

COMPOSITION OF THE XYLEM SAP OF TEA PLANTS (*CAMELLIA SINENSIS* L.)

R. R. Selvendran and S. Sabaratnam

A modified method for the collection of xylem sap from tea plants is described. In multi-stemmed tea plants a marked difference in the composition of the sap collected from different branches of comparable diameter on the same plant was found. The possibility that the variation could be due to root disease or mechanical damage on one side of the tree was eliminated by using V-stems from a main branch. The sap contained a small quantity of a substance which showed gibberellin-like activity.

In an earlier paper (Selvendran 1970) a simple technique for the collection of xylem sap from the stems of tea plants and the changes in the composition of the sap during post-prune growth were described. The sap was collected by applying gentle suction to the cut end of the stems of pruned plants using a filter pump. Although this technique was useful for potted plants which could be moved to a water tap, it was not suitable for field plants. A slightly modified procedure was therefore developed for the collection of sap from field plants and some analytical studies were made on the sap.

MATERIALS AND METHODS

Plants used in the investigation

Multi-stemmed, 5-year-old field plants of Clone TRI 2024 were used.

Modified Procedure for the collection of xylem sap

Fig. 1 illustrates the apparatus used for the collection of xylem sap from a field plant. It differs from the earlier method only in the method of application of suction to the cut-end. Tap A was closed and lead B was connected to an Edwards vacuum pump and the whole system evacuated in the laboratory. After evacuation, taps C and E were closed and the unit disconnected from the pump and removed to the field. With a rubber tube, one end of the capillary tube D was connected to the cut-end of the stem. On opening taps A and E, the sap rose in the capillary and collected in the test-tube. About 30 ml sap could be collected in an hour from a 5-year-old field plant. The sap collected was removed from the field as soon as possible and stored at -10°C .

Analysis of sap

The sap was concentrated by lyophilization and the concentrate was passed through ion-exchange columns to separate the charged and uncharged compounds.

* Reprinted from *Annals of Botany*, 35, 679-682. 1971, by courtesy of the Editors.

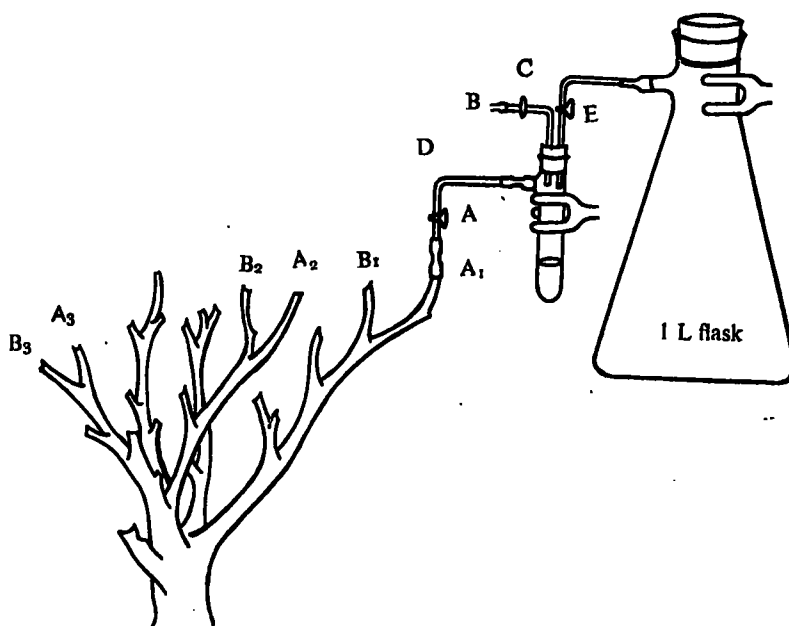


FIG. 1—Apparatus used for the collection of xylem sap from a plant in the field.

Amino acids

The amino acids were separated on paper chromatograms and estimated as described earlier (Selvendran, 1970).

Potassium

The potassium content of the sap was estimated using an Eel Flame Photometer.

Gibberellins

The following procedure was used for the partial purification and detection of gibberellin-like substances in the sap. Two litres of sap were collected from six 5-year-old plants and lyophilized. The residue (1.01 g) was taken up in 20 ml water, passed through a cation-exchange column (15 cmX1 cm diam) of Dowex AG-50 (8-10 per cent cross-linked H⁺ form, 200-400 mesh), and the resin bed was washed with two bed volumes of distilled water. The combined effluent (50 ml, pH 2.5) was extracted five times with 20-ml portions of ethyl acetate. The organic layer was evaporated to dryness under reduced pressure and the residue taken up in 0.5 ml of distilled water. The concentrate was chromatographed in one direction on Whatman No. 4 paper using the solvent isopropanol/ammonia/water (80:1:19 v/v). The chromatogram was cut into several pieces (5), eluted with aqueous ethanol, and the eluates after removal of ethanol were bioassayed for gibberellins with lettuce seedlings (Frankland and Wareing, 1960). Authentic GA₃ was chromatographed for comparative purposes and its position detected as described by Harada and Nitch (1967).

RESULTS AND DISCUSSION

The method described in this paper is suitable for the collection of xylem sap from field plants because it obviates the use of a filter-pump and is easy to carry out. The rate of collection of sap from a stem could be made comparable to the transpiration rate of the branch borne by the stem by adjusting the pressure in the evacuated system. This would make the sap study somewhat physiological.

Variation in the composition of the sap

Preliminary experiments with multi-stemmed field plants, showed that there were marked differences in the composition of the sap collected from different branches of comparable diameter on a single plant. As the tea plant is a perennial, the observed variation does not result from the phenomenon reported by Bollard (1958) and 1970 (personal communication). Bollard found variation from branch to branch in trees in spring when they were coming into blossom. If one branch blossomed before another then that branch yielded sap with markedly higher concentration of nutrients. This situation is not comparable with the present study. In Table 1 the composition of the sap collected from seemingly comparable branches of a plant is given.

TABLE 1 — *Quantities (μ moles ml⁻¹) of amino acids¹ and potassium ions in the xylem sap of seemingly comparable branches of a tea plant*

Constituent analysed	Description of branch					
	A ₁	B ₁	A ₂	B ₂	A ₃	B ₃
Glutamine	0.41	0.68	0.11	0.23	0.39	0.11
Theanine	0.62	1.06	0.42	0.89	0.62	0.36
Glutamic acid	0.19	0.18	0.20	0.31	0.06	0.13
Aspartic acid	0.01	0.02	0.01	0.02	0.02	0.03
Total amino acid	1.75	2.38	1.44	2.46	1.98	1.16
K ⁺	1.51	2.05	1.31	1.72	1.41	1.67

* The values were obtained using alanine as the standard.

The results indicate that the composition of the sap moving through different branches of the same plant do not seem to have any relationship to one another. However, on applying the same suction, the rate of collection of sap (ml h⁻¹) from the different branches was comparable. The possibility that the variation could be due to root disease or mechanical damage on one side of the tree was eliminated by using V-stems from a main branch (see Fig. 1).

In view of these findings, it would be unwise to study the seasonal changes in the composition of the sap of a multi-stemmed plant by collecting sap from a single branch of the plant. However, these studies could be carried out more systematically with free-growing plants to find out whether the composition of the sap has any bearing on apical dominance. That is, whether the actively growing apical shoots receive a markedly higher concentration of nutrients in the sap compared with side branches.

Gibberellin-like substances in the sap

A careful check showed that a very small quantity (approximately 0.04 μ g GA₃ equivalents l⁻¹) of gibberellins was present in the sap. The active spot co-chromatographed with authentic gibberellic acid on paper. This finding compares well with that reported by Sitton, Richmond, and Vaadia (1967) for the gibberellic-acid content (approximately 0.08 μ g GA₃ equivalents l⁻¹) in the sap of decapitated sunflower plants.

The presence of other growth factors in the sap is being investigated.

Rate of sap collection

As observed in earlier studies (Selvendran 1970), the rate of sap collection from lung-pruned field plants was considerably greater compared with that from clean-pruned plants, suggesting that the root-system of lung-pruned plants is more active during post-prune growth.

ACKNOWLEDGEMENTS

We are grateful to Dr S. Kulasegaram for his interest in the work and to Mr A. Kathiravetpillai for helping with the bioassay of gibberellins.

LITERATURE CITED

- BOLLARD, E. G., 1958. Nitrogenous compounds in tree xylem sap. In *The Physiology of Forest Trees*, ed. K. V. Thimann, 83-93. The Ronald Press Company, New York.
- FRANKLAND, B., and WAREING, P. F., 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. *Nature, Lond.* 185, 225-6.
- HARADA, H., and NITSCH, J. P., 1967. Isolation of gibberellins A₁, A₃, A₉ and of a fourth substance from *Althaea rosea* Cav. *Phytochemistry* 6, 1695-1703.
- SELVENDRAN, R. R., 1970. Changes in the composition of the xylem exudate of tea plants (*Camellia sinensis* L.) during recovery from pruning. *Ann. Bot.* 34, 825-33.
- SITTON, D., RICHMOND, A., and VAADIA, Y. 1967. On the synthesis of gibberellins in roots. *Phytochemistry* 6, 1101-5.

Date received, — 21st November 1970.