STUDIES ON PHLOEM EXUDATION FROM YUCCA FLACCIDA HAW.

VII. THE EFFECT OF COOLING ON EXUDATION*

P. M. L. TAMMES, C. R. VONK and J. VAN DIE

Laboratorium voor Insecticiden onderzoek, Wageningen
Centrum voor Plantenfysiologisch Onderzoek, Wageningen
en Botanisch Laboratorium, Utrecht.

SUMMARY

Bleeding inflorescence stalk tips of 10–15 cm length, still attached to the plant, were cooled in melting ice. The result was a considerable prolongation of the exudation period. Apparently the wound-sealing process was strongly retarded. Although the rate of bleeding seemed slightly lower, the dry-matter content of the exudate was 10–20% higher at 0°C than at 20–25°C.

When excised inflorescence tops were cooled as a whole, exudation gradually declined and stopped within 45 minutes. It recommenced within a few minutes when brought to room temperature.

It is concluded that both the sugar secretion and sugar uptake by cells surrounding the intact sieve-tubes stops at a low temperature. The secretion process is assumed to be the driving force of the exudation. As the bleeding continues at low temperatures, its driving force will be localized mainly outside the cooled inflorescence.

1. INTRODUCTION

The influence of a low temperature on the translocation of assimilates is not yet clear. WEBB (1967) found that translocation in *Cucurbita pepo* was almost completely arrested during a 45 minute period at 0°C. BOWLING (1968), on the other hand, found no inhibition of translocation in *Helianthus annuus* after such a cooling. FORD & PEEL (1966) did not find much temperature influence on longitudinal movement in willow stems, while cooling experiments of SWANSON & GEIGER (1967) with sugar-beet petioles showed only an initial decrease in the assimilate movement.

The flowering period of *Yucca flaccida* offered a good opportunity to investigate the influence of a low temperature on the various translocation processes which seem to be involved in the phloem exudation. In *Yucca* (VAN DIE & TAMMES, 1966; VAN DIE, 1968) the secretion of assimilates into the sieve-tubes provides the pressure needed for their movement. Cells around the sieve-tubes in receiving organs – such as the inflorescence – which normally absorb sucrose from the stream of assimilates, may eventually secrete this sugar into the sieve-tubes when the solute concentration in the latter drops as a result of wounding.

The aim of the present study was to investigate the influence of a low tem-

* Dedicated to Professor Dr. W. H. Arisz.

perature on the movement of the assimilate stream through the inflorescence stalk, as well as on the secretion/absorption process near the site of bleeding. The experiments were carried out in June/July of 1966–1968 at the Centre for Plant Physiological Research, Wageningen.

2. MATERIAL AND METHODS

A. Cooling of bleeding inflorescence stalks, still attached to garden plants, was carried out by bending them with the tip in a dewar bottle containing crushed ice and water at 0 °C. Over the tip was a glass tube, held in place by a plastic cover. The situation is illustrated in *fig. 1*. The length of the cooled tip was at least 10 cm.

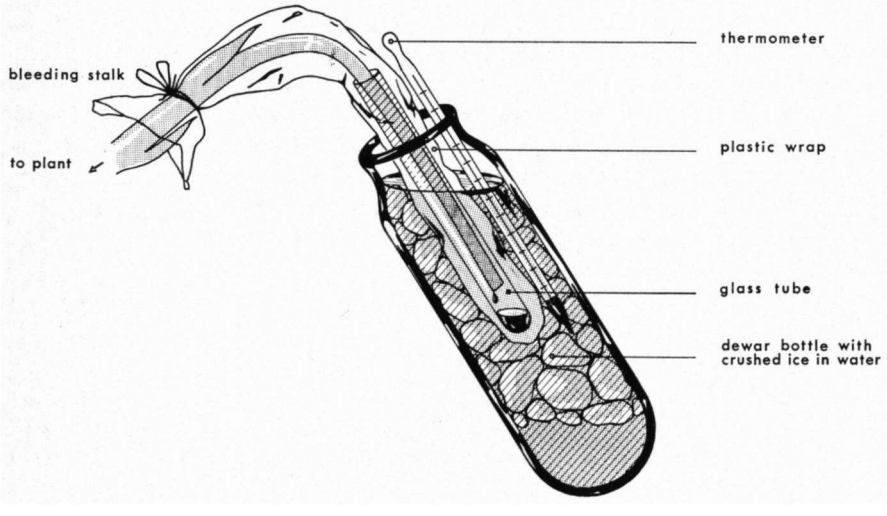

Fig. 1. Cooling of the end of an inflorescence stalk still attached to a plant.

B. In a wide-mouthed dewar bottle excised parts of stalks were inserted in the cooling mixture of ice and water, leaving about ½ cm of the tip above the fluid so that exudation could be observed from above, after removing the lid of the bottle; afterwards the tips were exposed to higher temperatures.

C. Isolated stalk parts were kept in the dewar bottle at 0 °C with a twice daily renewal of the wounds by cutting off a thin slice at both ends. Sap was collected for analysis after exposing to room temperature.

D. The sugar concentration was determined as total hexose according to MORRIS (1948) and the dry-matter content of the sap was determined after drying at 100 °C.

3. RESULTS

3.1. Cooling of tips of inflorescences, still attached to plants

Several series of experiments were performed at normal outside temperature conditions (20–25 °C) and at 0 °C. They all showed (1) a considerably higher dry-matter content in the exudate when the bleeding took place at a low temperature and (2) a greatly prolonged bleeding period.

Fig. 2 demonstrates a typical experiment in which all the data come from one inflorescence stalk.

At a normal temperature exudation stops within 7–8 hours but at 0 °C a continuous bleeding takes place for 24–36 hours. Apparently the sealing of the wound proceeds about 4 times slower at 0 °C than at 20–25 °C. The increase in dry-matter content of the sap collected at a low temperature is considerable: From the data shown in *fig. 2* it can be inferred that at a normal temperature the bleeding sap contained 1667:12.4 = 134 mg/ml in one experiment and 841:6.3 = 133 mg/ml in the other. But at 0 °C the dry-matter content of the sap was 3843:21.4 = 180 mg/ml in one experiment and 3596:20.5 = 176 mg/ml in the other. The influence of the low temperature on the rate of bleeding is due mainly to its effect on the process of clogging of the bleeding phloem; there is no influence on the process that drives the exudation.

Table 1 shows that even a cooling period of 140 hrs does not seriously affect the rate of bleeding, provided wound renewal is regularly performed. In 140 hrs of cooling 62.5 ml of exudate has been collected containing 10.78 grams of dry

Table 1. The influence of wound renewal on the rate of exudation during a cooling period of 141 hours.
The asterisks indicate the time of wound renewal. At the beginning and at the end of the cooling experiment exudate fractions were collected at the outside temperature of about 20 °C (control)

Period of the day	hrs of bleeding	exudate volume, ml	exudation rate, ml. hr^{-1}	dry weight %
control				15.6
19– 8	13	9.4	0.72	15.7
8–17	22	1.4	0.15	17.1
17– 8	37	1.9	0.13	19.4
8–20	49*	0.1	–	–
20– 8	61	8.0	0.66	18.3
8–20	73*	1.1	0.10	19.2
20– 8	85	9.0	0.75	17.8
8–20	97*	0.8	0.07	17.4
20– 8	109*	7.2	0.60	17.6
8–20	121*	7.2	0.60	17.7
20– 8	133	11.0	0.91	17.0
8–16	141	6.1	0.76	16.0
16–18 (control)	143*	2.5	1.25	15.4
18–20 (control)	145	2.7	1.35	15.1

weight (17.2% of dry-matter). Leaving 3 exudate fractions out of consideration because they were collected just before the renewal of the wound (from 38–50, from 62–74 and from 86–98th hr) the average rate of exudation at 0 °C is 0.6 ml/hr, a value also regularly encountered normally.

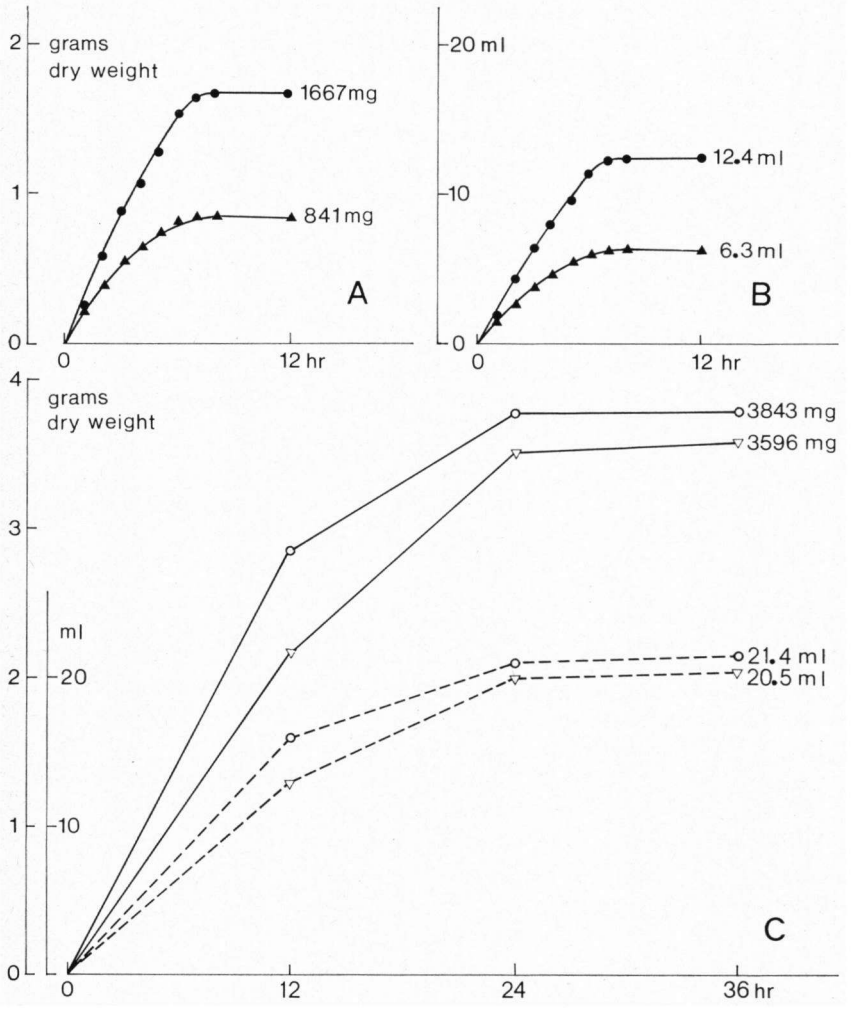

Fig. 2. Exudate production and the progress of wound-sealing at 20–25 °C and at 0 °C. In each experiment the collection of exudate started by cutting a thin slice from the bleeding site of the inflorescence stalk. All data are obtained from the inflorescence stalk of one plant.

2A and 2B represent the time-course of exuded total solutes and the exudate volume at normal temperature in two series of measurements. 2C represents these relations – also in two series of measurements – at 0 °C. In the cooling experiments only the proximal 10–15 cm of the bleeding stalk were kept at the low temperature.

3.2 Cooling of excised inflorescence stalk parts

When isolated parts of the stalk are cooled as a whole, the exudation gradually decreases and is hardly visible after 45 minutes. Only when the wound is renewed does a trace of sap appear. When a stalk is taken from the cooling vessel after one hour of cooling and subsequently brought to room temperature, exudation starts again within 4 minutes without renewal of the wound. When placed in water of 35 °C the first sap can be observed after 2 minutes. It first appears from the peripheral vascular bundles and gradually proceeds to the more central ones.

Exudate from excised stalk parts have a lower sugar content than exudates from stalks still attached to the plant (VAN DIE & TAMMES 1966). Still lower sugar contents could be found in the exudates from stalks that were kept at 0 °C for a couple of days (with a twice-daily renewal of the wounds at both sides) when they were brought to room temperature *(table 2)*.

Table 2. The increase in sugar content of the exudate from excised inflorescence parts during several days and subsequently brought to room temperature.

period of cooling	% sugar in first exudate fraction	% sugar in second exudate fraction
4 days	1.9	5.5 after 10 minutes
2 days	1.6	3.7 after 3 hours
2 days	1.6	3.6 after 3 hours

4. DISCUSSION

Various metabolic and physical processes underlying the sieve-tube bleeding phenomenon should be considered separately in an attempt to explain the reported experimental results:

1. The wounding of the inflorescence causes a turgor-drop in the sieve-tube elements, resulting in an osmotic attraction of water from surrounding cells.
2. The sealing of the wound normally proceeds slowly. It takes several hours. To get a regular flow of exudate one has to renew the wound a few times a day. The sealing reaction is undoubtedly a chemical or a metabolic process. Its retardation at low temperatures is not surprising.
3. The exudation of the assimilates is assumed to be the result of a pressure-flow starting at the sites of assimilate production and moving to sites of assimilate consumption or accumulation. It might be considered as a Poisseuille flow through elastic capillaries. If, possibly, the rate of flow through the cooled inflorescence stalk is slightly lower than that encountered at a normal temperature this can be satisfactorily explained by an increase in viscosity: the viscosity of a 20% sucrose solution at 20 °C is 2.0 centipoise; at 0 °C it is 3.8 centipoise.
4. Sugar secretion and sugar uptake by cells surrounding the sieve-tubes – into or from the sieve-tubes – will be a metabolic, reversible process on which the

temperature has a considerable effect. The position of this uptake/secretion equilibrium in the normal, non-bleeding plant, will strongly favor the exit of assimilates from the sieve-tubes into the developing inflorescence tissues. Consequently the sugar concentration of the sieve-tube contents will diminish while it moves through the stalk. In experiments with *excised* inflorescence tops the secretion/uptake equilibrium apparently shifts toward secretion, as an exudate with a lower sugar content could be obtained (VAN DIE & TAMMES 1966). The present experiments demonstrate the complete arrest of this sugar secretion in excised inflorescence tops when brought to 0 °C. It may be concluded, therefore, that in cooling experiments with bleeding inflorescence stalks still connected to the plant, the radial movement of sugars will also very probably be negligible. While at a normal temperature this radial movement may cause a regular loss of sugars from the stream of assimilates, it seems logical that at a normal temperature also the sugar content of the exudate will possess a lower value than in experiments performed at 0 °C. The relatively high dry-matter percentages found under low temperature conditions can readily be explained in this way.

The present experiments clearly show that cooling stops sugar secretion into the sieve tubes. As this process is the driving force of exudation, it might be concluded from the observed bleeding at 0 °C that the driving secretion process is mainly localized outside the cooled inflorescence, probably in the photosynthesizing parts of the plant. The sieve-tube contents are apparently pressed through passively from below through the inflorescence stalk, at least during exudation.

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