MOVEMENT OF FOLIAR-APPLIED ^{45}Ca IN BRUSSELS SPROUTS

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Summary

^{45}Ca remained virtually immobile when applied to the surface of the middle leaves of Brussels sprouts in doses containing extra non-radioactive calcium plus either water, citric acid, malic acid, diphenylamine, dimethylsulphoxide, or oxalic acid.

By contrast, the mobility of ^{45}Ca injected in the midrib of corresponding leaves was markedly influenced by the nature of the additive. Water, diphenylamine, dimethylsulphoxide, or oxalic acid had little effect, while citric acid and malic acid resulted in acropetal and basipetal movement. Even after 7 days, the movement was largely restricted to the side of the plant with the treated leaf in the middle.

The movement of methylene blue added as a marker to the injected ^{45}Ca dose containing extra non-radioactive calcium plus malic acid remained largely confined to the xylem of the petiole and later up and down in the xylem of the stem. ^{45}Ca had moved ahead of the marker after 10 min and occurred in all vascular tissues and surrounding parenchyma of the petiole. Movement of ^{45}Ca away from the xylem was first apparent 20 min after injection. At 60 min ^{45}Ca occurred in fascicular parenchyma, phloem, and phloem parenchyma, but not in the xylem. Movement of ^{45}Ca in the stem occurred in phloem as well as in xylem.

These differences in the movement of marker and ^{45}Ca were recorded for "dry", "moist", and "saturated" plants.

I. INTRODUCTION

Millikan and Hanger (1965) have suggested that the extent of movement of ^{45}Ca injected into the leaves of common stock, pea, broad bean, or subterranean clover was dependent upon the saturation or bypassing of "fixation" or "exchange" sites by the addition of non-radioactive cations or a chelating agent or both to the radioactive dose. The occurrence of foliar-injected ^{45}Ca throughout the entire root systems of pea and subterranean clover also indicated the probability of transport in the phloem.

The work described herein was performed to determine whether comparable movement of foliar-applied ^{45}Ca occurred in Brussels sprouts — a plant with much larger leaves and main stem, and presumably a greater fixation capacity for calcium, than the species previously used. A study was also made of the pathway of transport involved in acropetal and basipetal movements of the isotope within the plant.

II. METHODS

The following general procedure was adopted for these experiments. All plants of Brussels sprouts (cv. Long Island) used had at least 15 well-developed leaves. A phyllotaxis diagram

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for each plant was prepared. The radioactive doses were applied to leaves in the middle of the plants either by injection into a midrib, or to the undamaged upper leaf surface around the distal end of the midrib.

For the leaf injections a short length of midrib was cut out of the lamina near the leaf apex to form a flap attached at its proximal end. The free end of the flap was inserted into a tube containing the radioactive dose.

At appropriate times after the commencement of treatment, selected leaves or petiole and stem sections were removed and immediately cooled to $-40°C$ and freeze-dried.

Gross autoradiographs of entire leaves were made by the method described by Millikan and Hanger (1964). For microautoradiographs of stem and petiole sections Kodak AR 10 fine-grain autoradiographic stripping film was used.

To assist in the interpretation of the microautoradiographs portions of stems and petioles of non-radioactive Brussels sprouts were embedded in paraffin, sectioned with a microtome at 10 μ, stained with Connants Quadruple stain (Johansen 1940), and vascular bundles photographed.

After autoradiography, selected tissues of leaves and stem sections were radioassayed as described by Millikan and Hanger (1964).

Further details of each experiment follow.

(*a*) *Experiment 1*

Each radioactive dose contained 0·1 ml of 1M $CaCl_2.6H_2O$ (i.e. 4008 μg Ca) plus 5 μCi of ^{45}Ca (as $^{45}CaCl_2.6H_2O$ and containing 1·5 μg Ca) in 0·05 ml water.

The following additions were made separately to this basal dose: (1) 0·1 ml H_2O; (2) 0·1 ml 1M citric acid; (3) 0·1 ml 1M malic acid; (4) 0·1 ml diphenylamine–ethanol (1 g/l); (5) 0·1 ml dimethylsulphoxide (0·20%); (6) 0·1M oxalic acid.

Two series of treatments were established:

A: injected series (treatments 1–6).

B: surface-applied series (treatments 1–5). Each dose of series B also contained 0·1 ml of the wetting agent BASF (0·1%).

All treatments were applied to leaf 7 and were duplicated. The series A plants were harvested 7 days, and the series B plants 5 days, after the commencement of treatments.

For the purpose of demonstrating the relationship between leaf phyllotaxis and ^{45}Ca movement following injection, the phyllotaxis diagram of each plant was divided radially into six equal segments, assuming that the treated leaf was in the middle of the front segment. The mean ^{45}Ca concentrations (as counts/min/mg dry matter) of the leaves in each phyllotaxis segment above and below the treated leaf were calculated.

(*b*) *Experiment 2*

Twenty-four hours before the commencement of injections three moisture series were set up as follows:

(1) "Dry" plants — pots were left unwatered, but the plants did not wilt.

(2) "Moist" plants — pots were watered and stood in a tray of water.

(3) "Saturated" plants — pots were treated as for (2) and covered with a large plastic bag to create a saturated atmosphere.

At the time of injection, the leaves of the saturated plants had droplets of water at the vein endings.

Each dose, which was injected into the midrib of a middle leaf, consisted of 0·1 ml 1M $CaCl_2$ plus 0·1 ml malic acid plus 0·1 ml of a 3% aqueous solution of methylene blue plus 30 μCi ^{45}Ca (as $^{45}CaCl_2$) in 0·2 ml water. There were three replicates for each moisture series at each time of sampling listed below.

The marker methylene blue was added to the dose to assist in identifying in each section the actual vascular tissues involved in the movement of the dose, and by comparison with the autoradiographs, and by means of radioassays, to determine whether the ^{45}Ca occurred in adjacent unstained tissues.

The times of sampling after the commencement of the injection were: saturated plants — 10 min, 20 min; moist plants — 10 min, 20 min, 60 min, 100 min; dry plants — 70 min, 100 min.

Thin transverse sections of the midribs and petioles of the injected leaves of the plants obtained at each time of sampling were cut at 1-cm intervals from the mouth of the tube used to hold the injected dose. Where methylene blue occurred along the whole length of the petiole, thin transverse sections of the stem were cut at successive leaves above and below the injected leaf until no methylene blue could be detected in the vascular tissues.

After autoradiography selected vascular tissues and matching autoradiographs were photographed.

In the stem sections the methylene blue occurred in very localized areas of xylem tissue only. In each of these sections the blue-stained xylem and the immediately adjacent unstained phloem tissue were dissected out separately and radioassayed for ^{45}Ca, using an automatic sample changer and associated scaler and recorder. All samples were counted three times on successive days, and in each case the mean count was used to calculate the ^{45}Ca concentration. A similar separation of the various tissues in the vascular bundles in the petiole sections was found to be impracticable.

III. Results

(*a*) *Experiment 1*

In the injected series A plants the doses with 4008 μg Ca plus water required approximately 36 hr to be absorbed, and caused no subsequent injury. The injections of all other doses were completely absorbed within 24 hr, but some phytotoxicity resulted. Thus, with citric acid, malic acid, oxalic acid, or diphenylamine, necrosis appeared within 24 hr and finally extended along the midrib flap, and an inch or so of the midrib and adjacent interveinal tissue. On the other hand, the injection with dimethylsulphoxide caused no injury.

The surface applications of doses (series B) containing added water, malic acid, or diphenylamine were almost completely taken up after 24 hr, and by 36 hr all treated areas were dry. However, the additives citric acid or malic acid caused extensive necrosis, diphenylamine less severe necrosis, and water or dimethylsulphoxide only slight necrosis in the treated areas.

The mean results of radioassays for ^{45}Ca made on duplicate plants of each treatment are presented in Table 1. The very low ^{45}Ca concentrations (less than 1 count/min/mg dry matter) recorded in some instances in this table were all confirmed by previous autoradiographs which showed faint images of the tissues concerned after long exposures (up to 43 days).

As the radioassays presented in Table 1 show the extent of movement of ^{45}Ca from the treated leaves, the autoradiographs of the plants are not presented, with the exception of those shown in Figures 1–6.

Irrespective of the additive in the dose, negligible translocation of ^{45}Ca to the base of the petiole of each treated leaf occurred following surface application of the dose (series B, Table 1).

By contrast, most midrib injections resulted in movement of ^{45}Ca out of the treated leaf. This movement occurred in both acropetal and basipetal directions and in each case was mainly restricted to the side of the plant with the treated leaf in the middle.

The lack of substantial lateral diffusion of ^{45}Ca is clearly shown by the autoradiographs presented in Figures 2 and 4. Leaf 7 was injected in each plant.

TABLE 1

RESULTS OF RADIOASSAYS FOR ^{45}Ca IN BRUSSELS SPROUTS LEAVES FOLLOWING TREATMENT WITH CALCIUM PLUS VARIOUS ADDITIVES

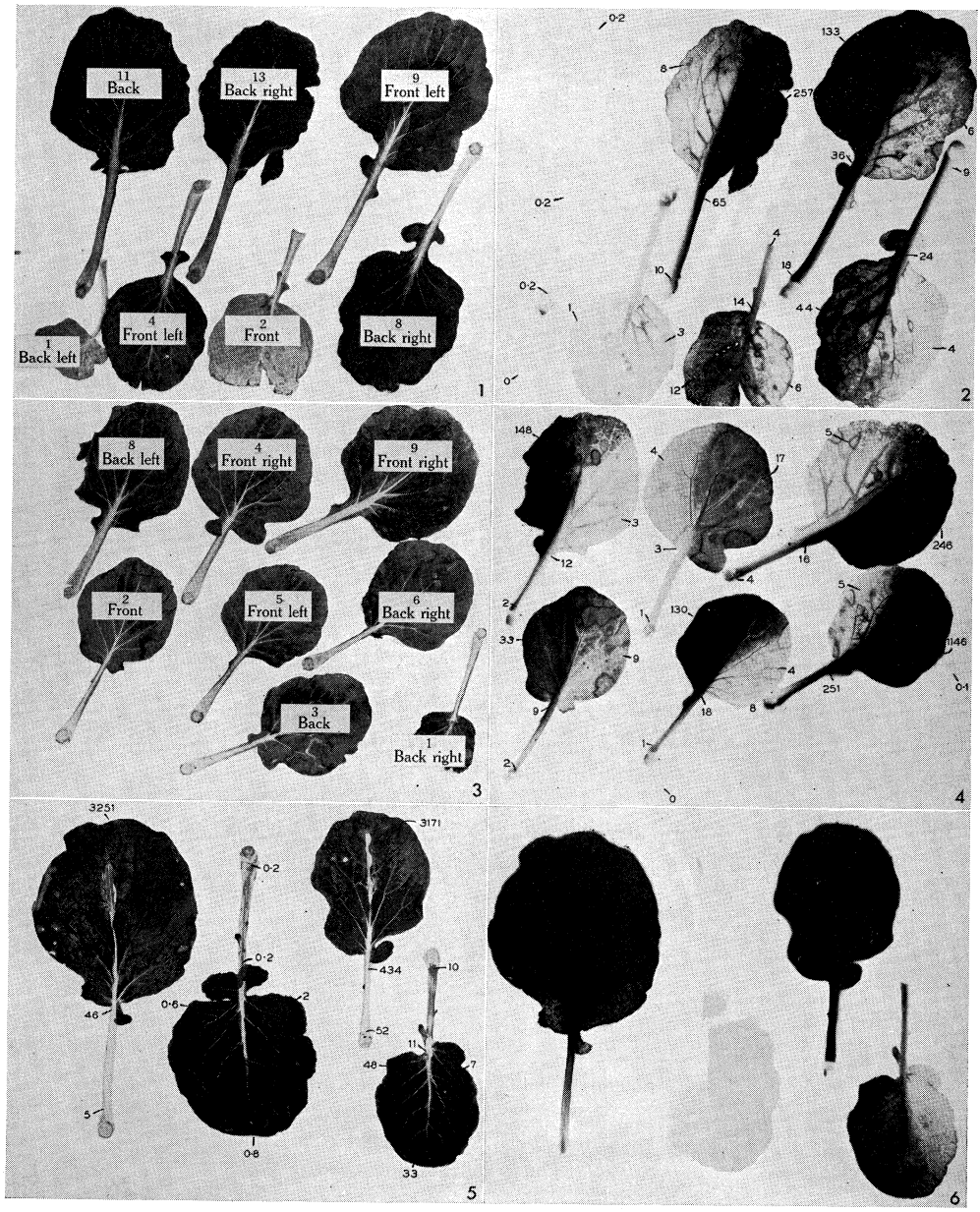
Results are means expressed as counts/min/mg dry matter. Measurements were performed on the treated leaf and on selected tissue of leaves grouped according to age and phyllotaxis above and below the treated leaf of duplicate plants. The composition of the basal radioactive dose of calcium and the concentrations of the various additives are given in Section II(*a*). PP, proximal end of petiole; MB, midrib base; LA, lamina apex

Additive	Leaves Included*	Series A — Phyllotaxis Segment† and Leaf Part																	Series B‡		
		Front			Front Left			Front Right			Right Left			Back Right			Back					
		PP	MB	LA	PP	MB	LA	PP	MB	LA	PP	MB	LA	PP	MB	LA	PP	MB	LA	PP	MB	LA
Water	Above	3	3	29	2	1	7	2	6	19	0·1	0·2	0·3	0·2	0·3	0·2	0·1	0·1	0·2	0·3	0·2	0·4
	Treated	37	1063	3421																0·5	260	1617
	Below	0·1	0·1	0	0·2	0·1	0·1	0	0	0	0	0	0	0	0	0	0	0	0			
Citric acid	Above	9	24	112	2	9	43	2	8	71	2	5	40	3	5	9	4	3	4	0	0	0
	Treated	516	1638	2692																0·1	17	1535
	Below	1	5	17	3	13	75	3	2	2	0	0	0	2	62	125	0	0	0			
Malic acid	Above	9	152	328	8	21	27	11	41	122	4	3	24	6	24	45	0·7	0·6	2	0	0	0
	Treated	82	672	2299																0·6	23	1922
	Below	6	43	55	4	18	45	6	9	9	4	4	5	0	0	0	0·2	0	0			
Diphenylamine–ethanol	Above	7	10	35	11	12	16	2	3	5	0·8	1	2	0·5	0·4	2	0·2	0·2	0·3	0	0	0
	Treated	36	187	2335																0	1	1219
	Below	2	7	8	1	2	2	10	16	41	0	0	0	0	0	0	0·5	0·3	0·4			
Dimethyl-sulphoxide	Above	1	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Treated	8	107	2309																0·3	5	2995
	Below	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
Oxalic acid	Above	4	4	17	1	1	4	2		3	0	0	0	0·3	0·3	0·4	0·3	0·2	0·4			
	Treated	28	240	3211																		
	Below	0·3	0·4	0·7	2	4	5	3	8	6	0	0	0	0	0	0	0·1	0·2	0·1			

* In relation to treated leaf.

† See Section II(*a*).

‡ In series B, leaves from the front segment only were radioassayed.



Figs. 1–6.—Photographs of (Figs. 1, 3, and 5) and autoradiographs of ^{45}Ca in (Figs. 2, 4, and 6) Brussels sprouts leaves sampled 7 days after commencement of the injection of the dose into leaf 7 in each case. Additives to each dose were malic acid (Figs. 1 and 2), citric acid (Figs. 3 and 4), and oxalic acid (Figs. 5 and 6). In Figures 1 and 3 the leaves were numbered up from the base of the plant, and the phyllotaxis segment is indicated on each leaf. Figures 5 and 6 show the injected leaf and leaf directly above from two plants. ^{45}Ca concentrations (expressed as counts/min/mg dry matter) in the leaf parts are shown on Figures 2, 4, and 5. Time of exposure: Figures 2 and 4, 43 days; Figure 6, 25 days.

The addition of water only to the dose resulted in some movement of ^{45}Ca into other leaves of the plant. Dimethylsulphoxide, diphenylamine, or oxalic acid did not enhance this movement. Most transport of ^{45}Ca resulted from the addition of either citric acid or malic acid to the basal dose (Table 1).

A feature of the radioassays in Table 1, and which was also evident in the autoradiographs, of which those presented in Figures 2, 4, and 6 are typical, was the low concentration of ^{45}Ca retained in the petioles as compared with that which accumulated in the laminae of the leaves above and below the injected leaf.

(*b*) *Experiment 2*

(i) *Petiole*

To assist in the interpretation of the microautoradiographs, the structure of typical vascular bundles in the petiole of Brussels sprouts is shown in Figure 7.

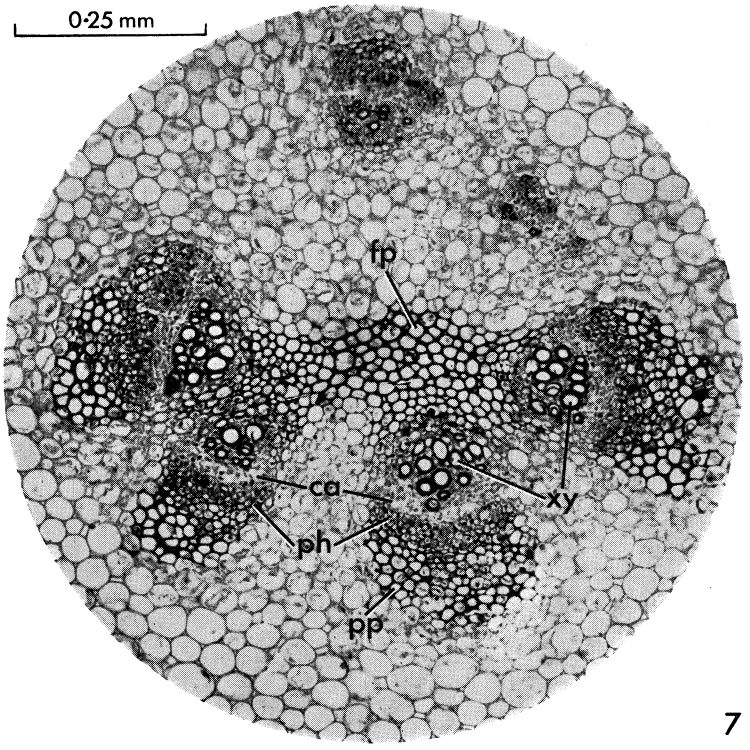


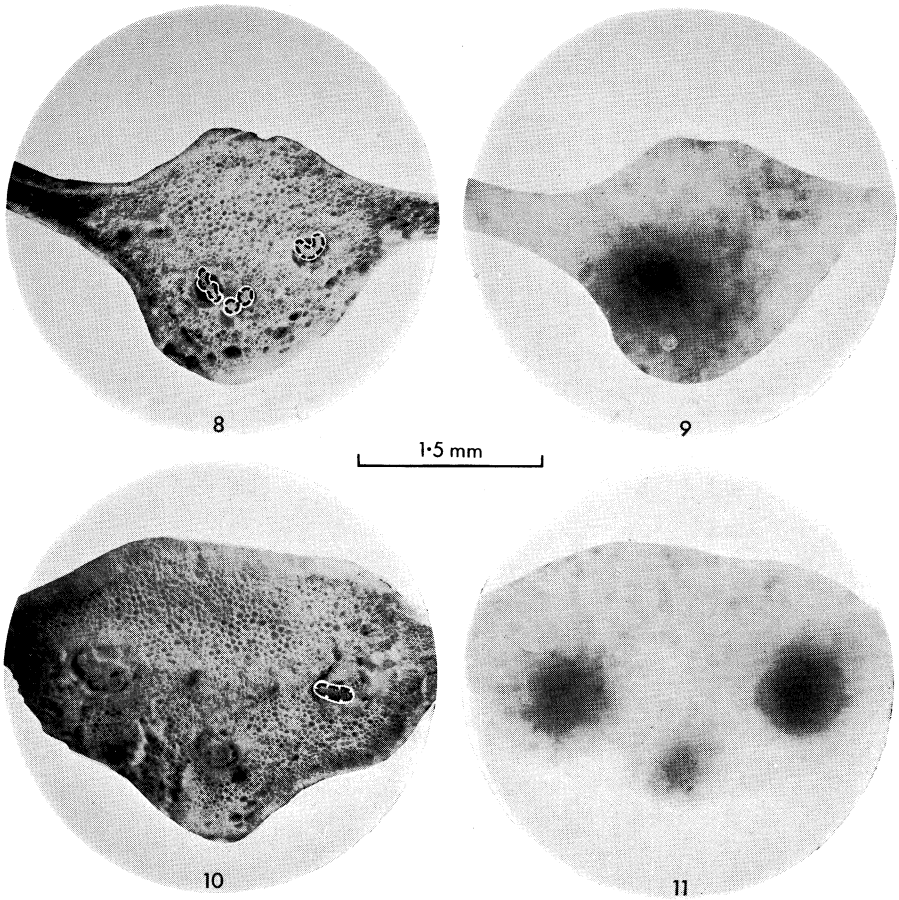
Fig. 7.—Transverse section through a vascular bundle in a petiole of Brussels sprouts. *ph*, Phloem; *ca*, cambium; *xy*, xylem; *fp*, fascicular parenchyma; *pp*, phloem parenchyma.

The main features are the occurrence of fascicular parenchyma connecting the xylem elements, and of phloem parenchyma exterior to the phloem. The individual bundles remain relatively discrete.

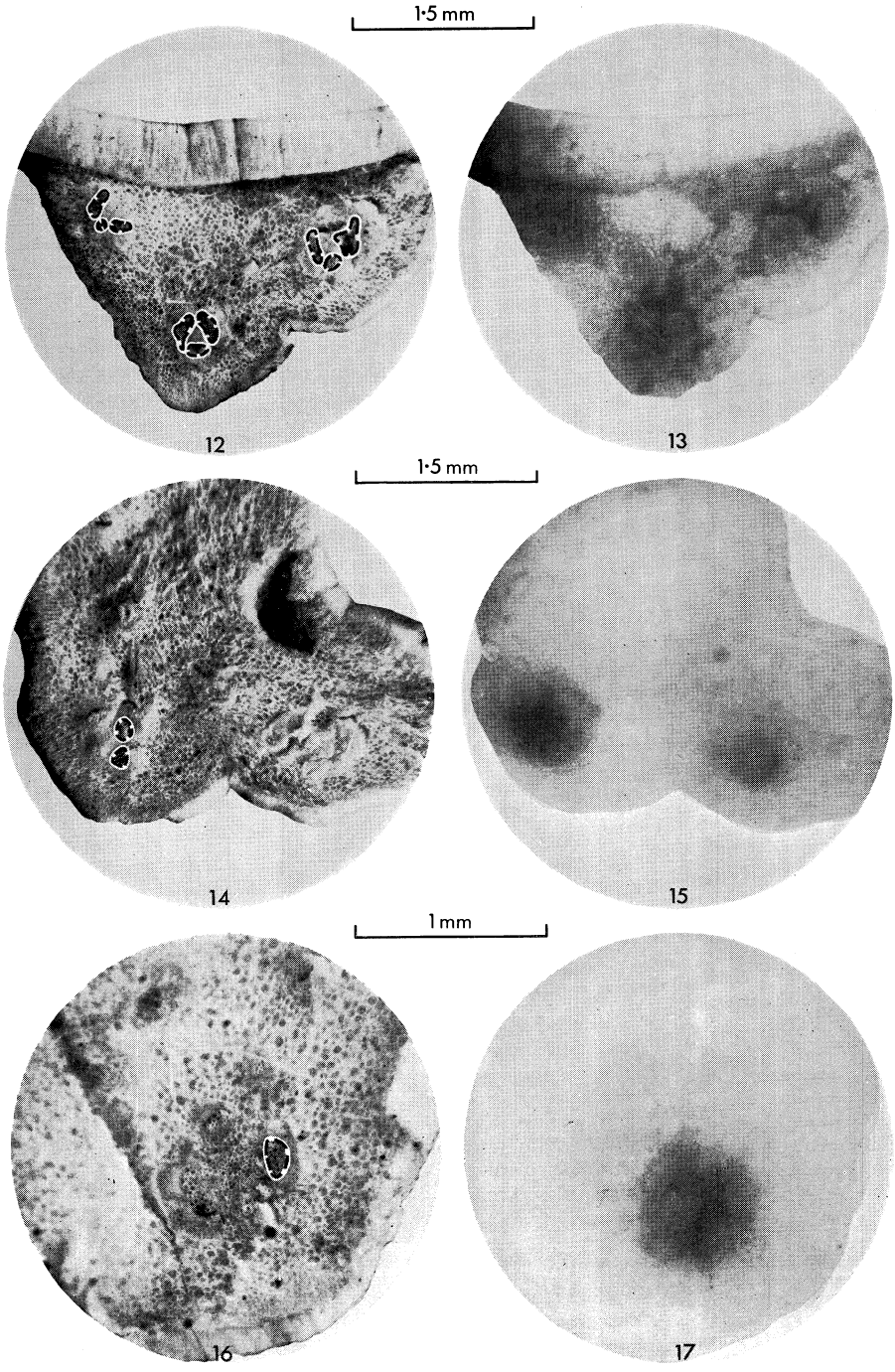
The mean distances which the ^{45}Ca and methylene blue had moved along the midrib and petiole after 10 and 20 min were found to be:

	In Moist Plants after:		In Saturated Plants after:	
	10 min	20 min	10 min	20 min
^{45}Ca	5 cm	8 cm	6 cm	8 cm
Methylene blue	2 cm	5 cm	4 cm	4 cm

These results, together with differences described below in their distributions within the petiole sections, showed that the ^{45}Ca and methylene blue moved independently of each other.



Figs. 8–11.—Microphotographs of (Figs. 8 and 10) and microautoradiographs of ^{45}Ca in (Figs. 9 and 11) transverse sections of the petiole of the injected leaf of a saturated plant sampled 10 min after the commencement of the injection. The sections were cut at 1 cm (Figs. 8 and 9) and 2 cm (Figs. 10 and 11) from the mouth of the injection tube. The encircled areas of xylem in Figures 8 and 10 were the only tissues stained with the methylene blue which was included in the injected dose. Exposure time for the microautoradiographs was 42 days.


1·5 mm
12
13
1·5 mm
14
15
1 mm
16
17

After the short (10- and 20-min) injection periods, and in both the moist and saturated plants, the methylene blue was restricted to the xylem tissues within the petiole of each injected leaf. It had not moved out of the petiole into the stem of any plant.

However, after injection periods of 60, 70, or 100 min, some lateral movement of methylene blue was apparent within the petioles of the moist and dry plants. Thus, within 1–2 cm of the mouth of the injection tube, all the vascular tissues except the phloem parenchyma were apparently uniformly stained with methylene blue, and there was also slight staining of the parenchyma surrounding the vascular bundles. Further down the petiole the staining was virtually confined to the xylem. It was much less intense in the phloem and fascicular parenchyma, and was absent in the phloem parenchyma.

These patterns of distribution of methylene blue contrasted sharply with that of the ^{45}Ca which was injected simultaneously.

Microphotographs of, and typical autoradiographs of ^{45}Ca in, petiole sections are presented in Figures 8–21. Moist or saturated conditions made no difference to the distribution of ^{45}Ca after injection periods of 10 or 20 min respectively.

After 10 min the ^{45}Ca occurred in all vascular tissues of individual bundles, even where there was no visual evidence of methylene blue (Figs. 8–11). Diffusion of ^{45}Ca into the parenchyma surrounding the bundles was also apparent, particularly within 1–2 cm of the mouth of the injection tube (Fig. 9).

After 20 min, and within 1–3 cm of the mouth of the injection tube, the lateral diffusion of the ^{45}Ca into surrounding tissues was more pronounced, and there was evidence of movement of ^{45}Ca out of the xylem (Fig. 13). Further down the same petiole (i.e. up to 10 cm) the ^{45}Ca occurred in all tissues of the vascular bundle, in contrast with the methylene blue which was confined to the xylem (Figs. 15 and 17).

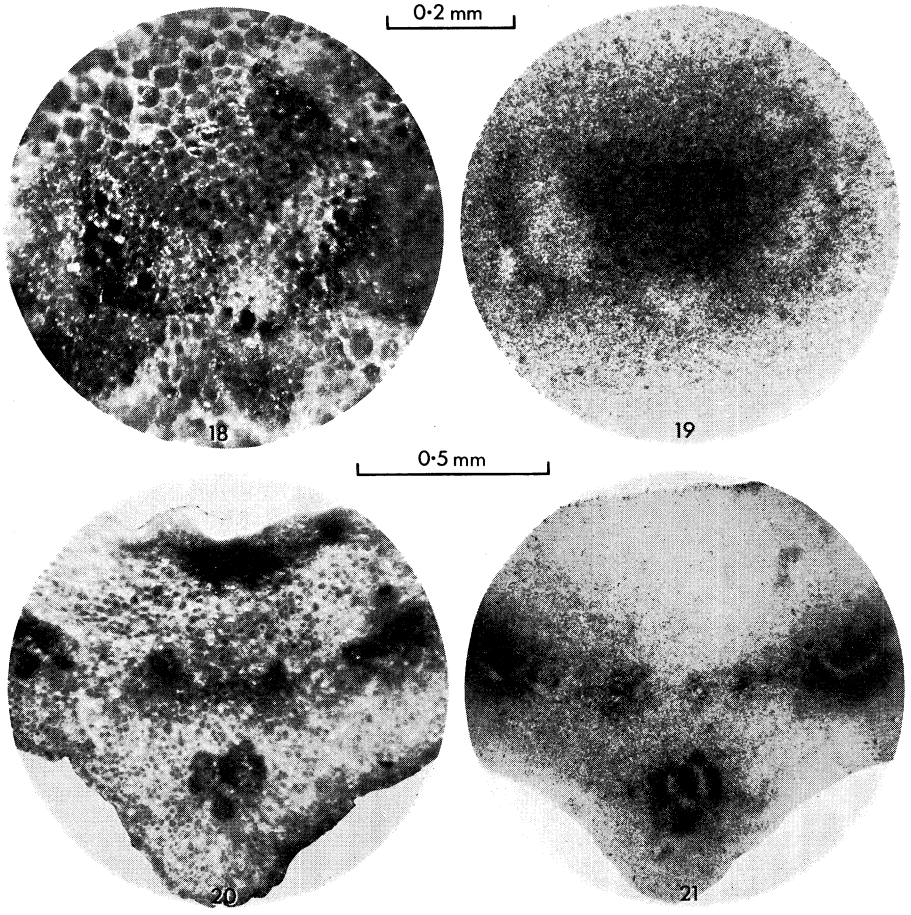
After 60–100 min, and irrespective of whether the plants were under moist or dry conditions, the autoradiographs consistently showed that the concentration of ^{45}Ca was relatively very much higher in the fascicular parenchyma, phloem parenchyma, and phloem than in the methylene-blue-stained xylem (Figs. 19 and 21).

(ii) *Stem*

Acropetal and basipetal movements of both methylene blue and ^{45}Ca in the stems occurred in plants sampled after injection periods of 60, 70, or 100 min, but not after 10 or 20 min. In all stem sections cut either above or below the injected leaf, the methylene blue was restricted to very localized areas in the xylem. Where the methylene blue moved into the petioles of leaves situated directly above or below the injected leaf, it again occurred only in the xylem.

Figs. 12–17.—Microphotographs of (Figs. 12, 14, and 16) and microautoradiographs of ^{45}Ca in (Figs. 13, 15, and 17) transverse sections of the petiole of the injected leaf of a moist plant sampled 20 min after the commencement of the injection. The sections were cut at 2 cm (Figs. 12 and 13), 7 cm (Figs. 14 and 15), and 10 cm (Figs. 16 and 17) from the mouth of the injection tube. The encircled areas of xylem in Figures 12, 14, and 16 were the only tissues stained with the methylene blue which was included in the dose. Exposure time for the microautoradiographs was 42 days.

The autoradiographs of the dry and moist stem sections, not presented, showed that in most instances the ^{45}Ca occurred in highest concentration in the methylene-blue-stained xylem tissue in the stems, whereas in the petioles referred to above the methylene-blue-stained xylem was very low in ^{45}Ca in comparison with the fascicular parenchyma and phloem parenchyma.



Figs. 18–21.—Microphotographs of (Figs. 18 and 20) and microautoradiographs of ^{45}Ca in (Figs. 19 and 21) transverse sections of the petiole of the injected leaf of a dry plant sampled at 70 min and cut at 3 cm from the mouth of the injection tube (Figs. 18 and 19) and of a moist plant sampled at 100 min and cut at 2 cm (Figs. 20 and 21). The areas of low ^{45}Ca activity in Figures 19 and 21 are xylem. Exposure time for the microautoradiographs was 18 days.

The methylene-blue staining made it possible to accurately identify and excise the radioactive xylem, and hence the adjacent unstained phloem tissues of the stem sections. The results of radioassays of ^{45}Ca in these tissues are presented in Table 2. These show that, in both the acropetal and basipetal sections of dry and moist plants, appreciable concentrations of ^{45}Ca occurred in both the methylene-blue-stained xylem and the unstained phloem. This is a further indication that the ^{45}Ca moved independently of the methylene blue.

TABLE 2

CONCENTRATIONS OF ^{45}Ca IN TISSUES OF BRUSSELS SPROUTS STEMS FOLLOWING TREATMENT WITH CALCIUM WHILE PLANTS WERE KEPT IN DRY OR MOIST CONDITIONS

Measurements (expressed as counts/min/mg dry matter) were performed on transverse sections of methylene-blue-stained xylem and adjacent unstained phloem tissues cut from stems above and below the injected leaf after the injection periods indicated in parentheses. P, phloem; X, xylem. Subscripts refer to the plant number

Site of Section*	Dry Plants (70 min)						Dry Plants (100 min)						Moist Plants† (60 min)				Moist Plants† (100 min)			
	P_1	X_1	P_2	X_2	P_3	X_3	P_1	X_1	P_2	X_2	P_3	X_3	P_1	X_1	P_2	X_2	P_1	X_1	P_2	X_2
4 above	—	—	—	—	—	—	250	370	—	—	—	—	—	—	—	—	70	80	—	—
3 above	100	280	—	—	—	—	180	360	440	470	—	—	—	—	—	—	180	140	—	—
2 above	210	580	140	200	—	—	280	1280	290	420	—	—	230	360	—	—	280	220	—	—
1 above	290	600	80	190	—	—	270	1620	280	680	—	—	130	380	—	—	290	190	80	190
1 below	310	700	170	210	30	50	250	240	1130	560	430	490	490	690	250	540	360	420	180	380
2 below	240	560	90	200	20	90	250	390	940	340	330	390	550	530	530	470	270	580	190	330
3 below	630	760	90	160	—	—	670	630	500	400	200	350	510	350	220	250	260	550	190	200
4 below	430	700	70	90	—	—	390	420	700	1060	350	230	160	420	180	150	580	140	100	150
5 below	310	780	30	80	—	—	510	670	400	590	90	10	50	60	50	60	500	320	150	80
6 below	410	930	—	—	—	—	340	‡	510	770	—	—	—	—	—	—	200	200	—	—
7 below	460	320	—	—	—	—	90	130	200	‡	—	—	—	—	—	—	—	—	—	—
8 below	50	60	—	—	—	—	60	100	220	400	—	—	—	—	—	—	—	—	—	—
9 below	—	—	—	—	—	—	—	—	290	280	—	—	—	—	—	—	—	—	—	—

* Numbers refer to successive leaves above or below the injected leaf. The section was cut immediately below the leaf indicated.

† No methylene blue or ^{45}Ca was detected in stem sections of plant 3 after either of the injection periods.

‡ Sample lost.

IV. Discussion

The restricted movement of ^{45}Ca within Brussels sprouts resulting from foliar injection contrasts with the extensive movement of ^{45}Ca observed by Millikan and Hanger (1965) throughout the plants of other species with comparable treatments.

It is suggested that this difference is due to the fact that the Brussels sprouts plants, being much bigger, contained many more sites for calcium fixation than the species used previously. Where comparable doses were injected into Brussels sprouts leaves of different size, there was invariably more movement of the isotope down the petiole of the smaller leaf (e.g. Fig. 6). This is illustrated by the following mean results of radioassays for ^{45}Ca of midrib and petiole sections of eight pairs of injected large and small leaves:

	Large Leaves	Small Leaves
Mean lamina area (cm^2)	81	43
Mean ^{45}Ca concentration (counts/min/mg dry matter):		
Petiole (proximal end)	32	164
Midrib base	352	877

The mechanism whereby citric or malic acids enabled the injected ^{45}Ca to bypass sites which normally would have fixed it is not known. Malic and citric acids have been correlated with soluble calcium in plants (Pierce and Appleman 1943; Clark 1968).

The barrier to the movement of surface-applied ^{45}Ca does not appear to be the leaf surface itself. Eynard (1961) and Bukovac and Wittwer (1961), working with grape vines and beans respectively, found that the absorption rate of ^{45}Ca into leaves was comparable with that of ^{32}P, but only ^{32}P was readily translocated. Chen (1964) even showed that tomato foliage was more efficient at absorbing calcium than the roots. By increasing the temperature of the roots, Phillips (1965) increased the rate of uptake of foliar-applied calcium into bean leaves, but failed to induce translocation.

As Crafts (1956) has pointed out, there are several steps between the absorption of molecules by the leaf surface and their final movement into the sieve tubes, and metabolic energy is required for some of them. It is not known at what stage of the process the blockage of calcium movement occurs in plants such as Brussels sprouts.

In spite of the apparent immobility of calcium within the leaf tissue, translocation of foliar-applied calcium has been recorded by Biddulph, Cory, and Biddulph (1959) in very small amounts in beans, by Krasinskii *et al.* (1958) in tomato, beet, and cucumber, and by Barinov (1959) in tomato. In addition, calcium applied as a foliar spray has been successfully used to reduce the severity of calcium deficiency disorders in some plants, e.g. black heart of celery (Geraldson 1954) and bitter pit of apples (Garman and Mathis 1956; Martin, Lewis, and Cerny 1960).

The movement of ^{45}Ca in the stems of Brussels sprouts following midrib injection of certain doses occurred in both acropetal and basipetal directions. Similar bidirectional movement of foliar-applied ^{45}Ca has been previously demonstrated by Krasinskii *et al.* (1958), Barinov (1959), and Millikan and Hanger (1965).

It is now generally recognized in the literature that the downward movement of solutions following leaf injections is in the xylem (Biddulph 1959). Thus, movement in petiole and stem of Brussels sprouts was found to conform with previous work in

respect to methylene blue, but not ^{45}Ca. Although within the first 10–20 min ^{45}Ca occurred in all vascular tissues including the xylem, the latter lost its original content of the isotope with time, even though the dose was still being taken up by the midrib flap and moving down the petiole.

It was also apparent from experiment 2 that internal moisture tension did not affect the pathway of movement of the isotope.

Outwards movement of ^{45}Ca in the petioles of leaves above or below the injected leaf also occurred in tissues other than the xylem. By contrast, in the stems the principal movement of the isotope was in the xylem, but its presence in the phloem was also demonstrated. These results have been confirmed by other unpublished experiments.

The independent movement of water and ^{45}Ca in the Brussels sprouts plants is in accord with the work of Bell and Biddulph (1963), who concluded that calcium movement is not the result of mass flow, but is due to the metabolic removal of the ions from exchange sites.

The occurrence of downward-moving ^{45}Ca in the phloem of Brussels sprouts is of interest, as it has been generally accepted that calcium is immobile in the phloem, and hence does not recirculate in plants (Biddulph 1959; Zimmermann 1960). Recent work has shown that calcium is evidently not completely immobile in plants, as recirculation of previously deposited ^{45}Ca has been reported by Ferrell and Johnson (1956) in western white pine, Vlasyuk and Grodzinskii (1958) in lupins, Kiselev (1961) in several plant species, Millikan and Hanger (1964) in subterranean clover, and Martin (1967) in apple trees. Also Biddulph, Nakayama, and Cory (1961) observed the limited transfer of ^{45}Ca from xylem to phloem in the old leaves of beans. Wiersum (1966), however, has stated that much of the evidence on the redistribution of calcium in plants may be explained by release into the xylem and translocation together with water.

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VI. References

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