FURTHER STUDIES ON EXUDATION IN CUCURBITS

ALDEN S. CRAFTS

Introduction

With DIXON'S recognition (7) that the movement of organic materials may take place through the phloem at rates sufficient to explain normal translocation, few advocates of the xylem theory remain, and attention is again centered upon the sieve tube hypothesis. The osmotic mechanism proposed by MÜNCH (14) to explain mass flow through sieve tubes is accepted by DIXON (7) and others (9). To some it may seem that the long search for a satisfactory explanation of the movement of organic materials in the plant is nearing an end.

There appears, however, to be a rational objection to the sieve tube theory. As early as 1835, VON MOHL (13) questioned the perforation of the sieve plate. KUHLA (10) and SCHMIDT (16) have cast further doubt on its structure. Faced by SCHMIDT'S work on the one hand and by RUHLAND'S (15) statement as to the impermeability of sieve tubes on the other, and feeling that I had substantiated the studies of both these men, I proposed in 1931 (2) that a mass flow of nutrients in solution might pass through the intermolecular spaces in the highly hydrated phloem walls.

My use of the Poiseuille formula for flow through capillaries indicated that resistance to such flow would be high. It seemed possible, however, that if the formula were altered to apply to flow between parallel plates, then the second power function of r, the radius of the capillary, as given in my modification of this relation (2, p. 14), might be replaced by a first-power function of d, the distance between the plates (4, p. 213). This was not the case; and, since careful consideration of the structure of cellulose as determined by x-ray analysis gave no hope for intermolecular spaces wide enough to accommodate mass flow under the existing gradients, this theory was abandoned (3, 4, 5).

In studying the mechanism by which assimilated materials enter the phloem and exit from it, I needed more information on the detailed structure of sieve tubes. Most of the descriptions in the literature were of mature elements, and those giving more complete details did not present a consistent picture of sieve tube ontogeny.

In studies on the sieve tubes of cucurbits (4), I attempted to describe the various stages in the development of these elements and soon realized that my early experiments checking RUHLAND'S work told only part of the story. By using a variety of methods, I studied not only the form and contents of the elements at different ages but also their osmotic properties, reactions to vital stains, protoplasmic properties, and general structural

relations. These studies on the sieve tubes of cucurbits (**4**), potato (**5**), and two *Nicotiana* species (**6**) have been described. The outstanding conclusion of this work was that, although the young sieve tube element has the properties of a normal living cell, with maturity it goes through a series of unique changes resulting in a cessation of protoplasmic streaming, a disappearance of all inclusions except sieve tube plastids, and, so far as could be determined from the few species studied, a loss of the property of semipermeability.

The implications of this finding are apparent. With the sieve tubes completely permeable at maturity, their vacuoles may constitute the principal channels for movement. Mass flow, which rates and gradients (**1, 2, 4, 5, 12, 14**) would seem to demand, could occur with only the end walls to offer resistance. One radical departure from the classic sieve tube theory as elaborated by MÜNCH, however, would be required. With the possibility of a certain amount of movement through the walls, a "limiting layer," as proposed in my earlier publication (**2**), would be required.

The weakness of my theory of mass flow through walls was obvious and was pointed out by many of my colleagues. The essence of these criticisms has been well presented by STEWARD and PRIESTLEY (**17**). At present there seems to be no doubt that the resistance to movement through the walls over the total distance traversed would be too great. I should like to point out in this connection that my "rejection of the sieve tubes as the path for translocation" (**17**) rested *principally* upon the data of SCHMIDT (**16**) and RUHLAND (**15**), and that choice of the walls *followed* a search for some other channel. Only after the sieve tubes appeared to be eliminated were the large cross-sectional area and the high hydration of phloem walls recognized and the theory proposed that these walls might constitute the actual conduits.

STEWARD and PRIESTLEY, going further, have questioned my use of certain data on exudation from cucurbits. In their words, "It seems much more probable that in a succulent plant of the type used the exudation from the phloem is not an indication of a normally occurring mass flow at all, either in the walls or sieve-tubes, but may be due rather to the turgor of surrounding tissues and the release of tissue tensions consequent upon cutting. A new cut then merely opens up a new length of stem." They question further, and with some justification, my comparison of "the rate . . . observed over a period not exceeding five minutes . . . with the rate calculated as necessary to account for uptake of dry matter by a pumpkin . . . assumed uniform over a period of 100 days." These questions are raised in the face of data (**2**, pp. 18, 19) indicating that exudation may occur at rates equivalent to flow of over 2 cm. per minute through the *total phloem* while the section removed each minute need not exceed 1 mm. in thickness;

and the statement that "a volume of solution equal to the *volume* of the *total phloem* in 13.7 cm. of stem flowed out during the 21 minutes, about 1 cm. of stem having been removed during the collection (**2**, collection C, table VII, p. 19).

The present paper concerns primarily this matter of the relation of phloem exudation in the cucurbit to normal movement of assimilated foods. It indicates that more than cell turgor and tissue tensions are involved in the exudation process, and that the flow, whether "normal" or not, may move sufficient food material over great enough distances to account for observed rates of growth and storage.

Experimentation

Since my previous work with cucurbits (**4**), several perplexing questions have arisen concerning exudation. Having an opportunity to study some squash plants growing in culture solutions in the greenhouses of the Plant Nutrition Division at Berkeley, I performed a number of experiments that should clarify some of these questions.

The first problem was to distinguish between xylem and phloem exudation and to find the conditions required for separating the two. This distinction proved rather simple with these plants. As they were growing in aerated full nutrient solution and were developing rapidly, root absorption was very active, and to collect xylem sap it was necessary only to cut the plant below the lowest leaf. As soon as the water deficit caused by transpiration was satisfied (within 5 minutes or less in these experiments), xylem sap flowed from the cut stump, and exudation continued for several days until food reserves in the root became exhausted. If it was desirable to obtain the xylem exudate free of contamination from the phloem, steaming of the hypocotylar region would coagulate the phloem sap *in situ;* and, after a few minutes had been allowed for washing the cut surface clean, xylem sap could be collected in a relatively pure form.

To obtain pure phloem exudate the excised portion of the same plant could be used. If a 1 mm. slice was cut from the internode every two minutes, phloem exudate could be collected over a period of half an hour or more. Since this excised shoot might be 8 feet or more in length, an appreciable volume of sap could be collected. It continued to flow long after the leaves had wilted, and a positive phloem pressure could be detected after the whole stem had become flaccid. It seems highly doubtful that this flow could be attributed either to cell turgor or to tissue tension.

For extended collections of phloem exudate, the stem had to be maintained in a more natural condition; another method, accordingly, was used. Transpiration from the leaves maintained a subatmospheric pressure in the xylem of these plants throughout the day and during most of the night.

If the initial cut was made at the tip of the shoot, therefore, pure phloem exudate could be collected with little effect upon the normal functioning of the plants.

Examination of the xylem pressure conditions, either by dissection under the binocular or by cutting under eosin solution, proved that at the tips of these plants xylem pressure was subatmospheric from early morning until well after sundown. Water was being lost at a higher rate than it was being absorbed, although root pressure could readily be demonstrated by removal of the transpiring surface.

The next problem concerned the duration of phloem exudation from healthy plants. Previous tests had shown that it could be maintained over a period of 4 hours (**4**). The first run in the present experiments covered a 12 hour period. At 9:34 A. M. on July 10, four plants were selected and collections started. Two of the plants were in aerated and two in non-aerated solutions. For the collections small glass test tubes, $\frac{3}{16}$ inch in bore and 3 inches long, were used, a tube being slipped over the end of the stem as soon as it was cut. In these collections a slice of stem about 1 mm. in length was removed every 2 minutes. Each time, before the cutting, the free sap adhering to the end of the stem was wiped off and was teased down the side of the tube with a small dissecting needle. The stems were placed in a sloping position so that the sap flowed downward and remained in the bottoms of the tubes. The four plants were handled simultaneously until 1:40 P. M., when exudation from the unaerated plants became so slow that collection was impracticable. The collections on the two plants in aerated culture solutions were then made every minute and continued until 10:00 P. M. During the collection several internodes were removed, and each node with a portion of the two accompanying internodes was saved. These nodes were kept in cold tap water and were sectioned on the following day for phloem measurements. The data from the collections are presented in table I.

Obviously the plants in unaerated culture solution suffered from slow water absorption by the roots. By noon they had both wilted, and phloem exudation had dropped to less than half of that of the other two plants. The latter maintained a fair rate throughout the afternoon until about 8:30 P. M., when, with a rapid drop in water loss, the rate increased appreciably. By 10:00 P. M. the water deficit had been completely satisfied, and xylem exudation had started. The collections were stopped, and a test tube was placed under the end of each stem. By morning there were about 10 cc. in each test tube. This was a watery solution of low concentration and, since the ends were not cut after 10:00 P. M., consisted principally of xylem sap.

According to these data (table I), exudation from the phloem may be

TABLE I

PHLOEM EXUDATION FROM SQUASH. VOLUME AND RATE OF FLOW FROM CUT STEMS. COLLECTIONS MADE JULY 10, 1934

PLANT	COLLECTION No.	COLLECTION TIME	WEIGHT OF SAP FRESH	WEIGHT OF SAP DRY	DRY WT. AS PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR* RATE OF FLOW PER HR.	TEMPERATURE OF GREENHOUSE
			gm.	*gm.*	%	*cm.*^{2}	*cm.*	°*C.*
A Not aerated	1	9:34–10:36	0.302	0.019	6.4	0.007	43.1	26.0
	2	10:36–11:39	0.167	0.008	4.7	0.015	11.1	27.5
	3	11:39–12:38	0.083	0.004	4.8	0.019	4.4	29.2
	4	12:38– 1:40	0.041	0.002	4.6	0.019	2.2	29.2
B Not aerated	1	9:38–10:37	0.156	0.013	8.1	0.010	15.6	26.0
	2	10:37–11:40	0.195	0.010	5.3	0.016	12.2	27.5
	3	11:40–12:40	0.117	0.005	4.6	0.020	5.9	29.2
	4	12:40– 1:37	0.058	0.002	4.2	0.024	2.4	29.2
C Aerated	1	9:40–10:43	0.354	0.026	7.1	0.008	44.3	26.0
	2	10:43–11:41	0.346	0.017	4.9	0.010	34.6	27.5
	3	11:41–12:40	0.265	0.011	4.3	0.014	18.9	29.2
	4	12:40– 1:39	0.137	0.007	5.0	0.017	7.9	29.2
D Aerated	1	9:42–10:44	0.197	0.011	5.5	0.006	32.8	26.0
	2	10:44–11:47	0.141	0.006	4.3	0.010	14.1	27.5
	3	11:47–12:45	0.118	0.004	3.8	0.013	9.1	29.2
	4	12:45– 1:42	0.128	0.005	4.2	0.015	8.5	29.2
C & *D* Aerated	1	2:00– 3:00	0.599	0.025	4.1	0.063	9.5	32.5
	2	3:00– 4:00	0.483	0.017	3.6	0.064	7.6	31.0
	3	4:00– 5:00	0.542	0.025	4.6	0.066	8.2	29.0
	4	5:00– 6:00	0.554	0.014	2.5	0.066	8.4	27.0
	5	6:00– 7:00	0.531	0.011	2.0	0.066	8.0	25.0
	6	7:00– 8:00	0.494	0.010	2.1	0.063	7.8	23.0
	7	8:00– 9:00	0.776	0.025	3.3	0.061	12.7	21.0
	8	9:00–10:00	1.155	0.041	3.6	0.064	18.1	20.0

* The values in this column are calculated rates based on the cross-sectional area of the total phloem. Since the sieve tubes occupy 25 per cent. or less of the phloem, these values must be multiplied by a factor of at least 4 to give actual rates.

maintained for many hours; and although the rate may fluctuate considerably, there is no indication of a complete stopping. During the 12 hour period, incidentally, there was collected from the plants in aerated culture solution an average volume of phloem sap equivalent to the *total volume of phloem* in 173 cm. of stem. About 36 cm. of stem were removed in the form of thin slices. The temperature and humidity in the greenhouse were such that even the plants in aerated culture solution were wilted slightly between 2:30 and 5:00 P. M.

In order further to confirm these results, a second test was made using two new plants in aerated culture solution. Two vigorous young plants, as

nearly alike as possible, were selected. Collections were started at 2:00 P. M. on July 21, 1934. One plant had sixteen nodes, the other seventeen, and each was about 8 feet in length.

At 7:00 P. M. the steam heat was turned on in the greenhouse, and at 7:30 four 500-candlepower floodlights were hung above the plants. Because of the steam and also the radiation from the lights, the greenhouse temperature remained relatively high; about midnight the leaves on one plant were slightly wilted. Two of the lights, consequently, were turned off; and at 2:00 A. M. a ventilator was opened. As the temperature was still 25° C. at 4:00 A. M., two more ventilators were opened; by 4:30 it had dropped to 20° C. Soon the sap started to come faster from both plants, and by 5:00 there was active xylem exudation. This continued for 2 hours and then stopped as the light intensity increased. Phloem exudation also dropped until 10:00 A. M., then it started to increase, and continued to gain in volume until the end of the run. The data from this test appear in table II.

The dry weight composition of the sap collected between 4:00 and 8:00 A. M. indicates that the sap was diluted with xylem exudate. To give a more correct picture of the rates of flow during this period, the values in the rate column have been corrected on the basis of a dry weight composition of 3.7, which is the average for the periods 3:00–4:00 A. M. and 8:00–9:00 A. M. Even with this correction the rates are high, as might be expected on the basis of the relative abundance of water. Under these conditions an osmotic system would tend to absorb water faster, flow would be increased, and solutes would be lost faster than they were being supplied. Next would follow a period of slow exudation as the water became less available. That this is the case from 8:00 until 12:00 is shown lower in this column (table II). These stems were about 240 cm. in length at the start of the experiment. Approximately 72 cm. were removed during the 24-hour collection period. A volume of sap equivalent to the *total phloem volume* in 189.9 cm. of stem was collected. This figure is based on the corrected values given in the rate column.

A third set of collections that was made seems to render even more improbable the idea that phloem exudation is caused by cell turgor or by the release of tissue tensions. In these tests the stems were first steamed below the cotyledons and cut from the roots, which again were in aerated culture solutions. Three groups of four plants each were used. In one group the excised stem of each plant was cut near the tip so that the apical portion represented about 25 per cent. of the stem and the basal portion 75 per cent. In the second group the apical portion was the larger and the basal portion relatively small. In the third group an attempt was made to cut the stem with two portions of equal weight. The stems were handled individually, and after they were cut, sap was collected from the apical and basal portions

simultaneously as long as it flowed freely. The cut was made in the center of an internode in each case. Data on these collections appear in table III.

TABLE II

PHLOEM EXUDATION FROM SQUASH. VOLUME AND RATE OF FLOW FROM TWO WATER CULTURE PLANTS FOR 24 HOUR PERIOD. PLANTS WITH SIXTEEN AND SEVENTEEN DEVELOPED NODES, APPROXIMATELY 8 FEET IN LENGTH. COLLECTIONS MADE JULY 21, 1934

COLLECTION		WEIGHT OF SAP		DRY WT. AS PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR* RATE OF FLOW PER HR.	TEMPERATURE OF GREENHOUSE
No.	TIME	FRESH	DRY				
		gm.	*gm.*	%	*cm.*^{2}	*cm.*	°*C.*
1	2:00– 3:00	0.788	0.055	6.9	0.044	17.9	26.0
2	3:00– 4:00	0.852	0.042	4.9	0.059	14.4	27.0
3	4:00– 5:00	0.710	0.032	4.5	0.060	11.8	22.5
4	5:00– 6:00	0.596	0.026	4.4	0.060	9.9	30.0
5	6:00– 7:00	0.437	0.019	4.3	0.073	6.0	26.0
6	7:00– 8:00	0.321	0.016	5.0	0.077	4.2	21.0
7	8:00– 9:00	0.499	0.020	4.1	0.077	6.5	24.0
8	9:00–10:00	0.671	0.024	3.6	0.077	8.7	23.5
9	10:00–11:00	0.725	0.023	3.1	0.084	8.6	24.5
10	11:00–12:00	0.584	0.019	3.3	0.087	6.7	23.0
11	12:00– 1:00	0.586	0.018	3.2	0.082	7.1	23.5
12	1:00– 2:00	0.689	0.021	3.0	0.082	8.4	25.0
13	2:00– 3:00	0.775	0.022	2.9	0.082	9.4	22.0
14	3:00– 4:00	0.644	0.021	3.3	0.086	7.5	25.0
15	4:00– 5:00	1.722	0.031	1.8	0.087	9.7	20.5
16	5:00– 6:00	2.622	0.037	1.4	0.087	11.5	23.5
17	6:00– 7:00	2.560	0.034	1.3	0.087	10.6	23.0
18	7:00– 8:00	0.507	0.014	2.8	0.087	4.4	23.0
19	8:00– 9:00	0.185	0.008	4.2	0.087	2.1	23.3
20	9:00–10:00	0.179	0.007	4.1	0.087	2.1	24.5
21	10:00–11:00	0.265	0.013	4.9	0.091	2.9	26.1
22	11:00–12:00	0.251	0.011	4.5	0.088	2.9	28.7
23	12:00– 1:00	0.648	0.023	3.6	0.086	7.5	29.5
24	1:00– 2:00	0.766	0.028	3.7	0.086	8.9	30.0

* The values in this column are calculated rates based on the cross-sectional area of the total phloem. Since the sieve tubes occupy 25 per cent. or less of the phloem, these values must be multiplied by a factor of at least 4 to give actual rates.

As these figures (table III) show, the volume of sap exuding from a portion of stem was roughly proportional to the weight of the stem; and it was not the nature of the local tissues but the mass of the stem acting as a reservoir and source of sap that regulated the volume delivered. The two halves of the cut internode were practically identical in every case, so that the first

TABLE III

RELATIVE VOLUMES FROM TIP AND BASAL PORTIONS OF EXCISED STEMS. COLLECTIONS MADE JULY 27, 1934. TEMPERATURE 22°–29° C. IN HEAD HOUSE

STEM NO.	TIME	LENGTH OF STEM		NO. OF LEAVES		FRESH WEIGHT		FESH WEIGHT OF SAP		DRY WEIGHT OF SAP		DRY WEIGHT AS PERCENTAGE OF FRESH	
		TIP	BASE	TIP	BASE	TIP	BASE	TIP	BASE	TIP	BASE	TIP	BASE
	min.	*cm.*	*cm.*			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%	%
1	68	60	160	4	13	52	479	0.191	0.452	0.016	0.037	8.4	8.3
2	45	40	200	2	12	20	322	0.048	0.186	0.005	0.016	9.6	8.5
3	30	40	135	3	9	26	327	0.117	0.438	0.010	0.035	8.6	7.9
4	30	45	170	3	9	20	305	0.061	0.193	0.006	0.017	9.7	8.7
5	22	190	10	24	1	630	68	0.239	0.075	0.018	0.005	7.7	6.9
6	30	210	50	11	3	236	114	0.322	0.164	0.025	0.012	7.7	7.1
7	50	180	40	10	2	204	62	0.274	0.136	0.024	0.010	8.7	7.3
8	30	200	25	12	2	288	75	0.260	0.071	0.023	0.006	8.7	8.5
9	30	140	20	14	2	153	100	0.189	0.177	0.014	0.012	7.5	6.6
10	30	90	40	9	4	112	93	0.137	0.101	0.012	0.008	8.5	7.9
11	30	110	75	6	7	190	190	0.218	0.222	0.020	0.018	9.1	8.5
12	30	80	70	8	5	95	121	0.245	0.269	0.026	0.023	10.6	8.7

5 cm. or more of stem from the cut was the same. It was the tissue beyond the first node, therefore, that determined the amount of sap collected.

In this series two additional points of interest were noted. Ordinarily the most rapid flow occurs from the young growing tissues near the apex of the stem (**4**). When the apical portions of these stems had been allowed to exude from their basal ends until flow had practically ceased, a cut near the tip did not result in exudation from that region. The stems acted as though they had been exhausted, and no more sap could be obtained by cutting.

The second point is apparent in the last two columns of table III. In every case the dry weight concentration of the exudate from the tip portion exceeded that from the base. This fact is further evidence for a concentration gradient in the actual conducting channels (**4**, table XIII, p. 210). There is no question of an admixture of saps in these experiments, since the shoots used were all excised and wilted soon after their removal from the root systems.

One further set of collections that was made gives some idea of the nature of xylem exudation. This experiment was run in conjunction with T. C. BROYER, Division of Plant Nutrition, who made most of the collections. The object of the experiment was to find the volume of xylem exudate that could be collected in relation to the size of the root system.

The plants were young squash grown in aerated, full nutrient culture solution. The four used in this test were removed from the culture solution, the roots washed, and the plants set up in tap water at 9:00 A. M., July 25. They were cut just below the cotyledons at 9:15, and the collections started. These continued until 9:00 A. M., July 26. The roots were then removed, centrifuged free of excess water, and weighed. Their total weight was 450 gm. During the 24 hours, 551.7 cc. of exudate were collected. These roots therefore exuded in this period a volume of sap greater than their own volume. Since less than 20 per cent. of this total volume is occupied by the lumina of the xylem vessels, evidently these conducting elements had their contents displaced more than five times during the test. The results of this experiment are shown in table IV.

It seems evident that large samples of xylem sap can be obtained from excised root systems of squash.

After these quantitative tests, a series of qualitative observations and microscopic studies were made in an attempt to explain the nature of stoppage of the phloem, together with the relation of sieve tube inclusions to flow and stoppage, and to compare in several ways phloem and xylem exudates.

In the comparisons the following points were observed: The dry weight composition of xylem exudate from a group of plants in aerated culture

TABLE IV

VOLUME AND COMPOSITION OF XYLEM EXUDATE FROM SQUASH COLLECTED JULY 7, 1934, AT BERKELEY, CALIFORNIA

No.	SAP COLLECTION		RATE PER PLANT PER MIN.	PERCENTAGE DRY MATTER	TEMPERATURE	
	TIME	VOLUME			CULTURE SOLUTION	GREENHOUSE
		cc.	*cc.*	%	°*C.*	°*C.*
1	9:15– 9:30	10.5	0.17	0.16	25.0	26.5
2	9:30– 9:45	28.0	0.47	0.16	.....	.....
3	9:45–10:00	30.0	0.50	0.16	.....	.....
4	10:00–10:10	24.5	0.61	0.15	.....	.....
5	10:10–10:15	10.2	0.51	0.13	.....	.....
6	10:15–10:20	12.5	0.62	0.16	.....	.....
7	10:20–10:25	12.0	0.60	0.18	.....	.....
8	10:25–10:30	12.5	0.62	0.18	.....	.....
9	10:30–10:35	12.5	0.62	0.19	.....	.....
10	10:35–10:40	11.5	0.57	0.22	.....	.....
11	10:40–10:45	10.5	0.52	0.18	25.0	30.0
12	10:45–10:50	11.0	0.55	0.19	.....	.....
13	10:50–10:55	10.0	0.50	0.20	.....	.....
14	10:55–11:00	8.5	0.42	0.23	.....	.....
15	11:00–11:05	9.5	0.47	0.21	.....	.....
16	11:05–11:10	9.5	0.47	0.23	.....	.....
17	11:10–11:20	16.0	0.40	0.21	.....	.....
18	11:20–11:30	14.5	0.36	0.19	26.0	30.5
19	11:30–11:40	12.5	0.31	0.21	.....	.....
20	11:40–11:50	10.5	0.26	0.22	.....	.....
21	11:50–12:00	9.0	0.22	0.21	.....	.....
22	12:00–12:15	11.5	0.19	0.20	.....	.....
23	12:15–12:30	9.5	0.16	0.18	28.3	32.0
24	12:30–12:45	8.0	0.13	0.16	.....	.....
25	12:45– 1:00	7.5	0.12	0.22	.....	.....
26	1:00– 1:30	10.5	0.09	0.18	.....	.....
27	1:30– 2:00	10.0	0.08	0.14	30.0	33.0
28	2:00– 2:30	9.0	0.08	0.15	.....	.....
29	2:30– 3:00	8.0	0.07	0.14	31.3	33.3
30	3:00– 3:30	8.5	0.07	0.13	.....	.....
31	3:30– 4:00	8.0	0.07	0.15	31.5	33.0
32	4:00– 4:30	7.0	0.06	0.15	.....	.....
33	4:30– 5:00	7.5	0.06	0.13	.....	.....
34	5:00–10:00	70.0	0.06	0.12	31.5	32.0
35	10:00– 8:00	20.5*	0.03	0.11	24.2	16.5
36	" "	13.0*	0.02	0.17	.....	.....
37	" "	24.0*	0.04	0.14	.....	.....
38	" "	29.0*	0.05	0.14	.....	.....
39	8:00– 9:00	6.5	0.03	0.10	18.0	23.0

* Volumes collected from the four separate plants from 10:00 P. M. on July 7 until 8:00 A. M. on July 8.

solution was 0.59 per cent.; from plants in non-aerated culture solution, 0.49 per cent.; and from a relatively pure tap water, 0.21 per cent.

Phloem exudate under these three conditions showed no consistent differences. On the other hand, from these young, vigorously growing squash plants in the blossoming stage the phloem sap averaged about 4 per cent. dry weight, whereas that from fruiting cucumber and pumpkin plants ran more than 10 per cent. in many cases during the first few minutes (**2, 4**).

Phloem sap has been found always to coagulate. This process takes place very rapidly upon heating or treating with alcohol; slowly in the cold in stoppered test tubes. The coagulum will not dissolve in mild acid or alkali. In no case has the xylem sap been found to coagulate.

Exudate collected from the phloem of cucurbits used in these experiments flowed out at rates of from 0.01 to 0.1 cc. per minute. While xylem sap from the same and similar stems commonly flows at many times this rate and will continue for hours with little change, the phloem soon plugs and must be reopened by cutting.

Phloem sap may be coagulated in the stem by heat; when the heated portion is cut, no phloem exudation occurs. Heating had no visible effect upon xylem exudation.

Exudation from the cut xylem depends upon conditions affecting water loss. Even when only the excised root system is involved, water deficit must be satisfied before an actual exudation occurs. Guttation occurs only when conditions tend to reduce transpiration to a value lower than that of water absorption by the roots.

Phloem exudation will occur from severely wilted plants. The volume is reduced in proportion to the water deficit, but the dry weight composition tends to increase in compensation.

Phloem sap from the plants studied at Berkeley had a pH of approximately 7.3, as shown by spot-plate tests with indicators. It is rather highly buffered. Xylem sap from the same plants had a pH of 5.5 and was practically unbuffered.

Additional information was gained from microscopic tests on phloem tissues. Slime plugs result from coagulation of the disintegration products of the nucleus and slime drops in cucurbits (**4**). Consequently, they can be found only in sieve tubes during a certain stage in their ontogeny. This stage corresponds, apparently, with that at which loss of the property of semipermeability takes place. Possibly, therefore, slime plugs may occur naturally in sieve tubes in which sap flow is very rapid. They always occur in these tubes in cut stems if exudation has been rapid and may be found at a distance of 10 cm. or more from the cut surface, lodged against the distal sides of sieve plates. An interesting fact is that although these structures are present for many centimeters from the cut, removal of a slice as thin as

0.5 mm. will cause exudation to resume after stoppage. Apparently they occur only in tubes in a certain stage of maturity and have little or nothing to do with stoppage.

When a stem of squash is freshly cut and is wiped repeatedly, the large central tubes of the phloem groups are clogged first, and flow continues longest from the peripheral and cortical tubes (**4**). If stoppage were caused by plugging of the sieve tube lumina, just the opposite would be expected. Repeated microscopic examinations have failed, furthermore, to show the lumina of cut sieve tubes, from phloem in which flow has ceased, to be filled with coagulated sap. In many cases the only evidence of stoppage was a peculiar staining of the walls of the *cut sieve tube elements.* Upon second thought, this seems to answer logically the question regarding the nature of phloem stoppage. From the standpoint of hydraulics it seems highly improbable that flow from a cut tube under a head of several atmospheres could be stopped within the lumen of the tube by a slow coagulation of the flowing sap. It would be washed away faster than it could possibly accumulate. Furthermore, stoppage of flow in intact stems with heat does not cause a plugging of the lumina, but on the contrary, usually fixes the contents in place, and in sieve tubes past the slime plug stage may leave them relatively free of coagulum.

The coagulated phloem sap has an affinity for aniline blue and gives a peculiar violet color reaction. In the walls of the actual cut elements this same color reaction occurs.

It seems, therefore, that stoppage results from coagulation of the phloem sap within the interstices of the walls of the cut elements. Again the possibility occurs that normal flow across end walls of the sieve tubes takes place not through tubules or perforations in the protoplasmic connections but through the intermolecular spaces of the cellulose walls.

Discussion

The experiments described indicate that phloem exudation, rather than being of local origin and caused by turgor or release of tissue tensions, is a direct manifestation of a process occurring throughout the plant. As a result of this process, organic foods are moved over long distances in relatively short times. The reactions of this conducting system to manipulation indicate it to be of the nature of an elongated, somewhat elastic osmometer working under a positive pressure and activated by a concentration gradient of its osmotically active contents.

The mechanism of xylem exudation, on the other hand, seems to be of a different nature. While the commonly pictured osmotic mechanism might explain a slow absorption of water, it cannot account for the rapid flow obtained from the plants used in the experiment reported in table IV. The

xylem vessels of these root systems had their total contents replaced many times within the period of 24 hours. Furthermore any theory of root pressure which fails to explain the simultaneous absorption and secretion into the non-living xylem vessels of water and inorganic ions is inadequate, for although these two functions may not be directly correlated, in the living plant they are both in process at the same time and they involve the same tissues.

While the xylem and phloem constitute two separate and distinct systems within the plant, either or both of which may exhibit exudation, the fact that the former may act as a source of water for the osmotic phloem system imposes upon them a certain interdependence. Situated as they are in close proximity over a relatively large area and separated by only a thin layer of cells, a very delicate water balance must necessarily exist between them. The open nature of the xylem should effect a rapid adjustment to fluctuating pressure conditions throughout the system. Such changes in pressure, therefore, would be transmitted to and effective upon the total phloem system at approximately the same time. Movement within the phloem, on the other hand, is relatively slow, with much internal resistance, and in addition the phloem gives every evidence of acting like an inflated elastic system. Consequently response to fluctuations in pressure in the phloem would be slower and changes in rate would be gradual.

With this relationship in mind, it might be interesting to consider briefly what happens when the cucurbit stem is cut and the phloem exudation occurs. Granting that translocation is occurring normally under a gradient of pressure within the phloem, and that transpiration is maintaining a subatmospheric pressure within the xylem, when the stem is cut a rapid readjustment must occur. First, cutting opens the phloem, allowing loss of sap against only atmospheric pressure. The gradient in pressure is greatly steepened and internal pressure rapidly lowered by exudation. In the xylem, resistance to uptake of water is removed and xylem sap flows rapidly away from the cut end until the whole xylem system comes approximately to atmospheric pressure. Water is relatively abundant for uptake by the phloem. Not only the xylem but all living cells in contact with the phloem serve as a source of readily available water because of the decrease in turgor pressure and consequent increase in suction force in the phloem.

The result of these combined changes is to produce an abnormally high rate of flow from the phloem which may be ten or more times the normal rate (**4**). With the deflation of the elastic system and the readjustment of water relations, this high rate falls off rapidly and at the same time concentration of the exudation sap decreases. Previous data (**4**, table X) indicate that 15 minutes or more may be required for the establishment of new equilibrium conditions; and the initial high rate and concentration of the exudate result in high average values during the first hour of collection, as shown in tables I and II.

With clogging of the xylem vessels with air, subatmospheric pressure is again established, and with lowered water availability pressure in the phloem is further lowered and several hours may be required for exudation flow to become normal again. If the supply of organic nutrients to the phloem is maintained there should be an increase in concentration to compensate for the lowered rate of flow.

In interpreting data on extended collections, as given in table II, even other factors must be considered. The lowering concentration of the exudate from sundown until daylight the following morning is probably related to the supply of soluble materials to the phloem. The great increase in rate shown in both tables I and II is related, on the other hand, to decreased transpiration. In both experiments water absorption by the roots exceeded water loss for a period and active xylem exudation resulted.

Following this, as transpiration again lowered water availability, the rate was greatly decreased and the concentration gradually built up. With increasing supply of osmotically active solutes, rate and concentration both approached again the values attained the previous day after the initial period. It should be pointed out that the initial rate of exudation from cut phloem is strictly abnormal owing to rapid deflation of an expanded system. The concentration at this time is very near normal. On the other hand, concentration after cutting cannot again attain the normal value so long as the system is kept open because of the new pressure conditions. Soluble materials are carried to the open end of the system as fast as they are supplied, and the original turgid condition with its attendant high concentration is not attained.

The interdependence of concentration and rate that is so intimately related to water availability is again brought out in a consideration of the effects of diurnal pressure variations in the xylem. With the lowering of xylem pressure by transpiration, flow of water to the phloem decreases, concentration of the phloem sap increases (if the supply of solutes is maintained), and translocation continues at somewhat lowered linear rates of flow. With increased xylem pressures at night, an increase in exudation pressure occurs and translocation takes place at a higher rate and lowered concentration. Because of this ready exchange of water across the intervening tissues, the osmotic phloem system responsible for phloem exudation can maintain translocation regardless of pressure conditions in the xylem, provided a supply of osmotically active materials is maintained.

Xylem exudation is of common occurrence in the spring. JAMES and BAKER (9) question its presence in any plants except the grape. I have found it easy to demonstrate in the common annuals that grow rapidly in March and April. *Brassica arvensis* (L.) B.S.P., *Malva parviflora* L., and *Lupinus bicolor* Lindl. make convenient demonstration material. Undoubt-

edly hundreds of other species, including many trees and vines, show this same phenomenon at certain times. That the flow actually occurs from the xylem can readily be shown by peeling back the cortical tissues and scraping off the cambium. In the vine it has been demonstrated even in some of the inner annual rings, while in the maple it occurs principally in the sapwood.

One might consider that in the trees and vines showing xylem exudation the situation is different from that in the leafy annuals mentioned. Examination of vines showed, however, that the roots of these plants were active and in some cases were actually producing many new feeding laterals, even though the buds were scarcely swelling. Apparently, therefore, the cases are similar; that is, an active root system is absorbing water faster than it is being lost, thus giving rise to a visible exudation of sap. In the leafy annuals the tops have to be removed before this exudation can be shown. In the woody species, complete removal of the top growth will eliminate the transpiration loss, and root pressure may be demonstrated well into the summer season. The fact that rapid hydrolysis of stored carbohydrates may result in a certain loss of sugars into this stream of xylem sap apparently has no direct bearing on the type of mechanism involved. The source of pressure seems to be in the active root tips, which are only remotely connected with this solution. The rate at which water may be absorbed and the location of the active organs seem to preclude the possibility that this sugar solution acts osmotically to cause water absorption from the soil.

There seems to be little justification for the attempt by JAMES and BAKER (**9**, p. 341) to assume an "essential unity of all sap movements in the plant" and to "include" these separate phenomena under "the common name of sap pressure." Such an assumption can only lead to error. Likewise the suggestion of STEWARD and PRIESTLEY that phloem exudation is strictly local in origin seems unsound in the light of the evidence set forth in this paper.

The recently proposed mechanism of VAN DEN HONERT (**18**) to explain translocation of foods on the basis of a surface tension phenomenon, although interesting for purposes of speculation, seems to have little support from the internal structure of phloem tissues. VAN DEN HONERT'S suggestion of the protoplasm vacuole interface as constituting the channel will scarcely bear scrutiny. When the sieve tubes are young and this interface is prominent, the cell has semipermeable properties, and movement from cell to cell would be difficult to explain. As the sieve tube elements mature, this interface appears to break down (**5**) and to give way to a region of indefinite properties. The protoplasmic strands of the sieve plate remain intact however, and the vacuoles consequently continue discrete in the separate elements, so that movement from cell to cell could not follow such an interface. The protoplasm ceases its streaming movements and apparently enters a phase of lowered activity.

Finally, from the standpoint of volume relations, such a mechanism seems highly improbable. Although it might explain movement of hormones in minute quantities, provided a continuous interface could be demonstrated, it would fail, as would protoplasmic streaming (5), to provide adequate transport for the quantities of food materials normally moved in plants.

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BOTANY DIVISION
UNIVERSITY FARM
DAVIS, CALIFORNIA

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