TEMPERATURE-INDUCED SAP FLOW IN EXCISED STEMS OF ACER[1]

JAMES W. MARVIN AND MARY T. GREENE

(WITH ONE FIGURE)

Received March 14, 1951

Introduction

The flow of sap from openings in the stems of Acer and some other woody genera has for many years received considerable attention and study. In maple, the flow is induced by temperature changes and a review of the literature indicates that practically all of the experimental work has been conducted in the field. It seemed desirable therefore to investigate some aspects of the flow mechanism in the laboratory where temperatures could be controlled.

Literature

The early observations on exudations from plant parts induced in various ways were comprehensively treated by PFEFFER (**21**). Recently KRAMER (**16**) and CRAFTS *et al.* (**10**) have published monographs relating to the subject.

Several different mechanisms have been proposed to explain maple sap flow. One of these is the thermal expansion of gases in the xylem which results in a pressure and a flow of sap if there is an opening in the xylem cylinder. CLARK (**8, 9**) made the first extensive observations on air temperatures, sap pressures, and sugar concentrations during maple sap flows. The simultaneous changes and apparent correlations between air temperature and sap pressures influenced Clark in considering flow to be the result of gas expansion. During the comprehensive and important investigations of JONES *et al.* (**14**), the question of gas expansion as a causative agent in maple sap flow was again considered and for the first time data on the gas content of the vessels were made available. These authors concluded that gas expansion alone was not sufficient to cause a flow. Shortly afterwards, WIEGAND (**24**) reviewed the Vermont data and added some of his own observations. He also concluded that neither gas expansion, water expansion, wood expansion, nor combinations of these could account for the flows observed. The valuable contribution of STEVENS and EGGERT (**22**) demonstrating sap flow from excised stems of red maple is interpreted in terms of another physical explanation: changes in volume may be associated with the formation of ice crystals in the xylem and phloem.

[1] Printed with permission of Vermont Agricultural Experiment Station; Journal Series Paper no. 14. This paper is also based upon work supported in part by a grant from the General Foods Corporation.

JOHNSON (**13**) has concluded from his studies of maple sap flow that the most probable effect of alternately high and low temperatures on flow is the effect on the oxygen content of the sap. He maintains that with high temperatures oxygen is less soluble and thus more is available for vital activities such as respiration. Therefore the period of increased respiration coincides with the period of greatest flow.

Many investigators have attributed the flow of sap to an osmotic effect. CLARK (**8, 9**) considered it but did not favor an osmotic explanation. On the contrary, JONES *et al.* (**14, 15**) conclude that the flow is the result of the "bleeding" of living cells and is essentially an osmotic mechanism. WIEGAND (**24**) is of the same opinion and he pictured in some detail the effect of a thermal gradient along a vascular ray which resulted in different temperatures at the two ends of a ray cell. This temperature unbalance would cause an osmotic unbalance and result in the movement of water or of a solution from the unwarmed to the warmed ray cells. He further postulated that the solution would either be secreted or moved by an osmotic gradient into the vessels.

Other investigators have considered the biochemical aspects of sap flow. The enzyme systems responsible for the hydrolysis of starch to sugars have been investigated by BOIS and NADEAU (**2, 3**) and BOIS and CHUBB (**1**) who postulate a starch to sucrose system. MEEUSE (**19**) also investigated the amylolytic properties of maple and birch sap and questioned the starch to sucrose system of BOIS *et al.* (**1, 2, 3**). They found amylase activity, but the concentration of the enzyme was very low. By incubating soluble starch with saps, maltose was produced as well as glucose. Seasonal changes in the carbohydrate content of the maple have been studied in great detail by JONES and BRADLEE (**15**).

The important problem of the composition of the vessel sap in the spring and the changes that occur in the composition of the sap at this time of year have been carefully investigated by BURSTRÖM and KRÖGH (**6, 7**) and BURSTRÖM (**4, 5**). These studies clearly show the rapid and profound changes occurring at the time of bud opening.

Although it has long been known that a flow of maple sap may be obtained from excised stems, recent work by STEVENS and EGGERT (**22**) demonstrates that excised stems absorb during periods of cooling between flows. Presumably in intact plants most of the absorption from roots also occurs on cooling. The mechanisms responsible for the accumulation of water and solutes in the vessels of roots are therefore of great significance. The discussions of EATON (**12**), CRAFTS and BROYER (**11**), LUNDGÅRDH (**17, 18**), VAN OVERBEEK (**23**), and NIE *et al.* (**20**) are pertinent.

Materials and methods

For many years casual observations have indicated that a flow of sap may be obtained from maple logs detached from the roots. STEVENS and EGGERT (**22**) have clearly demonstrated that such stems, if supplied with water, flow in what is apparently a normal manner.

There may be more than one kind of stimulus for the flow mechanism but, so far as is known at present, only a rise in temperature will produce a flow. It seemed desirable therefore, to have a controlled temperature apparatus in which the flow response of maple stems could be studied. An insulated chamber was constructed and supplied with refrigeration and heat. A fan with a 250 cubic feet per minute capacity circulated the air through the chamber and controlled the air temperature to $\pm$ 1° C. Temperatures in the experimental chamber and in the xylem of the stems were recorded by a recording potentiometer using copper constantan thermocouples. The thermocouple points measuring xylem temperatures were inserted obliquely two and a half to three inches in the tissues, approximately in the center of the stem, to reduce to a minimum the effect of thermal conduction along the wires.

The first experiments were on large stems 25 feet long and four to eight inches in diameter which weighed 45 to 55 kg. The cut ends were enclosed in metal caps with rubber gaskets and the flow from tap holes in the stems collected outside the box by means of a plastic tubing.

This procedure proved much too awkward and the apparatus and technique were redesigned to be used with much smaller stems. The stems used for the data reported here were four and a half feet long, 2 to 3 cm. in diameter, and weighed from 0.3 to 0.7 kg. These were from individual plants of *Acer saccharum* collected while frozen. They were either used immediately or collected in quantity in March and stored at -5° C. Such stored material was still usable after as long as four months under refrigeration. In nature, maple stems collected in Vermont show active flow from about November 15 to leaf emergence. Thus with the aid of frozen material in storage, experiments on flow could be conducted for nine months of the year.

The stems, which extended through both walls of the experimental box, had their ends enclosed with acrylic resin caps. The cap on one end was filled with distilled water and sealed off with glass stopcocks. The flow data were recorded from the other (basal) end of the stem with the apparatus illustrated in figure 1. The cap C and enclosed stem D was attached to a 10-ml. pipette A. At the start of an experiment the distilled water in the cap C was adjusted to form a meniscus near the bottom of the pipette and the stopcock E was closed. After warming the stem, the change in volume was measured in the pipette. In certain experiments solutions were perfused through the stem. These solutions were introduced through F with stopcock B closed. With pressures from 5 to 10 pounds per square inch the vessel sap could be replaced in from 8 to 28 hours depending upon the individual stem and its previous history. Burette G was used to supply the stem with distilled water or a sucrose solution during the cooling and freezing process.

Results

The data presented here are the results of experiments to explore the variations of flow responses from maple stems subjected to a variety of treatments under controlled temperatures. The flows were induced by rais-

ing the temperatures usually to 7° C, until flow ceased. In no experiment was a constant volume maintained at the end of a flow, but rather absorption followed immediately.

The experiments were usually run with six stems in replicate. These stems were collected from different individual plants and were selected for uniformity of shape and weight. The only source of material was naturally grown plants. Although grown in a fairly uniform stand, such plants probably show genetical differences as well as the effects of variation in the composition and moisture content of the soil.

At the outset it is of interest to compare the sap flow of sugar maples with that of another species of maple and other genera commonly associ-

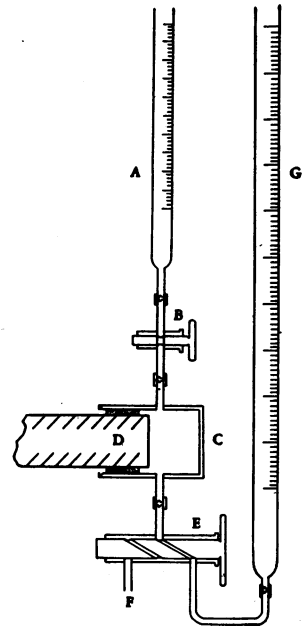

FIG. 1. Apparatus used to measure sap flow. See the discussion in the text.

ated with sugar maple in nature. Five stems, two from species of Acer and three from other genera, were given the same treatment—that is, frozen and then warmed to 7° C. Under these conditions both species of Acer gave a flow while the other genera absorbed water. These results are illustrated in table I.

In reviewing the results of a number of experiments in which the experimental conditions were essentially the same—that is, the stems, after being assembled in the apparatus and absorbing water on cooling, were frozen and then warmed to 7° or 8° C—a great variation in the flow pattern became apparent. It may be seen from table II that there is a variation in the duration of the flow from different stems from 1.25 hours to 53.25 hours. In general, freshly cut stems flow at a rapid rate although for a relatively short

TABLE I

FLOW RESPONSE (IN MILLILITERS) OF STEMS OF FOUR GENERA AND FIVE SPECIES AFTER FREEZING AND THAWING.

Experiment number	*Acer pennsylvanicum*	*Acer saccharum*	*Betula lutea*	*Prunus serotina*	*Fagus grandifolia*
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
1	+2.6	+2.1	−5.6	−0.5	−1.5
2	+1.5	+2.9	−10.9	−1.8	−8.8
3	+0.2	+1.8	−5.6	−5.2	−5.5
Average	+1.4	+2.3	−7.4	−2.5	−5.3

time as compared with stems which have been held frozen in storage for several weeks. In both types of material there is no apparent correlation between the duration of the flow and the total amount of sap produced. There may be both large and small amounts of sap produced during short flow periods and long flow periods.

Other variations observed were the differing times and temperatures at which flows began. Very commonly as the stems thawed there was an initial decrease in volume in the measuring system, perhaps the result of the change in state from solid to liquid in the vessels. If a flow occurred, this initial decrease was followed by an increase in volume in the measuring system. Table III gives the data from a typical experiment in which the experimental box was warmed to 7° C and held at that temperature. The data represent the cumulative changes in volume. During the three and a half hours required to warm the system, the stems warmed at a slower rate than the experimental box. Stem 3 began a flow after being warmed 1.0° C while stem 4 did not begin to flow until it had been warmed 5.5° C. Stems 1 and 2 were intermediate and, as often happened, stems 5 and 6 continued to absorb for the entire experimental period. Similar data are presented in table

TABLE II

VARIATIONS IN THE AMOUNT AND DURATION OF FLOW FROM DIFFERENT STEMS.

Stem weight	Month	Condition of stem	Temperature during flow	Duration of flow	Flow
gm.			*°C*	*hrs.*	*ml.*
459	December	Freshly cut	8	1.25	1.1
735	March	" "	8	1.50	6.8
915	March	" "	8	1.50	14.9
970	March	" "	8	2.00	17.3
627	November	" "	7	3.50	2.3
734	July	Stored stem	7	6.00	6.8
652	June	" "	7	14.75	7.2
583	July	" "	7	19.00	4.3
699	June	" "	7	26.50	21.6
621	June	" "	7	26.50	1.8
540	June	" "	7	28.00	1.0
477	June	" "	7	53.25	15.7

TABLE III

UNDER CONDITIONS OF RISING TEMPERATURE (TO 7°C) THERE ARE VARIATIONS BETWEEN STEMS IN THE TEMPERATURES AT WHICH FLOWS BEGIN (MAY).

Time	Box temp.	Stem temp.	Volume change in milliliters					
			Stem no. 1	2	3	4	5	6
min.	°C	°C						
0	+0.5	+0.5	0.0	0.0	0.0	0.0	0.0	0.0
15	+5.0	+1.5	−0.3	−0.1	+0.1	−0.1	−0.1	−0.5
30	+6.0	+2.0	−0.4	−0.2	+0.1	−0.1	−0.3	−0.7
45	+7.0	+4.0	+0.2	−0.3	+0.1	−0.2	−0.5	−1.1
60	+7.5	+5.0	+0.8	−0.3	+0.2	−0.2	−0.6	−1.3
75	+7.5	+5.5	+1.7	−0.1	+0.3	−0.2	−0.6	−1.5
90	+6.5	+5.5	+2.2	+0.1	+0.6	−0.1	−0.6	−1.7
105	+6.5	+6.0	+2.8	+0.2	+0.9	0.0	−0.6	−1.8
120	+7.0	+6.0	+3.3	+0.4	+1.1	0.0	−0.7	−2.0
135	+7.0	+6.0	+3.8	+0.5	+1.3	+0.2	−0.7	−2.1
150	+7.0	+6.5	+4.0	+0.7	+1.5	+0.2	−0.7	−2.3
180	+7.0	+6.5	+4.3	+0.9	+1.7	+0.3	−0.8	−2.4
195	+7.0	+6.5	+4.6	+1.1	+1.9	+0.4	−0.8	−2.5
210	+7.0	+7.0	+4.7	+1.3	+2.0	+0.5	−0.9	−2.6
225	+7.0	+7.0	+4.9	+1.4	+2.1	+0.5	−0.9	−2.7

IV, except that the final temperature was 29.5° C. Here stem 8 began flowing after a temperature rise of 5.5° C and continued to flow for 15 hours. Stem 10 also started to flow after a rise of 5.5° C but after two hours began to absorb. Stem 9 did not flow until after the temperature was elevated to 29.5° C. The other stems began to flow at temperatures between these

TABLE IV

UNDER CONDITIONS OF RISING TEMPERATURES (TO 29.5° C) THERE ARE VARIATIONS BETWEEN STEMS IN THE TEMPERATURE AT WHICH FLOW BEGINS (JULY).

Time	Box temp.	Stem temp.	Volume change in milliliters					
			Stem no. 7	8	9	10	11	12
hrs.	°C	°C						
.0	− 0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	+12.0	+ 5.5	−0.5	+ 0.6	−0.5	+0.1	−0.5	−0.8
1.0	+14.5	+13.0	−0.5	+ 1.7	−0.5	+0.6	+0.4	−0.6
1.5	+15.5	+15.5	−0.4	+ 2.6	−0.7	+0.7	+1.2	−0.6
2.0	+18.0	+17.0	−0.3	+ 3.4	−0.9	+0.6	+1.3	−0.7
2.5	+20.0	+18.0	−0.2	+ 4.3	−0.9	+0.5	+1.3	−1.0
3.0	+21.0	+21.0	+0.1	+ 5.3	−1.1	+0.3	+1.2	−1.1
3.5	+23.0	+23.0	+0.4	+ 6.3	−1.2	+0.2	+1.0	−1.0
4.5	+25.5	+25.5	+3.0	+ 9.4	−1.4	+0.2	+0.7	+0.9
8.0	+28.0	+28.0	+4.1	+11.1	−0.9	+0.3	+0.8	+2.9
9.0	+29.5	+29.5	+5.3	+11.8	0.0	−0.3	+1.0	+3.9
11.25	+29.5	+29.5	+5.4	+12.8	+1.6	−0.2	+0.9	+5.3
12.5	+29.5	+29.5	+6.2	+13.3	+2.1	−0.3	+0.7	+5.5
14.0	+29.5	+29.5	+7.1	+13.7	+2.6	−0.5	+0.4	+5.6
15.0	+29.5	+29.5	+7.5	+13.8	+2.9	−0.7	+0.3	+5.5

TABLE V

VARIATIONS IN FLOW BETWEEN TWO SECTIONS OF THE SAME STEM (DECEMBER).

Basal section			Upper section		
Stem weight	Volume change at 7° C	Flow per 1000 g. stem	Stem weight	Volume change at 7° C	Flow per 1000 g. stem
gm.	*ml.*	*ml.*	*gm.*	*ml.*	*ml.*
957A	+3.5	+3.7	609A	+1.5	+ 2.5
1007B	+1.3	+1.3	595B	+7.3	+12.3
987C	+7.0	+7.1	628C	+2.1	+ 3.3
736D	-1.0	-1.4	422D	+0.1	+ 0.2
853E	+1.7	+2.0	525E	-1.7	- 3.2
706F	+4.5	+6.4	1054F	-1.3	- 1.2
*874.3**	*+2.6*	*+3.3*	*638.8*	*+1.3*	*+ 2.3*

* Figures in italic represent averages.

extremes. From these data it is clear that all stems do not show the same response to changes in temperature.

Not only do the flow characteristics of individual plants differ when compared under uniform conditions, but sections of the same plant differ from each other. Six stems were selected and each stem cut into two sections, a basal section and an upper section. From table V, stem weights 957A and 609A are the weights of the basal and upper sections of the same stem. When the flows from the two sections, as for example stem C, were compared on a weight basis the flow for the basal section was 7.1 ml./1000 g. while for the upper section it was 3.3 ml./1000 g. In contrast, the upper section of stem B gave a higher yield than the basal section. A more striking difference than the quantitative one is the difference in direction of the response as in stems E and F. Here the basal sections gave a flow while the upper sections absorbed. It is clear that more must be known of the flow mechanism before reproducible results on a quantitative basis can be obtained.

TABLE VI

WITH STANDARD TREATMENT STEMS CUT DURING ACTIVE GROWTH (AUGUST) DO NOT FLOW.

Stem weight	Water absorbed on freezing	Volume change at 7° C
gms.	*ml.*	*ml.*
522	100.0	-14.0
567	51.2	- 6.0
515	65.8	-16.3
430	91.6	- 9.8
652	70.6	-11.1
530	103.2	-19.7
*536**	*80.4*	*-12.8*

* Figures in italic represent averages.

TABLE VII

A SECOND FLOW PRODUCED BY RAISING THE TEMPERATURE AFTER A FLOW AT A LOWER TEMPERATURE.

Log weight	Volume change at the lower temperature	Volume change at the higher temperature
gm.	*ml.*	*ml.*
	Experiment 9 December	
Duration	10 hrs.	3.5 hrs.
Temperature	7° C	17° C
642	- 5.4	- 4.5
667	+ 12.3	+ 1.7
632	+ 6.5	- 1.2
565	+ 7.7	- 1.0
681	+ 12.3	+ 1.3
558	+ 4.2	+ 1.3
*624.1**	*+ 6.3*	*- 0.4*
	Experiment 10 December	
Duration	6.5 hrs.	3 hrs.
Temperature	7° C	17° C
636	+ 0.9	+ 0.1
748	+ 4.9	+ 2.9
636	+ 6.1	+ 1.9
677	+ 4.2	+ 0.4
791	+ 0.6	- 1.4
515	+ 1.4	+ 1.4
667.1	*+ 3.0*	*+ 0.9*
	Experiment 11 December	
Duration	6 hrs.	3 hrs.
Temperature	7° C	17° C
697	+ 12.6	+ 2.2
617	+ 3.9	+ 1.2
608	+ 8.0	+ 2.0
577	+ 4.6	- 3.2
700	+ 2.9	- 1.3
459	+ 1.1	+ 0.4
609.6	*+ 5.5*	*+ 0.2*
	Experiment 12 January	
Duration	3.5 hrs.	3 hrs.
Temperature	7° C	25.5° C
625	+ 1.9	+ 2.2
872	- 2.0	- 1.6
819	+ 0.1	+ 0.3
627	+ 2.9	+ 2.3
518	+ 2.7	+ 1.4
872	+ 1.0	+ 2.5
722.1	*+ 1.1*	*+ 1.2*

* Figures in italic represent averages.

As a consequence, in the following experiments the interpretations of the results will be based on qualitative differences—that is, whether flow or absorption occurs—rather than on the quantitative measure of the flow observed.

The question arose whether under experimental conditions a flow might be obtained from stems collected in midsummer. Six stems were collected in August and on freezing and thawing they absorbed (table VI). Two possible explanations of these results have been suggested: first, that the cells in active growth were injured by freezing; second, that the starch con-

TABLE VIII

THE INACTIVATION OF THE FLOW MECHANISM BY STEAM.

Log weight	Volume change in normal flow	Volume change after exposure to steam at 1 p.s.i. for 2 hours
gms.	*ml.*	*ml.*
	Experiment 13 December	
Duration	7.5 hrs.	5.5 hrs.
514	- 4.9	- 8.4
611	+ 10.0	- 0.6
593	+ 6.8	- 1.6
559	+ 2.1	+ 0.3
571	+ 11.6	- 0.9
459	+ 6.0	- 0.4
*551.1**	*+ 5.3*	*- 1.9*
	Experiment 14 December	
Duration	5.75 hrs.	10 hrs.
697	+ 13.5	- 1.6
617	+ 4.9	- 7.3
608	+ 8.0	- 5.0
577	+ 4.6	- 8.0
700	+ 3.0	- 3.2
459	+ 1.1	- 6.5
609.6	*+ 5.9*	*- 5.3*

*Figures in italic represent averages.

tent is high and the sucrose content low in the wood at this time of year. Either or both of these factors might account for the lack of flow observed. Other observations indicate that maple stems will not flow well until four to six weeks after leaf fall.

In a consideration of the flow mechanism two points of view have been expressed in the literature: first, that the flow is a thermal response of a physical system or systems; second, that it is a temperature response of a biological mechanism or mechanisms.

In nature the flow of maple sap occurs during the time of year when the diurnal temperature range is frequently above and below 0° C. Does the

flow mechanism require freezing temperatures? Previous workers have suggested that it does. The question was reinvestigated in four experiments. The stems were set up in the usual manner in the experimental box, frozen, and allowed to flow at 7° C after freezing. At the end of the flow, when all of the stems had begun to reabsorb as with tree 10, table IV, the temperature was raised to 17° C in three of the experiments and to 25.5° C in the fourth. From table VII it is evident that a second flow occurred in many stems. Thus it was clear that freezing is not a necessary requirement for the flow mechanism. Field observations during the spring of 1949, based on the thermocouple temperatures of the phloem and outer xylem of a number

TABLE IX

THE FLOW RESPONSE FROM MAPLE STEMS PERFUSED WITH DISTILLED WATER AND WITH SAP FROM OTHER TREES.

Stem weight	Flow before perfusion	Flow/kg. log weight	Ml. water perfused	Flow	Sap perfused	Flow
gms.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
			Experiment 15 March			
Duration	3.5 hrs.		26.5 hrs.	3.3 hrs.	22 hrs.	2 hrs.
970	+ 17.3	+ 17.8	532	- 14.3	202	+ 4.4
771	+ 9.6	+ 12.5	188	- 7.7	44	+ 1.5
735	+ 6.8	+ 9.3	292	- 12.1	88	+ 4.1
860	+ 8.4	+ 9.8	445	- 18.3	144	+ 1.0
895	+ 13.9	+ 15.5	373	- 14.9	180	+ 3.7
915	+ 14.9	+ 16.3	409	- 13.9	154	+ 2.2
*858**	*+ 11.8*	*+ 13.5*	*373*	*- 13.5*	*135*	*+ 2.8*
			Experiment 16 November			
Duration	6 hrs.		17 hrs.	15 hrs.	24 hrs.	3 hrs.
648	- 1.3	- 2.0	184	- 15.4	40	- 0.3
627	+ 2.2	+ 3.5	200	- 4.4	40	+ 0.4
677	+ 7.2	+ 10.6	190	- 14.0	122	+ 0.3
585	- 4.5	+ 7.7	220	- 9.1	76	- 0.4
584	- 5.0	- 8.6	650	- 16.9	150	+ 1.8
587	+ 3.6	+ 6.1	108	- 2.7	60	+ 2.9
618	*+ 0.4*	*+ 2.9*	*259*	*- 10.4*	*81*	*+ 0.8*
			Experiment 17 November			
Duration	6 hrs.		24 hrs.	21.5 hrs.	21 hrs.	5 hrs.
576	+ 3.3	+ 5.7	80	- 6.1	50	- 0.2
705	+ 11.3	+ 16.0	173	- 9.3	118	+ 2.9
774	+ 9.8	+ 12.7	318	- 17.9	146	+ 0.9
644	+ 9.1	+ 14.1	132	- 8.0	90	+ 1.6
740	+ 0.5	+ 0.7	56	- 9.3	68	+ 0.3
884	+ 8.6	+ 9.7	213	- 16.2	172	+ 7.2
721	*+ 7.1*	*+ 9.8*	*162*	*- 11.1*	*107*	*+ 2.1*

* Figures in italic represent averages.

of trees, clearly demonstrated that good flows occurred when the lowest recorded stem temperatures of the diurnal cycle were appreciably above 0° C.

The second point of view was investigated by treating the stems with steam after a normal flow. The stems were removed from the experimental box and treated with live steam at one pound per square inch for two hours in a steam box. This was considered sufficient to inactivate the living cells. The stems were then allowed to absorb, were frozen and warmed to 7° C as before. From table VIII it can be seen that after the steam treatment no

TABLE X

FLOW CHARACTERISTICS OF STEMS PERFUSED WITH A 5% SUCROSE SOLUTION.

Log weight	Sucrose perfused	% solids in perfusate	Volume change at 7° C
gms.	*ml.*	*%*	*ml.*
	Experiment 18 November		
Duration	22 hrs.		4 hrs.
632	315	4.6	+ 24.3
638	260	4.3	+ 23.7
670	380	4.5	+ 5.6
710	450	4.6	+ 30.5
688	305	4.3	+ 28.2
800	450	4.5	+ 7.4
*689.6**	*360*	*4.5*	*+ 20.0*
	Experiment 19 December		at 24° C
Duration	16.25 hrs.		3 hrs.
673	140	5.0	+ 10.8
731	225	5.0	+ 10.8
860	295	5.0	+ 0.3
844	600	5.0	+ 12.3
733	460	5.0	+ 0.8
708	260	5.0	+ 1.1
758.1	*330*	*5.0*	*+ 6.0*

*Figures in italic represent averages.

flow occurred except for one doubtful case; rather, the stems absorbed water. Thus, it is evident that the activity of living cells is necessary to produce a flow.

In an attempt to study the internal conditions necessary for sap flow, the stems were perfused with distilled water under pressure, after a trial run with no treatment. The perfusion was continued until there was a negative test for solids in the perfusate with a refractometer. For example, a stem weighing 771 grams gave a negative test after 188 ml. of water had been perfused (table IX, experiment 15). When stems were perfused under uniform pressure there was great variation in the rate of perfusion of different

stems. Thus, during a perfusion period of 26.5 hours (table IX, experiment 15), 188 ml. of water passed through one stem while 532 ml. was perfused through another. After perfusion the stems were frozen and warmed to 7° C. In contrast to the normal flow, all stems absorbed. Thus, replacing the vessel sap with distilled water reversed the direction of the flow mechanism. This experiment has been repeated several times with the same results. It should be pointed out that the period of perfusion is long, from 17 to 26 hours, and some materials may diffuse from adjacent cells into the vessels during the perfusion. However, the uniformly negative results indicate that

TABLE XI

FLOW CHARACTERISTICS OF STEMS PERFUSED WITH A MANNITOL SOLUTION.

Stem weight	Volume change without treatment 7 °C	Ml. mannitol perfused	% solids in perfusate	Volume change at 7° C
gms.		*ml.*	*%*	*ml.*
		Experiment 20 January 2.5% Mannitol		
Duration		24 hrs.		6.5 hrs.
859		425	2.5	- 22.0
892		165	2.2	- 20.5
747		230	2.4	- 13.2
877		490	2.5	- 20.2
828		290	2.4	- 16.6
838		360	2.4	- 25.7
*840.1**		*326.6*	*2.4*	*- 19.7*
		Experiment 21 January 2% Mannitol		
Duration		21 hrs.		2.5 hrs.
660	+ 3.4	62.5		- 16.2
665	+ 1.7	500		- 13.2
865	+ 2.3	590		- 17.0
920	+ 0.5	850		- 11.2
1070	+ 5.5	990		- 23.7
780	+ 3.9	940		- 12.3
826.6	*+ 2.9*	*749.1*		*- 15.6*

* Figures in italic represent averages.

they are not present in sufficient quantity to influence the response to elevated temperatures. The same stems were next perfused with freshly collected sap from other trees. After freezing and warming a flow occurred. The flow observed was, however, somewhat less than the initial one. It is interesting to note that one stem in experiment 16 which did not flow with its own sap did flow with the sap from another tree.

From these experiments it became clear that perfusing with a synthetic sap would be of interest and, since sucrose is the largest single component of natural sap, a sucrose solution was used. Previous experiments have demon-

strated that 200 ml. is a sufficient quantity of perfusate to replace the vessel sap (table IX). Twelve stems in experiments 18 and 19, table X, were perfused with a 5% sucrose solution. The stems were then frozen and in experiment 18 warmed to 7° C. In all cases a flow occurred and in some stems in a considerable amount. The conditions in experiment 19 were the same as in experiment 18 except that the stems were warmed to 24° C. Replacing the sap with a sucrose solution, therefore, does not inactivate the flow mechanism since an apparently normal flow occurs after such treatment.

The preceding experiment gives rise to the question of whether the presence of sucrose in the vessel sap is essential for a flow. To investigate this question stems were perfused in some cases with a 2.5% mannitol solution and in others with a 2% mannitol solution. These concentrations are essentially isosmotic with the sucrose concentration commonly found in the stems. In table XI, experiment 20, good perfusion was obtained in 24 hours and in experiment 21, in 21 hours. In both experiments, after perfusion and the subsequent freezing and thawing, there was a very considerable absorption rather than flow. This absorption was nearly twice that with distilled water in experiments 15, 16 and 17, table IX.

Discussion

The data clearly indicate that flows from comparable stems under uniform temperature conditions are not quantitatively reproducible. Repeated flows from the same stem under the same temperature conditions result in different amounts of flow. Flows from different stems or from sections of the same stem under the same temperature conditions are widely different quantitatively, when compared on a fresh weight basis, and may even differ qualitatively. In other words, some flow while others absorb. For the present the significance of the experiments is related to the direction of the flow mechanism—whether a flow or absorption occurs. A more complete understanding of the flow mechanism is necessary to interpret the quantitative differences.

Theoretical considerations of possible explanations of the flow mechanism fall into two groups. The first concerns the effect of the temperature change on a physical system—namely, thermal expansion of gases, of the vessel solutions, or of the cell walls. The other point of view is directed at the effect of temperature change on a biological system. A thermal gradient along a ray cell or between ray cells might result in differences in the concentration of osmotically active substances or differences in membrane permeability or both. It has also been suggested that temperature differences would change the oxygen concentrations in the vessel sap and in the cells thus influencing respiration which in turn would influence flow.

Some of the results in tables III and IV are of interest in considering thermal expansion of a physical component as a direct cause of pressure and flow. In table III, stem 4 did not begin to flow until it had warmed to within 1° C of the final elevated temperature while stem 3 began to flow

after a rise of 1° C. Stem 9, table IV, did not begin to flow until the stem was warmed to the holding temperature. If the cause of flow were the expansion of a physical system alone, more uniformity in the response would be expected. The data in table VII are of interest in that a flow may be produced by a rise in temperature from a point 7° C above 0° C. Thus freezing is not a necessary antecedent for a flow. Thermal expansion of the system undoubtedly plays a part but does not appear to explain the entire mechanism.

The suggestion of a thermal gradient along living ray cells as a causative factor in the flow mechanism is open to doubt when the temperatures at which flow occurred in stems 7, 9, and 12, table IV, are considered. The stem temperatures were recorded with thermocouple points inserted into the center of the stem. It may be seen that the internal temperatures were the same as that surrounding the stems—that is, stem temperatures were uniform throughout at the time flow began.

Treating the stems with steam and presumably inactivating the living cells reversed the direction of movement in the vessels, thereby suggesting that the flow mechanism is a biological phenomenon.

The composition of the vessel sap profoundly influences the flow mechanism: tables IX, X, and XI. The direction of movement was reversed when distilled water replaced the vessel sap; sap from other trees or a sucrose solution perfused into the vessels resulted in an outward flow. The amount of sap produced was not, however, directly correlated with the sucrose concentration in the vessel sap (experiment 15, table IX). Extensive field observations by F. H. Taylor to be published elsewhere are in agreement.

Perfusion with a mannitol solution isosmotic with the sucrose concentrations usually found gave results in flow behavior unlike that with sucrose but similar to that with distilled water. This result indicates that either there is rapid absorption of the mannitol solution from the vessel sap, or flow is not the result of a simple osmotic mechanism. Experiments are in progress in which stems are being perfused with a number of different sugars to determine whether sucrose is a specific requirement.

Summary

1. A method has been developed to measure the flow of sap from small maple stems under controlled temperatures.

2. There is great variation in the quantity of sap produced under uniform temperature conditions from similar stems, or even from sections of the same stem.

3. A second flow of sap may be obtained by a second temperature elevation after the first flow following freezing. This fact indicates that a freeze before each flow is not necessary.

4. Flows are produced by stems perfused with sap from other trees or with a sucrose solution.

5. Absorption occurs when the stems are perfused with distilled water or a mannitol solution isosmotic with the sucrose content of normal sap.

6. Thermal changes in a physical system alone are not sufficient to account for the flows observed.

7. Living tissues are necessary for the flow mechanism to function.

DEPARTMENT OF BOTANY
AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF VERMONT
BURLINGTON, VERMONT

LITERATURE CITED

1. BOIS, E. and CHUBB, W. O. The sap of the birch tree, *Betula papyrifera* Marsh. I. The amylase system. Canadian Jour. Res. **B20**: 114–120. 1942.
2. BOIS, E. and NADEAU, A. Contributions a l'etude d'*Acer saccharum*. II. La presence d'amylases dans la se've d'erable et les produits d'hydrolyse. Canadian Jour. Res. **B16**: 114–120. 1938.
3. BOIS, E. and NADEAU, A. Contributions a l'etude d'*Acer saccharum*. III. Action des amylases de la se've d'erable. Canadian Jour. Res. **B16**: 121–133. 1938.
4. BURSTRÖM, H. The rate of the nutrient transport to swelling buds of trees. Physiologia Plantarum **1**: 124–135. 1948.
5. BURSTRÖM, H. Studies on the water balance of dormant buds. Physiologia Plantarum **1**: 359–378. 1948.
6. BURSTRÖM, H. and KRÖGH, A. The biochemistry of the development of buds in trees and the bleeding sap. Kgl. Danske Videnskab. Selskab, Biol. Medd. **20**(2): 1–27. 1946.
7. BURSTRÖM, H. and KRÖGH, A. Bleeding and bud development in Carpinus. Svensk Botanisk Tid. **41**: 17–44. 1947.
8. CLARK, W. S. The circulation of sap in plants. Twenty-first Ann. Report Secretary of Agriculture, Massachusetts, 159–205; summarized in Eleventh Annual Report Massachusetts Agricultural College: 26–39. 1874.
9. CLARK, W. S. The flow of sap and the power of plant growth. Twelfth Annual Report Massachusetts Agricultural College: 18–53. 1875.
10. CRAFTS, A. S., CURRIER, H. B., and STOCKING, C. R. Water in the Physiology of Plants. Chronica Botanica, Waltham, Massachusetts. 1949.
11. CRAFTS, A. S. and BROYER, T. C. Migration of salts and water into xylem of the roots of higher plants. Amer. Jour. Bot. **25**: 529–535. 1938.
12. EATON, F. M. The osmotic and vitalistic interpretations of exudation. Amer. Jour. Bot. **30**: 663–674. 1943.

13. JOHNSON, L. P. V. Physiological studies on sap flow in the sugar maple, *Acer saccharum* Marsh. Canadian Jour. Res. **C–23**: 192–197. 1945.
14. JONES, C. H., EDSON, A. W., and MORSE, W. J. The maple sap flow. Vermont Agr. Exp. Sta. Bull. **103**. 1903.
15. JONES, C. H. and BRADLEE, J. L. The carbohydrate contents of the maple tree. Vermont Agr. Exp. Sta. Bull. **358**. 1933.
16. KRAMER, P. J. Plant and Soil Water Relationships. McGraw-Hill. 1949.
17. LUNDEGÅRDH, H. Absorption, transport and exudation of inorganic ions by the roots. Arkiv för Botanik **32a**: 1–139. 1945.
18. LUNDEGÅRDH, H. Growth, bleeding, and salt absorption of wheat roots as influenced by substances which interfere with glycolysis and anaerobic respiration. Kgl. Landbruks Hogskol. Ann. **16**: 339–371. 1949.
19. MEEUSE, B. J. D. Observations on the enzymatic action of maple and birch saps. New Phytologist **48**: 125–143. 1949.
20. NIE, R. VAN, HELDER, R. J., and ARISZ, W. H. Ion secretion into the xylem and osmotic regulation and exudation. Koninkl. Nederland. Akad. Wetenschap, Proc. **53**: 567–575. 1950.
21. PFEFFER, W. The Physiology of Plants. Oxford Univ. Press. 1900.
22. STEVENS, C. L. and EGGERT, R. L. Observations on the causes of the flow of sap in red maple. Plant Physiol. **20**: 636–648. 1945.
23. VAN OVERBEEK, J. Water uptake by excised root systems of the tomato due to non-osmotic forces. Amer. Jour. Bot. **29**: 677–683. 1942.
24. WIEGAND, K. M. Pressure and flow of sap in the maple. American Naturalist **40**: 409–453. 1906.