DET KGL. DANSKE VIDENSKABERNES SELSKAB
BIOLOGISKE MEDDELELSER, BIND XX, NR. 2

# THE BIOCHEMISTRY OF THE DEVELOPMENT OF BUDS IN TREES AND THE BLEEDING SAP

BY

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KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
1946

Printed in Denmark
Bianco Lunos Bogtrykkeri A/S

At an early stage in spring, different for different trees and shrubs, the buds, formed during the preceding summer and autumn, begin to develop and the problem to be studied is the quantitative aspect of the transference of substances to the buds during growth.

Chief among these substances is no doubt water, and in several trees water just at this time becomes available in abundance, filling up the xylem under pressure from the roots and causing the phenomenon of "bleeding" which makes it easy to obtain samples of the sap.

A great deal more than water appears to be necessary for the growth processes in the buds, and our chief interest when starting this research was the nature and origin of the osmotically active substances necessary to establish the turgor of the growing buds. Taking it for granted that the bleeding sap is the main source of these substances it was supposed that the expanding cells might take up ions and organic substances of low molecular weight (mainly sugars) leaving out most of the water, and we thought that comparative analyses of buds and of the sap, supposed to supply them, might yield information on this point.

On the advice of professor LUNDEGÅRDH a study of the supply of nitrogen to the buds was also included in our programme.

We are greatly indebted to professor LUNDEGÅRDH also for having the numerous determinations of a series of ions in our samples of buds and bleeding sap carried out by his spectrographic methods in his Institute at Ultuna.

The determinations of carbohydrates were carried out for us in the Botanic Institute by Miss M. ANDERSSON and the Kjeldahl determinations were undertaken by Miss INGA NILSSON and Mr. GUNNAR HANSSON in the Institute for Medical Chemistry of the late professor WIDMARK. We wish to record our gratitude to these conscientious assistants.

## Methods.

After preliminary trials a small number of individual shrubs and trees were selected for the study. Most of these were available in the Botanic garden of the University, but others were added in which it was found that spontaneous bleeding would take place. These latter were found in the public "Trollsjöpark" at Eslöv near Lund. Unfortunately several samples of sap from trees in this park were lost together with the sampling vessels.

The plants selected were:

Syringa chinensis, a tree near the main entrance to the Botanical garden which had been cut down a couple of years previously and now presented an abundance of young shoots. We never succeeded in obtaining any sap from this tree, neither from the trunk nor from any of the branches or shoots and not even by applying suction.

Cornus alba, a richly branched shrub in the Botanic garden. According to Miyoshi (1900) several species of this genus will bleed spontaneously and this shrub proved no exception, although the bleeding was never copious.

Betula verrucosa, a big tree just behind the Botanical Museum, bleeding copiously from a 12 mm. hole bored into the trunk about 1 m. above the ground on March 22. Buds were first collected (on March 2) from another large birch which proved less suitable.

Carpinus betulus, a big tree near the Botanic Institute, also found to bleed copiously from a hole bored into the trunk.

Corylus avellana. A large number of hazel bushes were tested for bleeding, but only in one specimen, growing on an incline in the park, spontaneous bleeding was observed from branches about 1 m. above the emergence of the stems from the soil.

Acer pseudoplatanus. The specimens of Acer in the Botanic garden were not considered suitable, as the buds were difficult to reach and many of them were flower buds. Suitable young trees were found in the park and samples were taken from several trees. In one of these (II) the budding was exceptionally early and in another (III) comparatively late.

Fagus silvatica, a magnificent beech just outside the Botanic Institute. The buds accessible on the North side began to swell

early and turned out to be flower buds. On the opposite side the leaf buds showed a much slower development. Spontaneous bleeding was never observed from this tree, but sap could be obtained by suction as described below. A few small beeches in the park were observed to bleed spontaneously and a few samples were taken from these (II and III).

The collection of sap. In the first trials samples were collected from 12 mm. holes bored into the trunk of trees. A glass tube covered with rubber tubing so as to fit snugly was inserted and a sampling vessel suspended on the tube by means of a two-hole stopper. In the second hole a capillary glass tube was inserted so as to minimize evaporation. A little later it was found that enough sap could generally be obtained from branches of about 10 mm. thickness which were cut and the remaining stump connected with the sampling vessel. Comparisons were made in several cases between samples of sap from the trunk and from branches.

When no spontaneous bleeding occurred it was attempted in several cases to collect sap by suction with a 100 ml syringe, provided with a three-way tap, either from a hole in the trunk or from a cut branch. Such suction always gave a great deal of air, but in a few cases also a little fluid so that it became evident that sufficient quantities could be obtained by a more efficient device for suction. This could be easily arranged in the case of the beech at the Botanic Institute by connecting the sampling vessel to a vacuum pump inside the building. A mercury vacuummeter was inserted to measure the suction necessary to obtain sap in this way. The pressure at which sap would begin to flow could usually be ascertained within a few cm of mercury.

The samples of sap were stored in 20 ml. bottles stoppered with 8 mm. corks.

Samples of buds—representative as far as possible of the different sizes present—were collected in cork stoppered wide-necked bottles, counted during the gathering and weighed as soon as possible, usually within less than 1 hour and thereupon dried in a vacuum desiccator over silica gel which was renewed whenever the blue colour began to fade. A constant weight was usually attained in 3—4 days at a temperature of about 25° C.

The samples were kept in cork stoppered bottles—as no others were available—and the weight increased slowly by absorption of water vapour, so that in many cases they had to be dried again before analysis. For the carbohydrate analyses this second drying was again performed in vacuo, but for the nitrogen and ion analyses at 105° in an oven. This does not make any perceptible difference, except in the case of the birch buds which contain a certain proportion of fat.

The results of the weighings are given in table 1 per 100 buds stating the fresh and dry weight and the water content as difference.

## The Analyses.

All analyses of buds were made on the dry weight basis, whether the buds were intact at the time of weighing or, in the case of a small number of large buds, were cut up into small bits before they were completely dry. In the intact buds calculation on a number bases will often give slightly different results.

Sugar analyses. Lots of 20 to 50 dry buds were weighed, ground in a mortar, and extracted on a water bath 2 to 3 times with 40 ml. 50 % alcohol. The extracts were joined together, the alcohol evaporated, and the watery residue precipitated with Pb-acetate in order to remove proteins. Then Pb was precipitated by excess of $Na_2CO_3$, the solution made up to 50 or 100 ml. and reducing sugars and sucrose determined in aliquots of 1—2 ml. according to PHILIPSON (1943). The analytical errors of this method are less than 1 %, but the total errors of the determinations also include inhomogeneity of the material, losses of sugars during the extraction etc. These errors could not be exactly determined owing to the short time during which material was available, but parallel determinations on especially selected unequal lots of the same sample show that the total errors probably do not exceed 5 %.

The bleeding sap was analyzed in the same way after precipitation of proteins.

In some earlier bud samples determinations were made also of starch by extracting the residues of the alcohol treatment

with 4-n HCl. These solutions were neutralized, deproteinized, and analyzed as described above, but since irregularities occurred and the time did not permit a thorough testing of suitable methods of extraction, these analyses were discontinued. The more so because starch would be produced by assimilation when the leaves began to unfold.

Analyses of mineral ions were carried out at the Institute of Plant Physiology Ultuna-Uppsala according to routine methods of spectral analyses (LUNDEGÅRDH 1945) except P, which was determined according to ZINDZADZE 1934. The errors of the spectral analyses usually average 3 % for all elements, but because in several instances the amounts found approached the lower limits of determination, the values suffer from somewhat higher errors, which may, also on account of inhomogeneity of the material, probably often reach 10 %.

Nitrogen analyses were performed on sap and dried buds according to the Folin modification of the Kjeldahl procedure (FOLIN & FARMER 1912). Analyses were carried out in macro by titrating with 0.1-n acid and in micro by replacing the latter by 0.01-n acid. The time of distillation was shortened to 20 min., when it was found that $NH_3$ was almost quantitatively distilled in the first 15 min. The analytical errors were estimated at about 1 mg. N per g. dry substance for the buds and 0.1 mg. for the samples of sap taken for analysis.

Qualitative tests for nitrates were made by placing drops of sap alongside drops diphenylamine in sulphuric acid on a slide and allowing them to mix slowly. Such tests, made on known nitrate solutions, showed that a concentration of not less than 1 mM was required to give a slowly developing faint blue colour, while 5 mM would give an immediate strong reaction.

## Results of the Determinations.

In table 1 we have put together the results of the weighings together with remarks on the condition of the buds at the dates of sampling and data concerning the samples of sap.

The weights are given per 100 buds and show a regular and in the later stages very rapid increase in fresh weight. This increase is largely water as shown in the two last columns, but

Table

| Genus | Date | Number taken | Notes |
|---|---|---|---|
| Syringa | 1/3 | 200 | Closed, faintly green |
| | 6/3 | 200 | Scarcely altered |
| | 15/3 | 200 | — |
| | 6/4 | 50 | Opening up, green |
| | 20/4 | 50 | Young leaves |
| | 20/4 | 25 | Flower buds, opened |
| Cornus | 3/4 | 150 | Leaf and flower buds, green |
| | 12/4 | 50 | — partly opened |
| Betula I | 2/3 | 200 | Closed |
| — II | 6/3 | 200 | Buds very small, closed |
| | 15/3 | 300 | — |
| | 17/4 | 200 | Definitely larger, greenish |
| | 25/4 | 200 | Opening up |
| | 3/5 | 100 | Young leaves |
| Carpinus | 22/3 | 200 | Completely closed |
| | 6/4 | 100 | Swelled, but still closed |
| | 12/4 | 75 | Opening up |
| | 23/4 | 50 | Young leaves |
| | 23/4 | 25 | Catkins |
| Corylus | 3/4 | 200 | Closed, greenish |
| | 17/4 | 150 | Very green |
| | 25/4 | 100 | Young leaves |
| Acer I | 5/4 | 150 | Closed, green, several trees I |
| — II | 17/4 | 50 | Advanced buds tree II |
| | 19/4 | 50 | — |
| | 23/4 | 10 | Young leaves II |
| — III | 20/4 | 100 | Closed buds, tree III |
| | 26/4 | 25 | Opening up III |
| Fagus I | 2/3 | 100 | Large flower buds, closed I |
| | 6/3 | 100 | — |
| | 15/3 | 150 | — |
| | 12/4 | 50 | — developing |
| | 23/4 | 50 | — opening up |
| | 3/5 | 25 | Catkins |
| | 24/4 | 100 | Leaf buds, slightly opening I |
| | 5/5 | 25 | Young leaves I |
| — II-III | 27/4 | 200 | Small closed buds II and III |

1.

| Weight of 100 buds | | | Water % | Sap |
|---|---|---|---|---|
| fresh | dry | water | | |
| 4.10 | 1.40 | 2.70 | 65.8 | |
| 4.64 | 1.64 | 3.00 | 64.7 | |
| 5.23 | 1.70 | 3.53 | 67.6 | No sap, obtainable even by suction. |
| 11.22 | 2.91 | 8.31 | 74.0 | |
| 10.30 | 2.82 | 7.48 | 72.6 | |
| 26.44 | 6.52 | 19.92 | 75.3 | |
| 2.97 | 0.85 | 2.12 | 71.5 | 1.$^{22\text{-}23}/_{3}$ about 10 ml, 2.$^{28}/_{3}$-$^{3}/_{4}$ 10 ml, 3.$^{1\text{-}3}/_{4}$—Another |
| 8.24 | 2.12 | 6.12 | 74.3 | branch, 4.$^{5\text{-}7}/_{4}$ about 5 ml. No more obtainable. |
| 2.34 | 1.37 | 0.97 | 41.5 | 1.$^{23}/_{3}$ from trunk, flowing fast, 2.$^{24}/_{3}$ do., 3.$^{26}/_{3}$ do., |
| 1.90 | 1.12 | 0.78 | 41.0 | 4.$^{26}/_{3}$ from branch 20 ml in less than 10 min., |
| 1.83 | 1.07 | 0.76 | 41.5 | 5.$^{17}/_{4}$ from branch, 6.$^{25}/_{4}$ from branch, flowing |
| 2.59 | 1.22 | 1.37 | 53.0 | more slowly, 7.$^{3}/_{5}$ branch, very slow, 8.$^{3}/_{5}$ trunk, |
| 4.81 | 1.59 | 3.22 | 67.0 | very slow. |
| 9.57 | 2.55 | 7.02 | 73.5 | |
| 3.52 | 1.49 | 2.03 | 57.6 | 1.$^{23}/_{3}$ from trunk about 20 ml/min., 2.$^{23}/_{3}$ do., |
| 10.17 | 3.17 | 7.00 | 68.8 | 3.$^{26}/_{3}$ do., 4.$^{26}/_{3}$ from branch, dripping freely, |
| 12.07 | 3.57 | 8.50 | 70.5 | 5.$^{6}/_{4}$ branch do., 6.$^{12}/_{4}$ branch 20 ml in 2 hours. |
| 16.36 | 4.88 | 11.48 | 70.3 | No more obtainable. |
| 20.56 | 4.16 | 16.40 | 79.9 | |
| 3.705 | 1.27 | 2.435 | 65.8 | 1.$^{29}/_{3}$-$^{3}/_{4}$ low branch, bleeding slightly, several |
| 7.16 | 2.30 | 4.86 | 68.0 | samples lost, 2.$^{19\text{-}20}/_{4}$. |
| 10.77 | 3.25 | 7.52 | 70.0 | |
| 16.30 | 4.51 | 11.79 | 72.4 | 1.$^{3\text{-}6}/_{4}$ trunk, I, 2.$^{5\text{-}7}/_{4}$ trunk I, 3.$^{5\text{-}7}/_{4}$ branch I, |
| 53.96 | 9.90 | 44.06 | 81.5 | 4.$^{8\text{-}16}/_{4}$ branch II, 5.$^{17}/_{4}$ trunk II, |
| 60.84 | 10.57 | 50.27 | 82.7 | |
| 159.3 | 29.7 | 129.6 | 81.5 | |
| 35.25 | 6.60 | 28.65 | 81.3 | 6.$^{19\text{-}20}/_{4}$ branch, small sample III. |
| 60.30 | 10.72 | 49.58 | 82.3 | 7.$^{21\text{-}23}/_{4}$ branch III. |
| 12.79 | 6.49 | 6.30 | 49.3 | From I no spontaneous bleeding, sap obtained by |
| 11.60 | 5.90 | 5.70 | 49.2 | suction. 1.$^{5}/_{4}$ flower branch—430 mm Hg, 8 ml/hour, |
| 10.43 | 5.14 | 5.29 | 50.7 | 2.$^{11}/_{4}$ flower br.—430 mm Hg, 8 ml/h, 3.$^{12}/_{4}$ root— |
| 24.98 | 8.18 | 16.80 | 67.4 | 450 mm, 88 ml/h, 4.$^{12}/_{4}$ trunk—350 mm, 100 ml/h. |
| 41.68 | 11.26 | 30.42 | 73.1 | 5.$^{23}/_{4}$ flower branch—560 mm, 17 ml/h, 6.$^{23}/_{4}$ trunk |
| 84.0 | 23.3 | 60.7 | 72.4 | —620 mm, 170 ml/h, 7.$^{24}/_{4}$ leaf branch—600 mm, |
| 15.36 | 5.20 | 10.16 | 66.2 | 36 ml/h. No more obtainable by suction. Trees |
| 81.1 | 19.8 | 61.3 | 75.5 | II and III bleed spontaneously. 8.$^{27\text{-}28}/_{4}$ low |
| 5.19 | 2.13 | 3.06 | 59.0 | branch II, 9.$^{27\text{-}28}/_{4}$ high branch, 10.$^{27\text{-}28}/_{4}$ low |
| | | | | branch III, 11.$^{30}/_{4}$-$^{1}/_{5}$ low branch III. |

also the dry weight shows in all cases a substantial increase. As discussed in some detail in the next section this increase is largely due to new formation of cellulose. As long as the buds remain closed the cellulose must be built up from materials stored in the buds or brought in with the sap, but when the buds open up assimilation may become an important factor. At the same time evaporation of water from the buds may also begin seriously to interfere with calculations of the supply of sap. We have made no determinations of the transpiration, but from the literature it can be inferred that in the closed buds the transpiration, although by no means negligible compared with the water content, is slight only when compared with the water supply.

HARTIG (1883) weighed bundles of small branches with buds, kept in the open air for a certain period in early spring, and recorded their loss of water in per cent of the original water content. He found that Betula branches lost 28 % of the water in 7 days and Carpinus and Fagus the same amount in 3 days. Accepting these figures also for the buds we calculate that 100 Betula buds taken on March 6 would lose in 7 days 28 % of 0.78 g. = 0.22 g., 100 Carpinus buds on March 23 in 3 days 0.57 g. and 100 Fagus flower buds on March 6 also in 3 days 1.7 g. Calculated per g. of fresh buds this would amount to 17 mg./day for Betula, 54 for Carpinus and 50 for Fagus.

KNY (1895) cut off very short endpieces of branches carrying buds and sealed the cut ends with wax. In one series of determinations he sealed also the scars of the older leaves which in another series he left open. This made no difference and it can be concluded that the weight losses took place mainly from the buds. From each species he weighed 10 buds and gives the average. The buds were weighed again after a certain number of days in the open, but protected against rain.

He finds:

| | | | | | Loss |
|---|---|---|---|---|---|
| Carpinus .... | $^{6}/_{3}$ | 45 mg. | $^{15}/_{3}$ | 29 mg. | 16 mg. |
| Acer..... | $^{27}/_{3}$ | 341 mg. | $^{3}/_{4}$ | 227 mg. | 114 mg. |

Our weights for the corresponding isolated buds are respectively 35 mg and 163 mg and we find the losses per g fresh buds per day to be for Carpinus $\frac{16}{9}\frac{1000}{35} = 50$ mg. and for Acer 100 mg.

In spite of the naturally varying external conditions the results of the two investigations tally very well with each other, and it may be assumed that also in our experiments the transpiration is of about the same magnitude. It can be even considerably higher without materially affecting our main results.

## The Carbohydrates of Buds and Sap.

The carbohydrate determinations are put together in table 2. All the buds examined contain a mixture of hexose and sucrose, although in very varying proportions, and these substances are in all probability present in solution in the water of the buds and make up a rather considerable fraction of the osmotic pressure of the cells. There is a definite tendency for the sugar concentration to fall off during development.

Starch was determined only on young buds showing at most a greenish tint on the outside and in which therefore only an insignificant assimilation could possibly take place. Starch seems to be present in very variable quantities. Conversions of sugar into starch and vice versa are no doubt possible at any stage.

Except in the earliest stages we must further assume a new formation of carbohydrate by assimilation, and during the whole period of bud development a formation of cellulose is certainly going on—at an increasing rate as judged from the increases in dry weight.

Sugars are present in all the samples of sap examined, but the quantities are very variable. In Cornus, Betula, Carpinus, Corylus and Fagus hexose is preponderant, but in most samples from Acer there is a large surplus of sucrose. It is a curious fact that in the buds of Acer there is a decided preponderance of hexose. Our sugar values for the sap are much smaller than those recorded for the sugar maple and several other species.

In two cases, concerning the buds of Carpinus between March 22 and April 6 and the flower buds of Fagus between March 15 and April 12, it can be made probable by calculations that large quantities of sap have passed through the buds and supplied the carbohydrates necessary for growth. During the periods in question the buds did not show any green so that assimilation cannot have yielded any significant increase in dry

Table 2. Determinations

| Genus | Date | Notes | Per 100 buds | | | |
|---|---|---|---|---|---|---|
| | | | Water g. | Hexose μM | Sucrose as Hexose μM | Starch as Hexose μM |
| Syringa ... | 1/3 | Greenish ..... | 2.70 | 340 | 260 | 1000 |
| | 6/3 | — ..... | 3.00 | 440 | 40 | 860 |
| | 15/3 | — ..... | 3.53 | 265 | 215 | ... |
| | 6/4 | Opening, green ..... | 8.31 | 970 | 270 | 1620 |
| | 20/4 | Young leaves ..... | 7.48 | 1190 | 230 | ... |
| | 20/4 | Flower buds ..... | 19.92 | 2290 | 790 | ... |
| Cornus ... | 3/4 | Green ..... | 2.12 | 420 | 49 | 160 |
| | 12/4 | Opening up ..... | 6.12 | 1110 | 25 | 1090 |
| Betula I... | 2/3 | Closed ..... | 0.97 | 230 | 75 | 440 |
| — II.. | 6/3 | Brown ..... | 0.78 | 135 | 250 | 560 |
| | 15/3 | — ..... | 0.76 | 63 | 310 | ... |
| | 17/4 | Greenish ..... | 1.37 | 222 | 182 | ... |
| | 25/4 | Opening ..... | 3.22 | 352 | 59 | ... |
| | 3/5 | Leaves ..... | 7.02 | 323 | 430 | .. |
| Carpinus.. | 22/3 | Brown ..... | 2.03 | 98 | 293 | 235 |
| | 6/4 | — ..... | 7.00 | 367 | 386 | 1790 |
| | 12/4 | Greenish ..... | 8.50 | 630 | 545 | 1835 |
| | 23/4 | Leaves ..... | 11.48 | 425 | 171 | ... |
| | 23/4 | Catkins ..... | 16.40 | 800 | 1125 | ... |
| Corylus ... | 3/4 | Greenish ..... | 2.435 | 250 | 156 | 166 |
| | 17/4 | Very green ..... | 4.86 | 426 | 246 | ... |
| | 25/4 | Leaves ..... | 7.52 | 810 | 38 | ... |
| Acer I .... | 5/4 | Green I ..... | 11.79 | 654 | 393 | 976 |
| | 17/4 | Very green ..... | 44.06 | 2150 | ? | ... |
| | 19/4 | do. ..... | 50.27 | 1205 | 280 | ... |
| — II ... | 23/4 | Leaves II ..... | 129.6 | 2585 | 1985 | ... |
| — III .. | 20/4 | Green III ..... | 28.65 | 940 | 330 | ... |
| | 26/4 | Opening III ..... | 49.58 | 905 | 1650 | ... |
| Fagus I ... | 2/3 | Brown I ..... | 6.30 | 470 | 1770 | 6780 |
| | 6/3 | — ..... | 5.70 | 540 | 1860 | 2240 |
| | 15/3 | — ..... | 5.29 | 480 | 960 | ... |
| | 12/4 | — ..... | 16.80 | 2025 | 1265 | ... |
| | 23/4 | Opening ..... | 30.42 | 2770 | 1025 | ... |
| | 3/5 | Catkins ..... | 60.7 | 4500 | 614 | ... |
| | 23/4 | Greenish ..... | 10.16 | 1100 | 260 | ... |
| | 5/5 | Leaves ..... | 61.3 | 4520 | 3070 | ... |
| — II-III | 27/4 | Brown II-III ..... | 3.06 | 264 | 238 | ... |

of Carbohydrate.

| Conc. in bud water mM/Liter | | Sap | | | |
|---|---|---|---|---|---|
| | | Date | Notes | mM/Liter | |
| Hexose | Sucrose | | | Hexose | Sucrose |
| 126 | 48 | | | | |
| 146 | 7 | | | | |
| 75 | 30 | | | | |
| 116 | 16 | | | | |
| 159 | 15 | | | | |
| 115 | 20 | | | | |
| 198 | 12 | $^{22-23}/_{3}$ | Branch | 20.6 | 14.1 |
| 182 | 2 | $^{28}/_{3}$-$^{3}/_{4}$ | — | 5.0 | 4.7 |
| 235 | 39 | $^{23}/_{3}$ | Trunk | 83.4 | 7 |
| 173 | 160 | $^{26}/_{3}$ | — | 79 | 0.25 |
| 83 | 205 | $^{26}/_{3}$ | Branch | 62 | 0.9 |
| 162 | 67 | $^{17}/_{4}$ | — | 117 | 0 |
| 109 | 9 | $^{3}/_{5}$ | Trunk | 82 | 1.5 |
| 46 | 30 | $^{3}/_{5}$ | Branch | 64 | 8 |
| 48 | 72 | $^{23}/_{3}$ | Trunk | 22.5 | 0.65 |
| 52 | 28 | $^{26}/_{3}$ | — | 29 | 0.1 |
| 74 | 32 | $^{26}/_{3}$ | Branch | 47 | 0.05 |
| 37 | 7 | $^{6}/_{4}$ | — | 20 | 1.4 |
| 49 | 35 | $^{12}/_{4}$ | — | 37 | 1.0 |
| 102 | 32 | $^{29}/_{3}$-$^{3}/_{4}$ | Branch | 29 | 1.5 |
| 87 | 25 | | | | |
| 108 | 2 | | | | |
| 55 | 17 | $^{3-6}/_{4}$ | Trunk I | 1.6 | 26.5 |
| 49 | (6) | $^{5-7}/_{4}$ | Branch I | 1.8 | 20.7 |
| 24 | 3 | $^{8-16}/_{4}$ | Branch II | 16 | 10.5 |
| 20 | 7 | | | | |
| 33 | 6 | $^{21-23}/_{4}$ | Branch III | 4 | 44.5 |
| 17 | 16 | | | | |
| 75 | 140 | $^{5}/_{4}$ | Flower Branch | 27 | 0.3 |
| 95 | 164 | $^{11}/_{4}$ | — | 34 | 0.4 |
| 91 | 91 | $^{12}/_{4}$ | Root | 8 | 1.4 |
| 121 | 38 | $^{12}/_{4}$ | Trunk | 3 | 0.5 |
| 91 | 17 | $^{23}/_{4}$ | Flower Branch | 15 | 0 |
| 74 | 51 | $^{23}/_{4}$ | Trunk | 4 | 1 |
| 108 | 13 | $^{24}/_{4}$ | Leaf Branch | 18 | 0 |
| 74 | 25 | | | | |
| 86 | 39 | $^{30}/_{4}$-$^{1}/_{5}$ | Branch III | 8 | 0 |

substance. From the figures given in table 1 the increases in dry substance of 100 buds were respectively: Carpinus 1.68 g., Fagus 3.04 g., which we assume to be made up of protein, salts, sugar, starch and cellulose. From table 3 we get the increases in nitrogen as 48 and 103 mg. respectively, corresponding to 300 and 650 mg. dry protein, in accordance with the usually accepted composition.

The increases in salts can only be calculated very roughly from table 4, but they are so small by weight that even large errors can have no appreciable influence. We calculate increases of about 100 mg. in both cases.

When the figures for proteins and salts are subtracted from the dry weights we find increases in carbohydrate of 1.28 g. for Carpinus and 2.3 g. for Fagus. In the case of Carpinus the increases in sugar and starch make up 0.34 g. and in Fagus the sugars alone account for 0.15 g., the starch not being determined.

The Carpinus sap contained according to the analyses in table 2 about 6.5 g. sugar/l. and the Fagus sap 4.5 g/l. 100 buds should therefore require the minimum supply of 0.2 and 0.5 l. respectively to account for the observed increases in carbohydrate, leaving out of account the supply necessary for building up proteins. Calculated per g. average fresh bud per day this works out as 2.0 ml. for Carpinus and 1.0 for Fagus and it is evident that a transpiration even amounting to 0.1 ml./g./day does not seriously affect the result.

The necessary supply of sap is further increased when the losses by respiration are considered. Determinations of the respiration of developing buds given by GARREAU (quoted from BARTON-WRIGHT 1941) show for Syringa, Ribes and Tilia respiratory exchanges corresponding to the combustion per g dry weight/day of 47, 64 and 89 mg. sugar. The Carpinus and Fagus buds of our experiments grow at average rates of 37 resp. 13 mg./g./day and these rates suggest sugar combustions of a similar magnitude, since the economic quotient at C-heterotrophic nutrition of the buds is probably not higher than 20—30 % and certainly does not exceed 50 %. The supply of carbohydrate must be increased to cover this and the required quantity of bleeding sap will be at least double the figures computed above. If the increase in water content of the buds + the amount

of water evaporated were replaced by a sap containing 30 % sugar the increase in dry substance and the respiratory loss could be, at least approximately, accounted for without assuming any circulation. Small amounts of sap of such high sugar content might possibly be available in the phloem.

More conclusive evidence for the circulation of sap through the developing buds will be provided by a study of the increases in organic nitrogen and in ions.

## The Nitrogenous Substances in Buds and Sap.

Kjeldahl determinations on the buds and the samples of sap are put together in table 3. In practically all cases the nitrogen found by Kjeldahl determinations will represent the total N present. The analyses of buds are fairly reliable, but in many cases the samples of sap analysed have been too small and the possible errors of the results are rather large. All figures the errors of which may exceed 20 % are indicated as uncertain.

A number of the sap samples were tested for the presence of nitrate. Nitrate was definitely absent (concentration below 1 mM) from almost all samples, but present in two from Acer. In one of these the concentration was probably only slightly above the limit detectable (1 mM), but in the other the reaction was immediate and strong and probably corresponded to 5—10 mM.

The presence of nitrate may be taken to indicate the soil as the immediate source, while the Kjeldahl N originates from stores in the branches, trunk or root system. In most cases the nitrogen is seen to be higher in the sap from branches than in the trunk (or roots).

In the buds a regular increase in total nitrogen is observed throughout the period of growth and, assuming this nitrogen to be supplied by the sap, we can calculate the amount necessary on the supposition that all the nitrogen offered is retained in the buds.

Again selecting Carpinus $^{22}/_{3}$—$^{6}/_{4}$ and Fagus flower buds $^{15}/_{3}$—$^{12}/_{4}$ we find increases of 48 and 103 mgN/100 buds. Assuming an average content of 100 mgN/l. sap in both cases the increases require respectively $^{1}/_{3}$ and 1 liter sap or for the Carpinus buds 4.8 and the Fagus buds 2 ml. sap/g./day. In all the

Table 3. Nitrogen

| Genus | Date | Notes | per 100 buds | | |
|---|---|---|---|---|---|
| | | | Dry weight g. | Kjeldahl-N mg. | Kjeldahl-N mg./g. |
| Syringa ..... | 1/3 | Closed ..... | 1.40 | 38 | 27 |
| | 6/3 | — | 1.64 | 23 | 14 |
| | 15/3 | — | 1.70 | 36 | 21 |
| | 6/4 | Opening up ..... | 2.91 | | |
| | 20/4 | Young leaves ..... | 2.82 | 119 | 42 |
| | 20/4 | Flower buds ..... | 6.52 | 254 | 39 |
| Cornus ..... | 3/4 | Green buds ..... | 0.85 | 19.5 | 23 |
| | 12/4 | Partly opened ..... | 2.12 | 74 | 35 |
| Betula I ..... | 6/3 | Closed ..... | 1.12 | 14.5 | 13 |
| — II ..... | 15/3 | — | 1.07 | 14 | 15 |
| | 17/4 | Larger ..... | 1.22 | 19.5 | 16 |
| | 25/4 | Opening up ..... | 1.59 | 56 | 35 |
| | 3/5 | Leaves ..... | 2.55 | 66 | 26 |
| Carpinus ..... | 22/3 | Closed ..... | 1.49 | 31 | 21 |
| | 6/4 | — | 3.17 | 79 | 25 |
| | 12/4 | Opening up ..... | 3.57 | 79 | 22 |
| | 23/4 | Young leaves ..... | 4.88 | 161 | 33 |
| | 23/4 | Catkins ..... | 4.16 | 112 | 27 |
| Corylus ..... | 3/4 | Closed ..... | 1.27 | 27 | 21 |
| | 17/4 | — | 2.305 | 67 | 29 |
| | 25/4 | Young leaves ..... | 3.25 | 124 | 38 |
| Acer I ..... | 5/4 | I Closed, green ..... | 4.51 | 112 | 25 |
| — II ..... | 17/4 | II Advanced ..... | 9.90 | 465 | 47 |
| | 19/4 | — ..... | 10.57 | 445 | 42 |
| | 23/4 | Young leaves ..... | 29.7 | 1310 | 44 |
| — III ..... | 20/4 | III Closed ..... | 6.60 | 251 | 38 |
| | 26/4 | Opening up ..... | 10.72 | 386 | 36 |
| Fagus I ..... | 15/3 | Flower buds ..... | 5.14 | 77 | 15 |
| | 12/4 | — ..... | 8.18 | 180 | 22 |
| | 23/4 | Opening up ..... | 11.26 | 350 | 31 |
| | 3/5 | Catkins ..... | 23.3 | 580 | 29 |
| | 23/4 | Leaf buds ..... | 15.36 | 160 | 31 |
| | 5/5 | Young leaves ..... | 81.1 | 650 | 33 |
| | 27/4 | II and III Closed ..... | 5.19 | 19 | 9 |

Determinations.

| Date | Notes | per 1 sap | | |
|---|---|---|---|---|
| | | Nitrate | Kjeldahl-N mg. | Analysed quantity, ml. |
| 22-22/3 | Branch | — | < 25 | 2 |
| 28-31/3 | — | | ca. 100 | 2 |
| 1-3/4 | — | | 75 | 4 |
| 23/3 | Trunk | — | ca. 25 | 4 |
| 26/3 | Branch | — | - 75 | 4 |
| 17/4 | — | — | 340 | 5 |
| 25/4 | — | | 225 | 4 |
| 3/5 | — | | 220 | 5 |
| 3/5 | Trunk | | 140 | 5 |
| 23/3 | Trunk | — | ca. 7 | 15 |
| 26/3 | — | | - 100 | 2 |
| 26/3 | Branch | — | - 200 | 2 |
| 6/4 | — | — | - 50 | 2 |
| 9/4 | Trunk | | - 150 | 2 |
| 42/4 | Branch | — | - 25 | 4 |
| 29/3-3/4 | Branch | | ca. 800 | 2 |
| 19-20/4 | — | | - 200 | 2 |
| 3-6/4 | Trunk I | — | 125 | 4 |
| 5-7/4 | Branch I | + | 125 | 4 |
| 8-16/4 | Branch II | +++ | 50 | 4 |
| 19-20/4 | — | | ca. 100 | 2 |
| 21-23/4 | Branch III | — | 175 | 4 |
| 12/4 | Root | — | 75 | 4 |
| 12/4 | Trunk | — | ca. 100 | 2 |
| 23/4 | Flower Branch | — | 400 | 2 |
| 23/4 | Trunk | | 300 | 2 |
| 21/4 | Leaf Branch | — | 225 | 4 |
| 27-28/4 | II Branch | — | 75 | 4 |
| 30/4-1/5 | III — | | ca. 100 | 2 |
| 27-28/4 | III — | | - 25 | 4 |

plants studied it is necessary to assume an abundant flow of sap through the buds in order to explain the increases in nitrogen found even if all the nitrogen offered is retained by the buds—a natural assumption if it is combined as protein.

From the sieve tubes an insignificant amount only can be supplied, as judged from the scanty analytical material (HARTIG 1858, 1861). On the other hand the work of MASON and his associates on cotton point to the phloem as the transport channel from the leaves to the stem both for nitrogen and for sugars (1937).

## The Inorganic Ions in Buds and Sap.

The results of the spectrographic determinations on the ash of buds carried out on the basis of unit dry substance have been recalculated and presented in table 4 as $\mu$M per 100 buds. In the same table the ion concentrations of the samples of sap are put together. Several interesting facts can be gathered from this table.

Generally there is a considerable increase in the ion quantities with the growth of the buds which in this table is represented by the increasing water content, but certain exceptions to this general rule are very conspicuous.

Sodium, which in most buds is below the determinable minimum, was found in the earliest samples from Betula and Carpinus; but apparently disappears during development thus involving a transport (with water) out of the buds. A calculation shows that the quantities of Na thus transferred to the sap (?) would not be sufficient to raise the concentration above the minimum.

Manganese while showing rather irregular increases in most cases (Syringa, Betula, Corylus, Acer) is present only in the first buds from Carpinus and absent from the buds of Cornus and those of the big beech in the Botanic garden.

Potassium is present in the large majority of sap samples. In the few in which it is below the minimum determinable it is natural to suppose that it has been depleted by the buds which at the same time show a rapid growth and increase in potassium content. Sodium is below the minimum determinable

in all samples of sap. Calcium is present in all samples and shows smaller variations than the other ions. Magnesium is below the minimum in a large majority of the samples of sap and found only in one sample from Cornus, one from Betula and two from the Carpinus trunk, but not in the samples from branches. This again may indicate a fairly complete absorption into the buds. The concentration of manganese is low in all cases and in the sap very often below the minimum determinable. The small beeches II and III from the park show fairly high concentrations of Mn in the sap, and in this case Mn is also present in the buds while absent from the buds of the large beech in the Botanic garden. Phosphorus is present in most samples of sap, but generally in quite low concentration and it is obvious that it is absorbed with avidity by the buds.

Generally the samples of sap from branches show lower total concentrations than the corresponding samples from the tree trunks and this again we would explain as due to absorption on the part of the buds.

Such absorptions are illustrated by the examples in table 5 in which the increases of single ions in 100 buds over a given period are compared with the corresponding concentrations in the sap of branches of 1 cm. thickness. It is to be remembered that sap samples taken closer to the buds might have shown different compositions.

If we accept the sap analyses as representative and assume further a complete depletion regarding the ion requiring the largest supply which can be calculated from the figures printed in bold type, we find that variable and on the whole very large quantities of sap must have passed through the buds, and in the earlier stages only an insignificant fraction of the supply can have evaporated while 98 to 99 % must have returned to the branches and perhaps down into the trunk. In the later stages a fairly large proportion of the water may have evaporated from the young leaves.

When we reduce the figures to a common unit viz. the passage of sap through 1 g. fresh buds per day the variations become much smaller and the sap exchanges calculated do not seem extravagant.

We assume that the sap enters the buds through their vessels and is present as an "extracellular" solution from which

| Genus | Date | Notes | Per 100 buds | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Water g | K µM | Na µM | Ca µM | Mg µM | Mn µM | P µM |
| Syringa ... | 1/3 | Closed ..... | 2.70 | 560 | — | 142 | 71 | 0.46 | 172 |
| | 6/3 | — ..... | 3.00 | 343 | | 181 | 96 | | 201 |
| | 15/3 | — ..... | 3.53 | 810 | — | 168 | 119 | 0.63 | 244 |
| | 6/4 | Opening up . | 8.31 | 1640 | — | 245 | 172 | 0.67 | 540 |
| | 20/4 | Leaves ..... | 7.48 | 1260 | — | 295 | 540 | 2.23 | 1484 |
| | 20/4 | Flower buds | 19.92 | 2050 | — | 620 | 705 | 2.68 | 1260 |
| Cornus ... | 3/4 | Green buds . | 2.12 | 196 | — | 175 | 106 | — | 191 |
| | 12/4 | Partly opened | 6.12 | 556 | — | 574 | 110 | — | 537 |
| Betula I... | 2/3 | Closed ..... | 0.975 | 109 | 57 | 270 | 110 | — | 90 |
| — II... | 6/3 | — ..... | 0.78 | 85 | 35 | 188 | 92 | 0.84 | 75 |
| | 15/3 | — ..... | 0.76 | 44 | 26 | 130 | 45 | 0.39 | 47 |
| | 17/4 | — ..... | 1.37 | 138 | — | 218 | 61 | 0.73 | 95 |
| | 25/4 | Opening up . | 3.22 | 294 | — | 200 | 132 | 3.10 | 266 |
| | 3/5 | Leaves ..... | 7.02 | 575 | — | 345 | 236 | 1.27 | 453 |
| Carpinus.. | 22/3 | Closed ..... | 2.03 | 155 | 37 | 330 | 119 | 2.79 | 121 |
| | 6/4 | — ..... | 7.00 | 703 | — | 480 | 362 | — | 382 |
| | 12/4 | Opening up . | 8.50 | 900 | — | 457 | 425 | — | 483 |
| | 23/4 | Young leaves | 11.48 | 1310 | — | 857 | 910 | — | 790 |
| | 23/4 | Catkins ..... | 16.40 | 1150 | — | 552 | 610 | — | 579 |
| Corylus ... | 3/4 | Closed ..... | 2.435 | 220 | — | 371 | 153 | 8.3 | 113 |
| | 17/4 | — ..... | 4.86 | 441 | — | 465 | 382 | 17.0 | 285 |
| | 25/4 | Young leaves | 7.52 | 835 | — | 637 | 558 | 35.4 | 501 |
| Acer I..... | 5/4 | Closed ..... | 11.79 | 1200 | — | 1790 | 659 | 8.1 | 717 |
| — II .... | 17/4 | Advanced ... | 44.06 | 4960 | — | 2120 | 910 | 17.8 | 2350 |
| | 19/4 | — ... | 50.27 | 5140 | — | 2000 | 1310 | 15.9 | 2820 |
| | 23/4 | Young leaves | 129.6 | 11050 | — | 6760 | 3560 | 71.0 | 6500 |
| — III ... | 20/4 | Closed ..... | 28.65 | 2900 | — | 1980 | 620 | 32.4 | 1620 |
| | 26/4 | Opening up. | 49.58 | 3120 | — | 3860 | 1800 | 76.6 | 2380 |
| Fagus I ... | 15/3 | Flower buds | 5.29 | 422 | — | 907 | 565 | — | 373 |
| | 12/4 | — | 16.80 | 932 | — | 883 | 532 | — | 922 |
| | 23/4 | Opening up . | 30.42 | 1960 | — | 990 | 1060 | — | 1603 |
| | 3/5 | Catkins ..... | 60.7 | 7700 | — | 1890 | 1310 | — | 3240 |
| | 23/4 | Leaf buds... | 10.16 | 900 | | 700 | ? | — | 680 |
| | 5/5 | Young leaves | 61.3 | 3350 | — | 1370 | 1625 | — | 3220 |
| — II-III | 27/4 | Closed ..... | 3.06 | 254 | — | 192 | 198 | 1.2 | 219 |

Determinations.

| Date | Notes | Sap mM/l = μM/ml | | | | | |
|---|---|---|---|---|---|---|---|
| | | K | Ca | Mg | Mn | P | Sum of kations × 2 |
| 22-23/4 | Branch | 12.1 | 3.2 | 0.85 | 0.11 | ? | 32.5 |
| 28/3-3/4 | — | 2.5 | 2.6 | — | — | 5.15 | 10.2 |
| 1-3/4 | Branch II | 3.7 | 2.2 | — | — | 0.79 | 11.8 |
| 5-7/4 | — | 4.3 | 1.8 | — | — | 1.21 | 12.2 |
| 23/3 | Trunk | 1.1 | 3.9 | — | 0.06 | 0.16 | 10.1 |
| 26/3 | — | 1.3 | 5.2 | — | 0.07 | 0.19 | 13.1 |
| 26/3 | Branch | 1.5 | 0.9 | — | — | — | 4.8 |
| 17/4 | — | 3.6 | 2.2 | — | — | 2.56 | 11.6 |
| 25/4 | — | 4.4 | 4.9 | — | — | 1.68 | 18.6 |
| 3/5 | Trunk | 3.4 | 9.3 | 3.2 | 0.15 | 1.02 | 32.1 |
| 23/3 | Trunk | 1.3 | 7.9 | 1.81 | — | — | 22.0 |
| 26/3 | — | 1.4 | 6.4 | — | — | 0.24 | 15.6 |
| 26/3 | Branch | 0.9 | 2.4 | — | — | 0.92 | 6.6 |
| 6/4 | — | 1.4 | 4.3 | — | — | — | 11.4 |
| 12/4 | — | 0.9 | 6.0 | — | — | 0.18 | 13.8 |
| 12/4 | Trunk | 0.5 | 2.9 | 0.96 | — | 0.50 | 8.7 |
| 29/3-3/4 | Branch | 0.8 | 1.1 | — | 0.02 | — | 3.8 |
| 19-20/4 | — | — | 2.4 | — | — | — | 4.8 |
| 20-23/4 | — | 1.5 | 3.0 | — | — | — | 9.0 |
| 5-7/4 | Trunk | — | 3.3 | — | — | — | 6.6 |
| 5-7/4 | Branch | — | 2.5 | — | — | 0.47 | 5.0 |
| 8-16/4 | II Branch | — | 3.4 | — | — | 0.41 | 6.8 |
| 17/4 | II Trunk | 1.9 | 5.9 | — | 0.02 | — | 15.6 |
| 19-20/4 | III Branch | 2.5 | 3.1 | — | 0.08 | 0.71 | 11.4 |
| 21-23/4 | III — | 4.0 | 4.6 | — | 0.09 | 0.91 | 17.4 |
| 11/4 | l Branch fl. | 1.2 | 3.1 | — | — | 0.57 | 8.6 |
| 12/4 | I Trunk | 1.6 | 6.3 | — | — | — | 15.8 |
| 23/4 | I — | 2.9 | 5.9 | — | — | 1.40 | 17.6 |
| 23/4 | I Branch fl. | 4.5 | 5.5 | — | — | 1.14 | 20.0 |
| 33/4 | I — leav. | 3.3 | 3.3 | — | — | — | 13.2 |
| 27-28/4 | II Low Branch | 3.0 | 4.3 | — | 0.14 | 0.56 | 14.9 |
| 27-28/4 | III High — | 2.3 | 3.0 | — | 0.12 | 0.25 | 10.8 |
| 30/4-1/5 | III Low Branch | 3.3 | 2.8 | — | 0.36 | 0.56 | 12.9 |
| 27-28/4 | III Low — | 3.1 | 0.5 | — | 0.04 | — | 7.3 |

Table 5. Absorption of ions from sap into buds.

| Genus | Dates | Average fresh weight 100 buds g. | Increases for 100 buds | | | | | | | Necessary quantity of sap | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Water g. | K µM | Na µM | Ca µM | Mg µM | Mn µM | P µM | ml/100 buds | ml/g/day |
| Carpinus..... | $^{22}/_{3}$-$^{6}/_{4}$ | 7 | 5 | 550 | —37 | 150 | 240 | —2.8 | **260** | 500 | 4.8 |
| Sap µM/ml..... | $^{26}/_{3}$-$^{6}/_{4}$ | | | 1.2 | 0 | 3.3 | 0 | 0 | c. **0.5** | | |
| Acer III..... | $^{20\text{-}26}/_{4}$ | 48 | 21 | 220 | 0 | 900 | 1200 | 44 | **760** | > 950 | > 3.3 |
| Sap µM/ml ..... | $^{19\text{-}20}/_{4}$ $^{21\text{-}23}/_{4}$ | | | 3.2 | | 3,8 | 0 | 0.09 | **0.8** | | |
| Acer II ..... | $^{17\text{-}23}/_{4}$ | 107 | 85 | **6000** | | 5600 | 2600 | 53 | 4150 | > 6000 | > 9.3 |
| Sap µM/ml..... | $^{8\text{-}16}/_{4}$ $^{17}/_{4}$ | | | **1** | | 4.3 | 0 | 0.01 | 0.2 | | |
| Fagus I Flower buds..... | $^{12\text{-}23}/_{4}$ | 33 | 13.5 | 1000 | | 100 | 500 | 0 | **700** | > 800 | > 2.2 |
| Sap µM/ml..... | $^{11}/_{4}$ $^{23}/_{4}$ | | | 2 | | 4.3 | 0 | 0 | **0.85** | | |
| Fagus I Flower buds..... | $^{23}/_{4}$-$^{3}/_{5}$ | 63 | 30 | 5700 | | 900 | 250 | | **1600** | > 1400 | > 2.5 |
| Sap µM/ml..... | $^{23}/_{4}$ | | | 4.5 | | 5.5 | 0 | | **1.14** | | |

the growing cells take up and concentrate as solutes in their vacuole and protoplasm certain inorganic ions. Most of the water is either not absorbed or is absorbed and again excreted and leaves the buds. The mechanism and channels of this return transport of water is unknown and should be investigated. This power of absorbing ions against the gradient is very common in the vegetable kingdom and probably an attribute of most living cells at one stage or other of their life (Comp. KROGH 1946).

On the assumptions that all the ions found in the buds are dissolved in the water and that the extracellular fluid present at any moment in the xylem system of a bud is a small fraction only of the intracellular we can figure out the ionic concentrations in the cell water by dividing the quantities of ions given in table 4 by the quantity of water also given. The results are presented in table 6 in which we have also added up the kations multiplied by 2 and the soluble sugars to obtain the total osmotic concentration. We have every reason to assume that organic anions are present in sufficient quantity to balance the kations and that the salts of alkalies as well as the sugars are present as solutes and exert their normal pressure, but with regard to Ca, Mg and Mn the point is doubtful. Mn is present in such small quantity that it does not matter, but the actual pressures of Mg and Ca may be significantly lower than the concentrations given.

The table shows on the whole a pronounced fall in total concentration throughout the period of development. Both the sum of ions and the sugars participate in this reduction and it is at least a possibility that the high values found in the early spring is connected with the winter conditions of the buds.

Regarding the concentrations of single ions the following facts should be noted:

Potassium is present in all buds in fairly high concentration. In Syringa the concentration falls during development, especially in the flower buds. In Betula there is little change except for the very low value found in the buds collected on March 15. In Carpinus the concentration rises in the leaves, but not in the flowers, so far as determinations go, but in Fagus the flowers reach a higher concentration than the leaves.

Table 6. Concentration of ions in bud-water mM/l.

| Genus | Date | Notes | K | Na | Ca | Mg | Mn | P | Sum of Kations×2 | Sugar | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Syringa.... | 1/3 | | 208 | 0 | 53 | 26 | 0.2 | 64 | 574 | 174 | 748 |
| | 6/3 | | 115 | 0 | 61 | 32 | 0 | 67 | 416 | 153 | 569 |
| | 15/3 | | 229 | 0 | 48 | 34 | 0.2 | 69 | 622 | 105 | 727 |
| | 6/4 | | 197 | 0 | 29 | 22 | 0.1 | 65 | 496 | 132 | 628 |
| | 20/4 | L..... | 169 | 0 | 39 | 72 | 0.3 | 65 | 560 | 174 | 734 |
| | 20/4 | Fl..... | 103 | 0 | 31 | 35 | 0.1 | 63 | 338 | 135 | 473 |
| Cornus .... | 3/4 | | 93 | 0 | 83 | 50 | 0 | 90 | 452 | 210 | 662 |
| | 12/4 | | 91 | 0 | 94 | 18 | 0 | 88 | 406 | 184 | 590 |
| Betula..... | 2/3 | I..... | 113 | 59 | 280 | 114 | 0 | 93 | 1132 | 274 | 1406 |
| | 6/3 | II..... | 109 | 45 | 241 | 118 | 1.1 | 96 | 1028 | 333 | 1361 |
| | 15/3 | | 58 | 34 | 171 | 59 | 0.5 | 62 | 646 | 288 | 934 |
| | 17/4 | | 101 | 0 | 158 | 45 | 0.5 | 69 | 610 | 229 | 839 |
| | 25/4 | | 91 | 0 | 62 | 41 | 1.0 | 83 | 390 | 118 | 508 |
| | 3/5 | L..... | 82 | 0 | 49 | 34 | 0.2 | 64 | 330 | 76 | 406 |
| Carpinus .. | 22/3 | | 76 | 18 | 163 | 59 | 1.4 | 60 | 634 | 120 | 754 |
| | 6/4 | | 100 | 0 | 69 | 52 | 0 | 55 | 442 | 80 | 522 |
| | 12/4 | | 106 | 0 | 54 | 50 | 0 | 57 | 420 | 106 | 526 |
| | 23/4 | L..... | 114 | 0 | 75 | 79 | 0 | 69 | 536 | 44 | 580 |
| | 23/4 | Fl..... | 70 | 0 | 34 | 37 | 0 | 35 | 282 | 84 | 366 |
| Corylus ... | 3/4 | | 90 | 0 | 152 | 63 | 3.4 | 46 | 616 | 134 | 750 |
| | 17/4 | | 91 | 0 | 96 | 79 | 3.5 | 59 | 540 | 112 | 652 |
| | 25/4 | L..... | 111 | 0 | 85 | 74 | 4.7 | 67 | 550 | 110 | 660 |
| Acer..... | 5/4 | I..... | 102 | 0 | 151 | 56 | 0.7 | 61 | 620 | 72 | 692 |
| | 17/4 | II..... | 113 | 0 | 48 | 21 | 0.4 | 53 | 364 | 55 | 419 |
| | 19/4 | | 102 | 0 | 40 | 26 | 0.3 | 56 | 336 | 27 | 363 |
| | 23/4 | L..... | 85 | 0 | 52 | 28 | 0.5 | 50 | 332 | 27 | 359 |
| | 20/4 | III.... | 101 | 0 | 69 | 22 | 1.1 | 57 | 386 | 39 | 425 |
| | 26/4 | | 63 | 0 | 78 | 36 | 0. | 48 | 354 | 33 | 387 |
| Fagus ..... | 15/3 | I Fl.. | 80 | 0 | 171 | 107 | 0 | 71 | 716 | 182 | 698 |
| | 12/4 | | 56 | 0 | 53 | 32 | 0 | 55 | 282 | 159 | 441 |
| | 23/4 | | 65 | 0 | 33 | 35 | 0 | 53 | 266 | 108 | 374 |
| | 3/5 | | 127 | 0 | 31 | 22 | 0 | 54 | 360 | 125 | 465 |
| | 23/4 | L ..... | 90 | 0 | 70 | ? | 0 | 67 | .. | 121 | .. |
| | 5/5 | | 55 | 0 | 22 | 26 | 0 | 53 | 206 | 99 | 305 |
| | 27/4 | II-III.. | 83 | 0 | 63 | 65 | 0.4 | 72 | 422 | 125 | 547 |

Sodium is present only in the early developmental stages in Betula and Carpinus.

Calcium and magnesium are always present, but the concentrations very variable though on the whole falling during development.

The concentration of manganese is always low and often below the determinable minimum. It would appear that the plants from the park (Corylus, Acer and Fagus II and III) show higher values than the rest, but whether this is due to a possibly higher concentration in the soil we do not know.

Phosphorus is by far the least variable constituent of the buds. The lowest figure found is 35 mM in the catkins of Carpinus and the highest 96 in Betula buds from March 6, but most values for the single species are remarkably constant viz. Syringa 63—69, Cornus 88—90, Carpinus (excepting the catkins) 55—69, Acer 48—61, Fagus about 70 in the earliest stages, but later 53—55. (The phosphorus is present partly as organic phosphates of fairly small molecular weight and of first rate importance in the metabolic processes.)

As stated in the introduction to the present paper the study was undertaken on the supposition that the bleeding sap is the source of supply of nutrient material and ions for the buds. This supposition has not so far been definitely shown to be correct. It is admittedly very difficult to imagine a mechanism by which the flow of sap through the buds, which the analyses and calculations shows to be necessary, can be brought about.

Experiments are not lacking (Curtis 1935, Mason et al.), which tend to show that the phloem may be of more importance to the supply of nutriment to buds than the xylem.

If the supply is to take place from the phloem, without a recurrent flow of water we would have to assume

1. that the sap, present at a positive pressure only during the period of bud development, is nevertheless of very little if any importance to the developing buds except perhaps as a source of water supply,

2. that all the necessary substances viz. sugars, soluble nitrogenous substances, and ions become stored in the autumn in appropriately high concentrations in the phloem or cortex very

near to the buds, mobilized and transported up through the sieve tubes during the spring development.

While we find it very difficult to accept these assumptions as probable, a combination of both ways of supply is certainly within the range of possibilities, and we have planned experiments for the spring of 1946 to study these problems.

## Summary.

During the period of development in March and April samples were taken of buds from a small number of trees and shrubs and analysed for sugars, nitrogen and the ions K, Na, Ca, Mg, Mn and P. Samples were taken within the same period of bleeding sap from trunks and branches and likewise analysed. Sugars were present in all samples of sap, with a preponderance of hexose except in Acer. Organic nitrogen was regularly present in low concentration. Most of the ions were usually present in low concentration, but Na below the treshold in all cases. Quantitative results are given in the tables.

Even before any significant assimilation could take place the buds increased considerably in dry substance, nitrogen and most of the ions, but Na and Mn disappeared, when present initially.

Quantitative comparisons between the increases in carbohydrate, protein and ions, determined in the buds and the sap assumed to supply them, show that large quantities of sap are necessary, amounting to 2—10 ml./g. fresh bud/day, from which the growing cells take up the substances needed, while the water must be largely returned to the branches. This conception, which involves an active transference of sugars and ions into the cells, is discussed. The total concentration of osmotically active substances in the water of the buds is very high at the end of winter and decreases more or less regularly during development.

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Indleveret til Selskabet den 21. December 1945.
Færdig fra Trykkeriet den 23. Marts 1946.