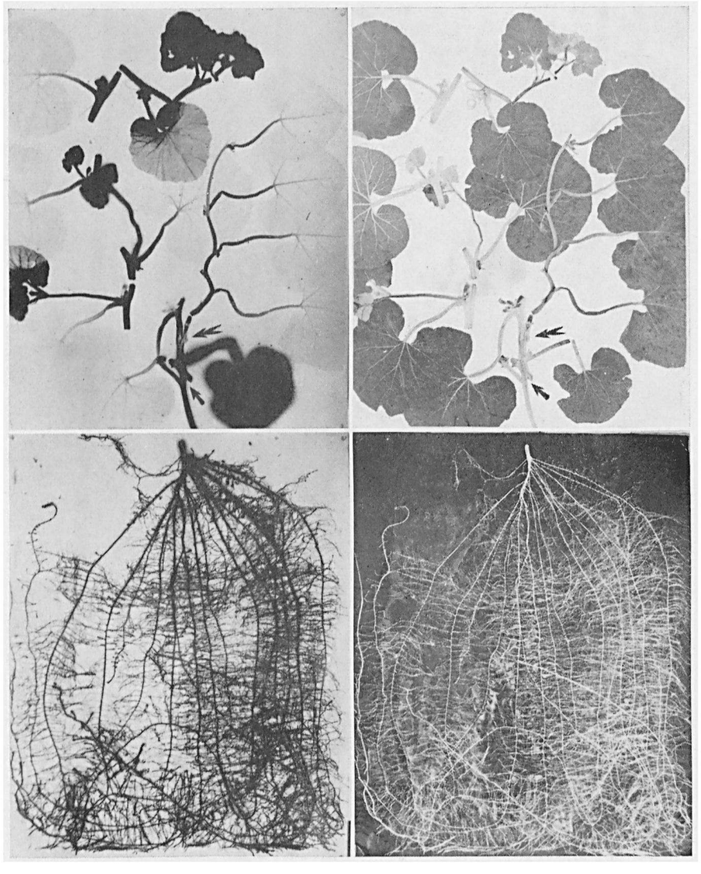


Plate 4. Same graft combination as in plate 3, but point of application of $C^{14}O_2$ is the leaf on the melon intermediate: F/M*/F—. April 6, 1959. Results are the same as in plate 3, except that the roots contain still more radioactivity.

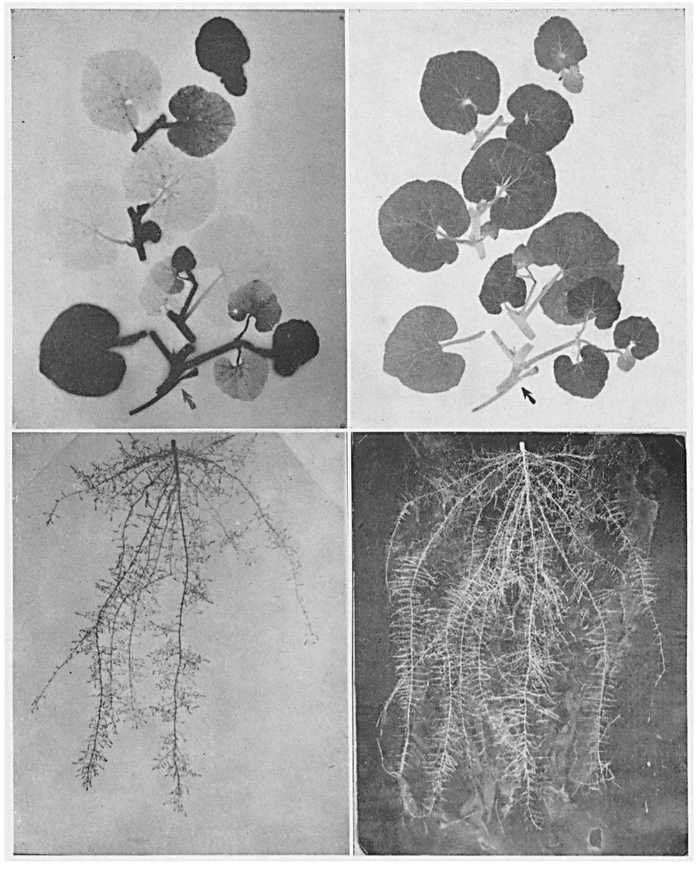
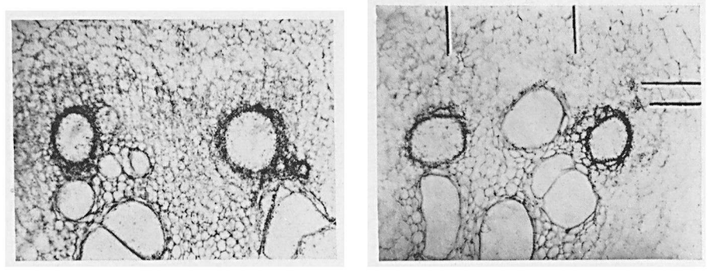
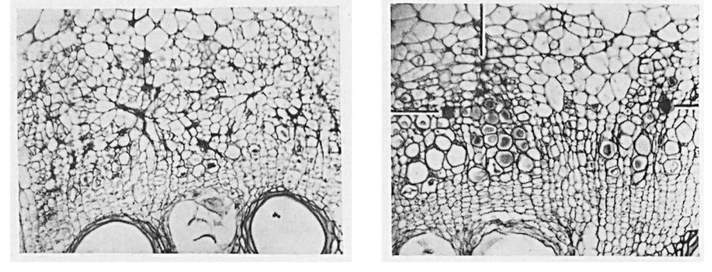


Plate 5. Melon/*Cucurbita ficifolia*, without stock leaves: M*/F—. Exposure to $C^{14}O_2$ (melon leaf at lower left) 10 days after stock shoot was removed (stump of shoot extends to upper right of arrow which indicates graft union). During these 10 days after removal of stock leaves and prior to exposure to $C^{14}O_2$, the plant went through a serious condition of incompatibility, followed by the onset of a gradual recovery. In the stage shown, recovery is most prominent in the new root growth, the above-ground parts still looking rather bad (this does not show up in the picture!). Especially the exposed melon leaf was still badly yellowish-green. In this recovering plant, the labelled photosynthates readily travelled into the roots, even in such quantities that the film had to be exposed much shorter than usually: only 1 day instead of 14 days; the latter period produced a fully over-exposed, blurred image. Note that complete, apparently dead, roots are missing in the autoradiograph.



a *b*

Plate 6, *a* and *b*. Microautoradiographs of transverse sections from the stock of recovered M*/F— grafts. In both, radioactivity is concentrated in two of the youngest fully developed xylem vessels. In *b*, the horizontal lines indicate two newly-formed sieve tubes with high concentrations of radioactivity; the vertical lines point toward groups of collapsed sieve tubes.



c *d*

Plate 6, *c* and *d*. Micrographs of transverse sections from the stock of M/F— grafts. Plate 6, *c*: plant not yet recovered, but recovery just beginning. Most of the sieve tubes have completely collapsed, only near the cambium a few new sieve tubes are being formed. Plate 6, *d*: plant in a farther advanced stage of recovery. Vertical line points to a series of collapsed sieve tubes. Horizontal lines: sieve plates with solid (blue) callose deposits. Toward the cambium the sieve tubes become progressively wider, and the callose deposits progressively less heavy. The dark masses not filling the entire sieve tube are plugs of contracted sieve-tube contents stained dark brown by tannic acid and ferric chloride.

respectively. Although the results with both F*/F± and M*/M± can be described in the terms of source and sink, the marked difference in degree to which this explanation applies cannot be accountde for as yet.

It should be kept in mind that a sharp distinction must be made between cases in which the source-and-sink terminology applies, and cases in which a condition of incompatibility occurs, resulting in an absence of translocation. In the latter, the roots should fundamentally act as sinks, but in one way or another, *i.e.*, structurally or functionally, the route between source and sink is blocked.

Returning to the failure of M*/F— to show any appreciable translocation across the graft union, the question should be raised whether there is a lack of union between the partners, at least as far as the phloems are concerned, the direct xylem connections having been definitely proven, DE STIGTER (1956). In this connection it is relevant to refer to MCCLINTOCK (1948) who found a discontinuity of the phloems in the graft combination of peach on Marianna plum rootstock. This graft, too, will stay alive if some branches with leaves are maintained on the stock.

In order to check the M/F phloem union, double grafts were made by inserting a cucurbit seedling laterally into the stem of the melon scion. After this second graft had established itself, the stock leaves were removed, and $C^{14}O_2$ was administered to the lowermost leaf of the cucurbit insertion. Plate 3 shows that in this F*/M/F— combination photosynthates did travel downwards into the roots, now even passing two graft unions. The conclusion can only be that the M/F graft union is very well able to function properly. The possibility should be considered, however, that only cucurbit photosynthates are "admitted" by the cucurbit rootstock tissues. To find this out, the same double graft combination was used, but now the leaf on the melon intermediate was exposed to $C^{14}O_2$. From plate 4 it is evident that here, too, considerable amounts of radioactivity have travelled into the roots. Thus the possibility of specific photosynthates has been ruled out.

Up to this point everything appears to support our original hypothesis that some factor originating from the cucurbit leaves is required to keep the stock phloem functioning, so that, in its absence the sieve tubes immediately stop functioning and collapse. A more prolonged observation of M/F— plants, however, showed that matters are still more complex. In our special system of water culture the plants started showing every symptom of incompatibility after removal of the stock leaves: complete cessation of root growth, die-back of large parts of roots, stagnation of top growth, yellowing of leaves, sieve tube collapse in the stock (plate 6*c*), but the plants just failed to die altogether, as they invariably will do when grown in soil. Instead, after some 8–10 days, tiny lateral rootlets began to appear higher up the main roots, so tiny indeed that at first they were taken for fungal outgrowths. Gradually these rootlets began to grow, after some time at

ever increasing speed. Shoot growth also resumed, in short, the plants showed a gradual but complete recovery. Once this process of recovery has started, C^{14}-photosynthates readily travel downwards across the graft union, as can be seen from plate 5. The plant from which this autoradiograph was made, even contained so much radioactivity in its roots that a film exposure of only one day was sufficient to produce the image presented here; the normal period of 14 days which was first tried, gave a completely over-exposed, blurred image.

Microautoradiographs, some details of which are reproduced in plate 6, *a* and *b*, showed that the bulk of the radioactivity in the stock is present in one or more of the youngest fully developed xylem vessels, some activity also being found in the newly-formed sieve tubes, and in the starch, if present.

Histological examination of recovered plants revealed a wide range of phloem conditions in the stock, from fully collapsed sieve tubes in the older parts, *via* stages of relatively narrow sieve tubes with variable, frequently heavy callose deposits, to big healthy sieve tubes with wide sieve perforations and little callose, near the cambium; see plate 6*d*.

General discussion

From the experimental results it is evident that one of the main questions of our problem concerns the exact nature and functioning of the graft union. As a first approach, histological examination showed, De Stigter (1956), that in the course of the process of uniting, a cambium is formed, continuous with the cambia of stock and scion. This cambium accomplishes the definitive union between stock and scion. Direct continuity of the xylems can be easily observed, even macroscopically, but is much more difficult to demonstrate for the phloems. As a result, the phloem continuity has not strictly been proven, but our conclusion was that "there is no reason to doubt the existence of a direct phloem connection between the melon top and the cucurbit stock". This phloem continuity was further substantiated by the remarkably good growth of the double graft F/M/F—. In our present study this has again been confirmed by F*/M/F— and F/M*/F—, in both of which rapid downward translocation took place irrespective of whether the labelled photosynthates originated from the cucurbit top or from the melon intermediate.

The results of these double graft experiments, with apparently well functioning unions, were considered to be conclusive proof that also the simple M/F graft union is perfectly in order, anatomically. Still, however, against this conclusion the criticism might be raised that the M/F graft union is *not* fully perfect originally, but that the laterally inserted cucurbit shoot in F/M/F— induces this first union to improve to such an extent as to become fully capable of functioning. While such an action of the second graft should not be considered impossible, it must be borne in mind that even the single M/F— graft turned out to be able to achieve this improvement (supposed we justly use this term), without the help of a second graft, as shown by its full

recovery. This spontaneous recovery of M/F—, now, is a most unexpected thing. If we continue, for the moment, thinking in terms of an imperfect graft union, this recovery implies that only defoliation of the stock will put the plant to complete its graft union (after having been on the verge of death). In the presence of stock leaves it would never have achieved this completion, for defoliation of the stock will bring about the full range of incompatibility reactions at any time, no matter how young or old the graft is. The definitive answer to the questions involved awaits a much more detailed and scrupulous examination of the graft union, at the exact site of transition. For the time being, however, we do not consider the "incomplete phloem hypothesis" to be very probable.

The alternative, by far to be preferred, is to approach the problem from a physiological point of view, by assuming that the biochemical functions of stock and scion are not fully complementary, or that a condition of biochemical unbalance exists. Our original hypothesis of a "specific substance", required for the proper functioning of the rootstock's translocatory system would fit this view. To reconcile this hypothesis with the spontaneous recovery of M/F—, we have to assume that the stock has to pass through a process of adaptation, as a result of which it becomes able to synthesize the "translocation factor" from the melon's supply of photosynthates. If this is true, it seems reasonable to assume that the stock will need some time to build up an enzymatic apparatus of sufficient capacity. This would account for the recovery being only slow and gradual.

The above considerations will suffice to show that in grafted plants one should not limit himself to thinking about the graft union as a mere mechanical barrier. Moreover, in translocation studies quite other factors may be involved, as demonstrated by the different behaviour of our F/F and M/M control grafts. The relative amounts of foliage present on scion and stock will greatly influence the distribution pattern of compounds applied to either one of the partners. These effects are best described as resulting from the degree to which the various parts act as "sources" and "sinks" with regard to each other. One should be aware of this in interpreting the results of DILOV, POPOV and KASSABONEVA (1959) on the distribution of P^{32} in grafted melons and pumpkins. They themselves ascribed their findings to a "barrier effect" exersized by the graft union. It is interesting to note that they, with P^{32}, do not mention any complete or nearly complete absence of downward translocation in their melon/pumpkin grafts like we did in ours with C^{14}. Still, of all the combinations made, the roots of melon*/pumpkin contained the smallest relative amount of radioactivity.

Finally, we should discuss why M/F— has always appeared to be completely incompatible, while it is now evident that the very same combination can make quite satisfactory growth. Our explanation is simply this, that in soil the weakened roots cannot withstand the

action of microorganisms attacking them *en masse*, while in our system of water culture conditions are much more favourable for survival and recovery.

SUMMARY

In this study, using autoradiographic techniques, our attention was primarily directed to the question whether or not, and under which conditions, the scion's labelled photosynthates would pass into the rootstock. Until recently, in the absence of stock leaves the melon/*Cucurbita* graft appeared to be completely incompatible. In a specially devised system of water culture, this turned out not to be true, however, since stock-defoliated grafts after an extremely critical period are able to recover gradually but completely. From then on they show a remarkably good growth. Microorganisms attacking the roots are taken to be responsible for recovery not taking place if the grafts are grown in soil.

Trying to account for this apparent incompatibility and subsequent recovery, the problem should be approached from different angles. The one way of approach concentrates upon the degree of perfection of the graft union, in the anatomical sense. The other major line of thought is biochemical in nature, and considers the question how far the biochemical functions of stock and scion are complementary, or balanced.

For the present, it seems justified to consider the graft union to be anatomically perfect. The "temporary incompatibility", then, should be due to the absence of a "translocation factor". The subsequent recovery can be visualized to be due to a process of biochemical adaptation.

Looking upon a graft union as a mere mechanical barrier in translocation studies, is to be critisized, the more so since still other factors may be involved, such as the degree to which the various parts of the graft act as "sources" and "sinks" with regard to each other.

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