TRANSLOCATION OF ASSIMILATES IN FRITILLARIA IMPERIALIS L.
1. THE SECRETION OF ^{14}C-LABELLED SUGARS BY THE NECTARIES IN RELATION TO PHYLLOTAXIS

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SUMMARY

^{14}CO$_2$ was supplied to single leaves at various heights along the axis of flowering *Fritillaria imperialis* plants. The movement of the ^{14}C-labelled assimilates from the lower leaves was mainly towards the bulb, and from the higher ones mainly towards the flower-bearing shoot part.

The nectar always contained equal amounts of glucose and fructose, of which the ^{14}C-labelled forms were predominantly collected from the nectaries of the flower that was on the same orthostichy as the ^{14}CO$_2$-treated leaf. It is concluded that the five orthostichies which can be distinguished morphologically reflect five largely, but not completely, separated pathways of translocation of assimilates in the shoot of this plant species.

1. INTRODUCTION

Translocation in dicotyledonous plants in relation to phyllotaxis has been a subject of several studies reported in literature (*e.g.* CALDWELL 1930; PRO-KOFIEV *c.s.* 1957; SHIROYA *c.s.* 1961). Recently Ho & PEEL (1969) reported two main pathways, which operate in the longitudinal translocation of assimilates exported by the leaves of *Salix viminalis*. In short-term experiments they found the transport of foliar applied tracers to be restricted to leaves of the same orthostichy as the treated leaf and to those of both orthostichies which were at an angular distance of 72° from that leaf. This despite the fact that in the stem of these shoots a complete ring of phloem was present.

Of monocotyledonous plants – with their quite different vascular pattern – such studies are unknown. Moreover, information about translocation in general in this group of plants in relatively scarce. The present study on assimilate distribution in flowering *Fritillaria imperialis* demonstrates a close relationship between phyllotaxis and the secretion of ^{14}C-sugars by the nectaries of the various flowers, following the application of ^{14}CO$_2$ to individual leaves along the shoot axis.

2. PLANTS AND METHODS

Plants were raised outside from bulbs planted in large pots in the autumn. In

spring they were allowed to grow until the onset of flowering. The pots were subsequently brought into a room kept at 4 °C. Flowering was greatly retarded by this treatment. Each time a plant was needed for an experiment it was brought at room temperature, resulting in resumption of growth and opening of the flowers. In this way the experiments could be conducted over a period of about 6 weeks. When raised under normal outside conditions all plants flower at the same time and can be used for experiments for a few days only.

$^{14}CO_2$ was evolved in a small tube of cellulose nitrate sealed with silicon rubber onto the lower side of a leaf. The tube contained 0.2 ml of 0.005 M Na_2CO_3 with 100 μC of ^{14}C. The CO_2 was liberated by injection of a small amount of N sulphuric acid. The tube was kept at its place for about 24 hours. The administration of $^{14}CO_2$ took place under the normal conditions of illumination occurring in the laboratory, with incidentally additional illumination from two incandescent lamps of 250 watts.

Nectar samples were withdrawn from the nectaries with self-filling pipets of 10 or 20 μl ("Drummond micro-caps").

Radio-activities of solutions were measured by liquid scintillation counting using as a counting solution a mixture of toluene + "Pre-mix P" (5.3 g/l) and methanol (3:1 v/v). On chromatograms the amounts of ^{14}C were measured by gas-flow counting with a Packard chromatogram scanner.

Carbohydrates were separated by descending paper chromatography on Whatman no. 3MM with N-propanol–benzyl alcohol–formic acid–water (50/72/17/20 v/v) as a solvent.

3. RESULTS

3.1. The composition of the nectar

Paper chromatography showed the presence of glucose and fructose, and traces of sucrose, in the nectar samples. Direct quantitative analysis of a number of

Table 1. The distribution of ^{14}C among glucose and fructose in the nectar of *Fr. imperialis* after the administration of $^{14}CO_2$ to various leaves.

$^{14}CO_2$ supplied to:	^{14}C-glucose cpm	^{14}C-fructose cpm	$\dfrac{^{14}C\text{-fructose}}{^{14}C\text{-glucose}}$
a top leaf of the leafy shoot	2117	2243	
(in quadruplicate)	2270	2279	
	2120	2313	
	2152	2163	
(average)			1.04
a top leaf (leafy sh.)			1.14
a middle leaf (leafy sh.)			1.16
a bract			1.2
a terminal leaf			1.0

nectar samples, using standard laboratory methods, gave equal amounts of glucose and fructose (in a typical analysis: sucrose 0.6 mg/ml, glucose 22,2 mg/ml and fructose 23,7 mg/ml). When $^{14}CO_2$ was supplied to a leaf the nectar contained ^{14}C-glucose and ^{14}C-fructose with an activity ratio close to 1.0 (*table 1*).

3.2. Phyllotaxis

The leaves of the leafy shoot part of *F. imperialis* are inserted in a helix; the phyllotaxis was found to be 2/5. Although the stem of the plant is often strongly twisted, ribs running lengthwise on its surface exhibit the same torsion and so enable to establish the course of the orthostichies. Although *F. imperialis* plants regularly have more than five flowers, the 80 plants used in the present experiments always had five, or incidentally less flowers. The two coils of the helix onto which these flowers were inserted, were so close together that actually the five flowers stood in one circle. The sequence of flowering was difficult to establish as the flower buds no. 3, no. 4 and no. 5 mostly opened simultaneously (the first flower that opened is indicated as no. 1, the second as no. 2, etc.). Assuming a phyllotaxic configuration of 2/5 the sequence of the flowers in the whorl would be: 1–4–2–5–3– (*fig. 1*). As the sequence of flowering was often more or less a guess, no value has been attributed to deviating sequences regularly encountered.

3.3. Experiments with acid fuchsin

A plant was cut off above the bulb and left for a few hours as such. No severe wilting did occur in this period. Hereafter one of the flowers was severed – under water – from its peduncle and the peduncle part still attached to the plant was brought into a 2 % solution of acid fuchsin. While keeping the plant under normal room conditions the dye could be seen to be sucked into the vessels which turned red. It mainly moved into those leaves which were inserted on the same orthostichy as the dye-absorbing peduncle. Some of these leaves, however, had uncoloured regions, while in some of the leaves that belonged to neighbouring orthostichies (at an angular distance of 1/5) the dye appeared in a few vascular
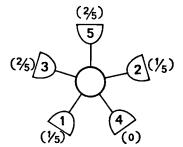

Fig. 1. The arrangement of the flowers around the axis of *Fr. imperialis* in case of a 2/5 configuration. The numbers indicate the sequence of flowering. In brackets the angular distances of the flowers of the orthostichy of the flower no 4.

bundles. Apparently the leaves mainly belong to a particular orthostichy, but regularly have a few vascular connections with vessels of a neighbouring one.

3.4. The distribution of ^{14}C among the various flowers following the administration of $^{14}CO_2$ to leaves

A typical experiment is shown in the *tables 2* and *3*. Here $^{14}CO_2$ is administered to a middle leaf of the leafy shoot-part of the flowering plant. The treated leaf was of the same orthostichy as the flower no. 2. 20 µl samples of nectar were collected and their radio-activities measured. The data show that the flower no. 2 has received most of the ^{14}C translocated from the leaf to the nectaries. Flowers that were at an angular distance of 1/5 (72°) of the treated leaf also got some ^{14}C, while those at an angular distance of 2/5 (144°) secreted only small amounts of radioactive substances. Similar experiments were carried out in which leaves situated at various other positions along the leafy shoot-part have been supplied with $^{14}CO_2$ (*tables 4–6*). For practical reasons the results are presented in a condensed form. *Tables 7* and *8* give the experimental results obtained after the $^{14}CO_2$ was fed to leaves inserted onto the flower-

Tables 2 and 3. The distribution of ^{14}C among the various nectar samples collected from the flowers 1-5 after the supply of $^{14}CO_2$ to a middle leaf of the leafy shoot part, belonging to the same orthostichy as flower no. 2.

hours after administration of the $^{14}CO_2$	cpm per 20µl of nectar collected from the flowers no:				
	1	2	3	4	5
1	7	8	8	–	–
3	9	1453	10	10	–
5	8	11161	69	119	–
23	11	38436	37	5244	–
25	20	29649	207	565	88
29	16	35689	188	1863	66
47	41	13168	114	4014	442
51	36	6344	48	1812	292
118	–	–	16	–	262
120	–	–	28	–	406

Table 3

	Flower number:				
	1	2	3	4	5
angular distance of the flowers from the treated leaf	2/5	0	2/5	1/5	1/5
µl of nectar collected	160	160	200	140	120
cpm/µl of nectar	1	849	4	97	13
% of total nectar ^{14}C	0.1	89	0.5	9	1

Table 4. $^{14}CO_2$ was administered to a leaf of the leafy shoot, situated at approximately 1/3 of its height (measuring from the bottom) on the same orthostichy as flower no. 4.

	Flower number				
	1	2	3	4	5
angular distance of the flowers from					
the treated leaf	1/5	2/5	1/5	0	2/5
μl of nectar collected	140	160	180	180	140
cpm/μl	20	2	2	28	20
% of total nectar ^{14}C	29	3	4	54	10

Table 5. $^{14}CO_2$ was administered to the bottom leaf of the leafy shoot part, inserted on the same orthostichy as flower no. 4.

	Flower number				
	1	2	3	4	5
angular distance of the flowers from					
the treated leaf	1/5	1/5	2/5	0	2/5
μl of nectar collected	160	160	100	100	80
cpm/μl	0.9	0.9	0.7	1.8	0.4
% of total nectar ^{14}C	25	25	12	30	8

Table 6. $^{14}CO_2$ was administered to the top leaf of the leafy shoot part, belonging to the same orthostichy as flower no. 5.

	Flower number				
	1	2	3	4	5
angular distance of the flowers from					
the treated leaf	1/5	2/5	1/5	2/5	0
μl of nectar collected	140	160	160	100	120
cpm/μl	9	2	24	5	818
% of total nectar ^{14}C	1	0.3	4	0.4	94

Table 7. $^{14}CO_2$ was administered to a bract of flower no. 2. This plant only possessed 4 flowers.

	Flower number				
	1	2	3	4	5
angular distance of the flowers from					
the treated leaf	1/5	1/5	2/5	2/5	0
μl of nectar collected	180	180	200	200	160
cpm/μl	21	199	221	209	1241
% of total nectar ^{14}C	1	11	14	13	61

Table 8. $^{14}CO_2$ was administered to one of the terminal leaves, situated above flower no. 5.

		Flower number		
	1	2	3	4
angular distance of the flowers from the treated leaf	2/4	0	1/4	1/4
μl of nectar collected	260	300	300	260
cpm/μl	1	5840	3	332
% of total nectar ^{14}C	0.02	96.6	0.05	3.3

bearing shoot-part. The tables show that during translocation the label re-mained largely confined to those vascular bundles that had anatomical con-nections with the treated leaf. Neighbouring orthostichies at an angular distance of 1/5 from the treated leaf apparently also got some activity, while those at a distance of 2/5 (or 2/4) got less. Only when the treated leaf was one of the bottom leaves the picture was less pronounced (*table 5*).

In general the time needed to reach the maximal level of ^{14}C in the nectar, and also the height of that level, depended on the distance between the flowers and the treated leaf (*fig. 2*).

3.5. The distribution of the photosynthates among the various plant parts

After the nectar samples had been taken the plants were divided in three parts: (1) the bulb, (2) the leafy shoot, and (3) the flower-bearing shoot-part. The plant parts were grinded and extracted with 70% ethanol. *Fig. 3* demonstrates the relative distribution of soluble activity among these plant parts in relation to the position of the leaf treated with $^{14}CO_2$ along the stem axis. The bulb contained relatively high amounts of radio-active solutes when the treated leaf occurred in the lower half of the leafy shoot part. In these cases low percentages of total radio-activity could be detected in the flower-bearing part of the plant. But that part, on the other hand, contained considerable amounts of soluble ^{14}C-compounds when leaves situated at larger distances from the bulb had been fed with $^{14}CO_2$.

4. DISCUSSION

The five orthostichies which can be distinguished in the leafy shoot-part of *Fr. imperialis* apparently reflect the presence of five anatomically more or less independent pathways of translocation to the nectaries. The conducting strands in the stem virtually connect leaves and flowers of the same orthostichy. The experiments with the dye, however, demonstrated that often a particular leaf has a few connections with the bundles of an adjacent orthostichy. The experimental results, therefore, may be explained more accurately by assuming a translocation of ^{14}C in bundles running through a one-fifth sector (of the
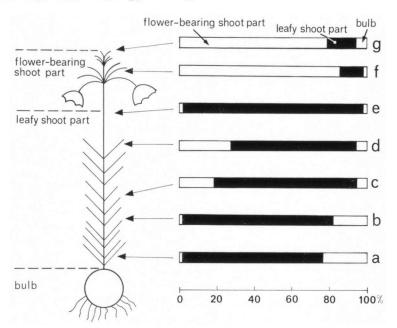
Fig. 2. The time course of the secretion of ^{14}C-nectar substances following the administration of $^{14}CO_2$ to leaves at various heights. The leaves were situated at approximately 5 cm (A, a bract), 60 cm (B, at $\frac{1}{2}$ of the leafy shoot), 70 cm (C, at $\frac{1}{3}$ of the leafy shoot) and 90 cm (D, the bottom leaf) from the nectaries.
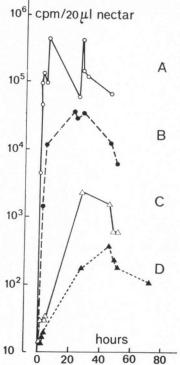
Fig. 3. The relative distribution of 70% ethanol-soluble ^{14}C-assimilates in *Fr. imperialis* plants 4–5 days after the supply of $^{14}CO_2$ to the following plant parts (indicated by arrows):
(a)–(d) single leaves at various heights along the leafy shoot-part
(e) the bare stem part
(f) a bract
(g) one of the terminal leaves

cross-section) of the stem. These bundles are not exclusively connected with the row of leaves of a particular orthostichy, nor necessarily with one flower either. If this assumption is right it might be expected that only one-fifth of all the nectaries secrete ^{14}C-sugars after a single leaf has been fed with $^{14}CO_2$. These nectaries may belong to one flower only, but also to some adjacent ones.

The experimental results clearly demonstrate that the flower-bearing shoot part is an important "sink" for products of photosynthesis. Besides its own photosynthates it also attracts considerable amounts of these products from the upper leaves of the leafy shoot-part. But even from a bottom leaf small amounts of photosynthates move to the nectaries. The developing bulb, on the other hand, is able to attract small quantities of assimilates from the terminal leaves, in spite of the vicinity of the flowers.

The glucose and fructose of the nectar apparently find their origin in sucrose: The equal amounts of both reducing sugars in the nectar and the 1:1 ratio of their labelling unequivocally point in that direction.

This could mean that sucrose is the translocated sugar in *Fritillaria*, as has been demonstrated for many other plant species.

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