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STUDIES ON PHLOEM EXUDATION FROM *YUCCA FLACCIDA HAW.*

I. SOME OBSERVATIONS ON THE PHENOMENON OF BLEEDING AND THE COMPOSITION OF THE EXUDATE

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Abstract

After young inflorescent stalks of intact, actively transpiring *Yucca flaccida* Haw. plants were wounded an exudate was obtained. Its flow continued for several weeks provided there was regular re-wounding.

The exudate had a pH of 8.0–8.2 and contained about 18 per cent of total solids. Of these, sucrose constituted about 80–90 per cent. Glucose and fructose were minor constituents. The amino acid concentration was as high as 0.07 molar. Glutamine appeared to be the main nitrogenous constituent.

The exudate only contained small amounts of inorganic solutes of which potassium was the main kation. The calcium content was relatively low.

There appeared to be a great similarity between this phenomenon of bleeding and the composition of the exudate and that observed by one of the authors (P.M.L.T.) in previous years in several palm species.

Various arguments are adduced in favour of the view that the exudate has a phloem origin.

Introduction

Sugar-rich saps can be tapped from the young inflorescent stalks of several species of palms (e.g. Prinsen–Geerligs, 1893; Gibbs *et al.*, 1913; Browning and Symons, 1916). According to Tammes (1933; 1951; 1958) these saps are phloem exudates.

After incisions have been made in the bark, sugar-rich exudates can also be obtained from many tree species growing in temperate regions. The phloem origin of these fluids seems to be of little doubt (Dixon, 1933; Huber, 1953) although some kind of contamination is unavoidable. With the exception of the "manna" from *Fraxinus ornus* and *Fr. exelsior*, in general only small quantities of sap can be obtained from these trees. Although these exudates are very useful for paper chromatographic analyses of sieve tube sap (Ziegler, 1956; Zimmermann, 1957, 1958), the trees themselves are unpracticable for more extensive studies on phloem translocation in which controlled environmental conditions are required.

Both for plant physiological studies and more applied research on the translocation of biocides in plants, it seemed important to find a plant species from which sieve tube sap could be obtained in millilitre quantities and over a period of weeks, without being too unpractical for laboratory work. The first author investigated many plant species for this property, but without success.

In the summer of 1962 an exudate was obtained from the stalk of a young inflorescence of *Yucca flaccida Haw.* and analyses showed a great similarity with the composition of palm saps and other phloem exudates. In the following year exudates from *Yucca* plants growing in the Wageningen Arboretum were collected and analyzed. In the present paper general observations on the phenomenon of bleeding and analytical results are reported and briefly discussed.

Materials and methods

Collection of the exudates. When the inflorescence of the *Yucca* plant had reached a height of about 30–40 cm it was bent downward and tied with string. A few bracts with buds on the base of the inflorescence were removed. On the following day the stalk was cut through the soft lower part of the inflorescence. In general bleeding started immediately or during the following day (Fig. 1).

It was always necessary to renew the wound twice a day by cutting off a slice about 1 mm thick. In this way bleeding was enabled to continue for a few weeks. During tapping the inflorescences were covered with plastic to prevent raindrops from entering the vial.

Carbohydrates. Reducing sugars were determined by the colorimetric method of NELSON (1944)–SOMOGYI (1945), before and after acid hydrolysis. Free and combined fructose were determined by the methods of ROE (1934) and ROREM, WALKER and McCREADY (1960). Descending chromatograms were run for 24 hours and 48 hours, using the solvent system of GIOVANNOZI–SERMANNI (1956).

Organic nitrogenous compounds. Total protein was estimated by the method of MEJBAUM–KATZENELLENBOGEN and DOBRYSZYCKA (1959), using crystalline egg albumin as the standard. The total amino acid content was estimated as described in a previous paper (VAN DIE, 1959). Two-demensional descending amino acid chromatograms were run in the solvent system of ROCKLAND and UNDERWOOD (1954). The amounts of ninhydrin positive spots on the paper were measured after treatment with copper (III) nitrate and subsequent elution with methanol. Urea derivatives were detected on the chromatograms by a modification of the EHRLICH test (SMITH, 1953), replacing p-dimethylaminobenzaldehyde by p-dimethylaminocinnamaldehyde. The latter compound, generally used for the detection of indole derivatives (HARLEY–MASON and ARCHER, 1958), proved to be a convenient reagent for the detection of citrulline, allantoic and urea.

Macro and micro elements. These substances were determined by Messrs. H. J. IMMINK and O. SPOELSTRA of the Institute for Biological and Chemical Research of Field Crops, Wageningen, using standard laboratory methods. Sodium and potassium were determined by

TABLE 1

The composition of the exudate from the inflorescent stalk of *Yucca flaccida Haw.*

The substances listed in the first part of the table were determined in portions of freshly tapped exudate obtained a few hours after the removal of a slice of tissue from the cut inflorescent stalk.

The substances in the second part of the table were determined in a large volume (ca. 100 ml) of exudate collected over a period of 10 days.

total dry matter	17.1– 19.2	%	
electric conductivity (20° C)	1.03	mMho/cm	
pH	8.0– 8.2		
sucrose	150 –165	mg/ml	
fructose	2 – 4	mg/ml	
glucose	2 – 4	mg/ml	
glucose-1-phosphate	ca. 1	mg/ml	
total amino acids (as glutamine)	6.3– 10.1	mg/ml	
glutamine			58 %
valine			10 %
serine/glycine			7 %
(iso)leucine			6 %
arginine			7 %
lysine			5 %
glutamic acid			4 %
α-alanine			2 %
asparagine			+
aspartic acid			+
proline	++		
allantoic acid	+		
allantoine	+		
urea	absent		
total protein	0.5– 0.8	mg/ml	
invertase	absent		
total phosphorus	0.310	mg/ml	
inorganic phosphorus	0.105	mg/ml	
nitrate	absent		
magnesium	0.051	mg/ml	
calcium	0.014	mg/ml	
potassium	1.680	mg/ml	
sodium	0.0041	mg/ml	
zinc	0.0021	mg/ml	
iron	0.0014	mg/ml	
manganese	0.0005	mg/ml	
copper	0.0004	mg/ml	
molybdenum	0.00001	mg/ml	

flame photometry, calcium was determined as oxalate by titration with permanganate. Colorimetric methods were used for magnesium (titanium yellow), manganese (formaldoxime), copper (diethyldithiocarbamate), zinc (dithizone), molybdenum (rhodanide complex, dissolved in isopropylether), and iron (O-phenanthroline).

TABLE 2

The variability in the composition of a series of bleeding sap fractions collected from a single plant at irregular intervals

Exudate No.	Time of collection	Reducing sugars mg/ml	Sucrose mg/ml	Amino acids (molar)	Proteins mg/ml
1	13/6–14/6 (night)	2	165	0.058	0.80
2	14/6 (afternoon)	2	190	0.063	0.70
3	14/6–15/6 (night)	3.5	165	0.044	0.47
4	15/6 (evening)	2	150	0.063	0.53
5	15/6–16/6 (night)	3	150	0.043	0.61
6	16/6 (morning)	5	165	0.069	—
7	16/6 (evening)	4	165	0.056	0.64
8	25/6 (afternoon)	2	165	0.044	—

RESULTS

1. *The composition of the bleeding sap*

A number of exudate fractions were collected from a single plant. Special precautions were taken to prevent microbial contamination of these exudates. They were collected a few hours after a new slice had been cut off from the stalk and immediately frozen and stored at — 25° C. The analytical data presented in Table 1 are, as far as the organic constituents are concerned, all obtained from such exudate fractions. For the inorganic micro-constituents a large volume of sap was needed and obtained from several plants over a period of ten days. It was stored in a polyethylene bottle and preserved with toluene.

The composition of the bleeding sap was not constant. Carbohydrate, amino acid and protein content varied slightly and independently of each other. Table 2 demonstrates this variability for a series of bleeding sap fractions collected from a single plant. Their size very much depends on the rate of wound renewal. This had not been kept constant in these experiments.

2. *The phenomenon of bleeding*

a. To a certain extent bleeding is a local process. It especially occurs at the distal part of the young florescence, near the developing flowers. When a bleeding inflorescence was cut off at its basal part, no bleeding was observed from the stalk part remaining on the plant.

On the other hand it also appeared to be possible to obtain bleeding sap from other plant parts. After all the aerial parts of the plant had been removed a small amount of exudate (about 0.5 ml) could be collected from the stem stump. This bleeding started two days after the removal of the leaves. Sucrose was the only detectable carbohydrate in this exudate and its concentration was about 15 per cent. Consequently the bleeding can not be considered as strictly limited to the developing inflorescence.

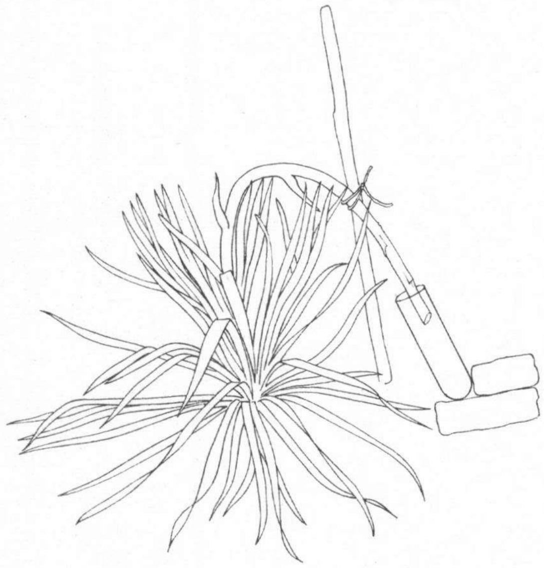

Fig. 1

b. Actively bleeding *Yucca* plants have a xylem vessel system which is under a negative tension. This could easily be demonstrated. After opening the xylem system of a leaf of a bleeding plant by a small incision at the leaf tip, it was possible to observe a distinct intake of water through the wound into the bleeding plant.

c. Some attempts have been made to investigate why the exuding sap of the inflorescent stalk is not sucked back by the xylem vessel system of the plant. Microscopic observations of cut-off slices of bleeding inflorescences showed that most of their xylem vessels were clogged near the wound surface, so that the sap is probably not sucked back. Clogging of xylem vessels is of rare occurrence at some distance of the wound surface.

Discussion

The rapidly developing inflorescence of *Yucca flaccida* undoubtedly needs considerable amounts of organic materials which have to be supplied through the stalk tissues. Although the greater part of the mass of the inflorescent stalk consists of parenchyma cells it is difficult to see how a diffusional movement of water and solutes, known to occur in parenchyma cells, can be responsible for the observed rates of solute translocation. Hence it can only be explained by translocation through the vascular bundles. In the very similar bleeding of palm inflorescences TAMMES (1933) observed that exudation occurred from the vascular bundles. According to MEYER–MEVIUS (1959) the exudate from the fruit stalk of *Clivia* also originates in the vascular bundles.

A considerable number of arguments can be adduced in favour of the view that the exudation from *Yucca* inflorescences should be considered as a phloem bleeding.

1. The bleeding cannot be explained as a xylem bleeding. Use was made of plants with abundant foliage growing under conditions of relatively high air temperature and low air humidity. In order to obtain xylem sap from such plants it is necessary to overcome a negative pressure. The existence of such a negative pressure in the xylem of a bleeding *Yucca* could be demonstrated by experiment.

2. Xylem exudates are known to be slightly acidic, whereas phloem exudates are slightly alkaline (FIFE and FRAMPTON, 1936; MOOSE, 1938; CRAFTS, 1954). The high pH of the exudate distinctly points to a phloem origin.

3. The calcium content of the exudate from *Yucca* is very low. This was also found for the exudates of *Arenga* and Coconut palms (TAMMES, 1958) and that of *Agave* (TAMMES, unpublished). Although MOOSE (1939) reported relatively high calcium values in phloem exudates of *Robinia pseudo acacia*, *Fraxinus* and *Platanus* species, there seems to be general agreement on the immobility of this element outside the xylem vessels (ARISZ, 1952; BIDDULPH, 1959; ESAU, 1961; ZIEGLER, 1963).

4. The low salt content of the exudate from *Yucca* does not suggest that the xylem vessel solutes make an important contribution to it. The electrical conductivity of xylem exudates from the cucumber and the tomato plant is several times as high (VAN DIE, 1959) as that of *Yucca*, although the total solute concentration in the *Yucca* exudate is about 60 times as high.

5. The high dry matter content found for the *Yucca* exudate is one of the most characteristic features of a phloem exudate. Xylem exudates, as obtained in spring from such trees as *Betula* and *Acer* species only contain a few per cent of dry solutes; those from herbaceous plants generally only a few tenths of a per cent (cf. CRAFTS, 1954).

6. The exclusive position of sucrose in the *Yucca* exudate also suggests a phloem origin. Exudates obtained from many tree species (WANNER, 1953; ZIEGLER, 1956; ZIMMERMANN, 1957; SWANSON and EL-SHISHINY, 1958; MEYER–MEVIUS, 1959) and also sap exuding through the severed oral parts of various types of sucking aphids (ESAU, 1961) are all characterised by their high concentration of

total solutes (ca. 15–25 per cent) of which the majority consists of sucrose. Sucrose is often the exclusive carbohydrate present, sometimes together with certain oligosaccharides (ZIMMERMANN, 1957). Free monoses are only incidentally mentioned as major constituents in the phloem exudates of certain plant species (MEYER–MEVIUS, 1959).

The high amino acid content of the exudate from *Yucca* is striking. Except during growth and scenescence of the leaves the organic nitrogen content of phloem exudates is usually relatively low (MITTLER, 1953; HUBER, 1956). It seems probable that the high amino acid and amide content of the *Yucca* exudate is in some way connected with the mobilization of the soluble nitrogenous substances, required by the developing flowers.

There is a great deal of similarity between the exudation and exudate composition of *Yucca* and that of various palm trees (TAMMES, 1933, 1951, 1958), but unlike palm trees and the trees of temperate climates, *Yucca* species may be used for phloem translocation studies which have to be carried out under controlled conditions or otherwise inside the laboratory.

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