Botany. — *On the transport of introduced nitrogenous substances in the leaves of Vallisneria spiralis.* By W. H. ARISZ and J. OUDMAN [1]). (Communicated by Prof. J. C. SCHOUTE).

(Communicated at the meeting of April 24, 1937).

§ 1. *Introduction.*

The object of this investigation is the quantitative determination of the transport in leaves of Vallisneria spiralis. The BIERBERG experiments with regard to the influence of the protoplasmic streaming on the transport in the leaves of this plant were repeated at this laboratory by A. KOK. She found that caffeine and lithium salts taken up by the leaf from outside show a retarded diffusion. No acceleration caused by the protoplasmic streaming was demonstrable. The transport substances employed were substances which do not naturally occur in Vallisneria leaves, whilst the method of analysis used did not admit of a quantitative determination. In view of our experiments with Drosera tentacles, in which an accelerated transport for asparagine with a different course from the transport of caffeine was found, it seemed advisable to investigate quantitatively the transport of asparagine and caffeine in the case of Vallisneria leaves also. Asparagine is so suitable for transport tests owing to its being a substance which in natural conditions may occur in a plant.

§ 2. *Method of Investigation.*

The method employed in these investigations is based entirely on that of A. KOK with Vallisneria and on that of our Drosera tests. In the experiments of A. KOK, pieces of Vallisneria leaves took up from agar, in which the transport substance was to be found, the dissolved substance to a sufficient extent at one extremity (KOK, fig. 3, p. 54). In our experiments the transport substances used were asparagine and caffeine, both nitrogenous substances, which were applied in a 2 % agar. The concentration of both substances in all the experiments was 0.05 mol. It was possible to demonstrate absorption and transport by the increase in the nitrogen content of the leaves. All nitrogen determinations were carried out by the micro-Kjeldahl method with the Parnas-Wagner apparatus. An increase of nitrogen is only to be determined with sufficient accuracy if the nitrogen content of the leaf is known. Orientation tests showed the nitrogen content of the leaf not to be equal in all parts. The

[1]) Dr. OUDMAN wishes to acknowledge his indebtedness for a grant of the „Stichting tot verruiming van werkgelegenheid voor academisch gevormden".

content is greatest at the top, and gradually decreases towards the base, the greatest differences being more than 50 %. In order to eliminate these differences in one and the same leaf and those between various leaves, the following plan was adopted. (Cf. fig. 1). Leaves of suitable length

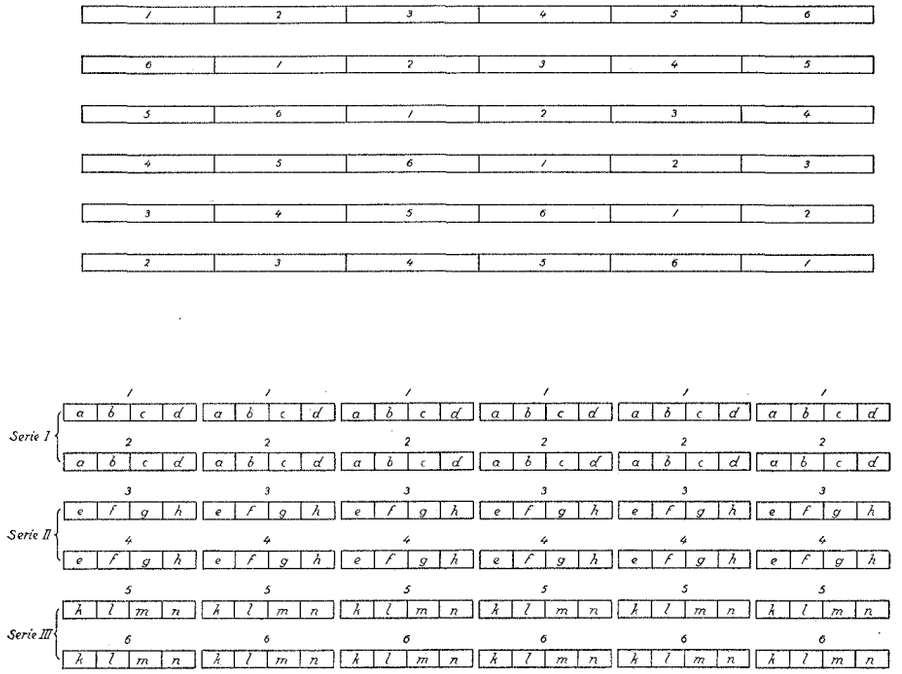

Fig. 1. Method of combination of pieces of leaves in order to eliminate the variability between the leaves and between different parts of a single leaf.

were cut off, divided into 6 equal parts of 3,5 cm. and numbered as shown in fig. 1. 6 Leaf pieces no. 1 together give a combination from all parts of the leaf, top, middle, and base, but at the same time composed of 6 different leaves. One series is formed by two such combinations of 6 leaf pieces. Series consisting of 12 of such leaf pieces still have too great a variability, which is caused by the leaves differing in breadth from one another and by their further being broader at the top than at the base. For this reason the leaves were cut off along the sides to a uniform breadth of 4 mm.

It was necessary for the transport experiments to apply the transport substance at one extremity of the leaf piece and to determine how the nitrogen of the rest of the leaf increased. For this purpose the 3.5 cm. long pieces were transversely divided into small pieces of 8 mm. by means of a brass plate in which grooves had been made, through which a razor-blade could be moved. Narrow strips were cut away at both extremities, so that 4 small pieces of 8 mm. came from one leaf piece (see fig. 1),

the small pieces *a*, *b*, *c*, *d* from series I, the small pieces *e*, *f*, *g*, *h* from series II, and the small pieces *k*, *l*, *m*, *n* from series III. In some experiments longer leafpieces were investigated; these, also, were divided into small pieces of 8 mm. The first series of 12 leaf pieces thus yields 12 small pieces *a*, 12 small pieces *b*, etc.; the second series 12 small pieces of *e*, *f*, etc., the third series 12 small pieces of *k*, *l*, etc. In each case 12 small pieces together were analysed to find their content of nitrogen. Series I served as check, series II and III served for transport tests; the strength of absorption and transport was found by determining the difference in nitrogen of the pieces of series II and III with the average amount of nitrogen in the pieces of series I. The application of the transport substances asparagine or caffeine took place in agar 2 %. Plates of about 3 mm. thickness were cast of this, and these were cut into strips of 8 mm. broad. With the experiments carried out later, the ends of the leaf pieces projected somewhat out of the agar, as they were found in that case better to remain turgescent (cf. p. 444). Both on the lower and on the upper side a strip of agar of this kind was applied over the first zone of 8 mm. of the leaf (see fig. 2). The other extremity of the leaf was in

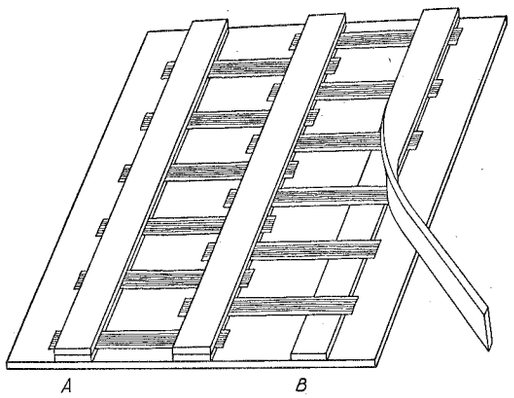

Fig. 2. Pieces of leaves of Vallisneria spiralis laid between agar strips for the diffusion tests. The agar strips A and B contain the transport substance.

contact with a strip of pure agar on the upper and lower side. On the conclusion of the transport experiment the leaf pieces were washed and divided as indicated above into pieces 8 mm. in length.

The reliability of the method described is satisfactory. The differences in the amount of nitrogen of the pieces *a*, *b*, *c*, *d* is at the most from 3 to 4 %. With experiment 32, for instance the amount is in γ nitrogen 144, 148, 142, 146, average 145 γ, with experiment 33 in γ nitrogen 138, 138, 134, 138, av. 137 γ, and with experiment 35 144, 138, 138, 142, av. 140.5 γ. With experiment 34 longer leaf pieces were divided into 7 small pieces of 8 mm. The checks not treated (8 small pieces) contained 110, 110, 108, 110, 112, 112 and 114 γ nitrogen. A difference greater than

6 γ nitrogen therefore indicates a transport in the leaf. As one series consists of 12 small pieces, the demonstrable increase per small piece is only $\frac{1}{2}$ γ.

The experiments were carried out from the month of November 1936 to March 1937. The plants grew much more vigorously in February and March.

§ 3. Transport tests with asparagine and caffeine.

A commencement was made with leaf pieces of 35 mm., from which 4 pieces of 8 mm. were cut. The experiments were carried out in a room in the laboratory in daylight. The leaves were cut into pieces, after which they were allowed to remain from 15 to 20 minutes in a Petri dish on filter paper and arranged for the transport tests in the manner indicated, in glass boxes with a diameter of 12 cm. and a cubic capacity of 500 cc., in which the air was saturated with water vapour. The boxes were placed during the transport in a room with a constant temperature of 25° C. in the dark.

The results of the asparagine tests are summarized in table 1. With each test the number is given. Tests with the same number are parallel tests, which are distinguished by the addition of A. B. C. etc. The duration of the test is shown, and usually amounts to from 18 to 24 hours. The increase of nitrogen is expressed in γ, and is calculated on a leaf surface of $12 \times 8 \times 4$ mm^2. $= 384$ mm^2. In some cases (tests 27, 34 and 42) 8 leaf pieces were analysed. These values have been converted to the same area as in the other experiments.

From table I it is seen that the total absorption of nitrogen in the leaf

TABLE I. Transport of asparagine 0.05 mol. at 25° C. in the dark. Amount of nitrogen taken up by pieces of leaves in γ. Pre-treatment 15—20 minutes in water with filter paper.

Number of experiment	15 A	21 A	24	26	30	34
Time of transport in hours	22 h.	22 h.	23 h.	24 h.	22 h.	22 h.
First piece of 8 mm.	85	96	54	82	86	73
Second piece of 8 mm.	41	48	34	36	52	52
Third piece of 8 mm.	31	40	18	16	20	25
Fourth piece of 8 mm.	39	48	34	26	30	19
Total amount	196	232	140	160	188	169

pieces was considerable, 140—232 γ nitrogen, i.e. 658—1090 γ asparagine. The distribution of the nitrogen over the leaf is not quite according to expectations. One would expect most would be found in the first piece which is in direct contact with the asparagine, and that the amount of nitrogen would gradually decrease towards the other end. It is true that the nitrogen increase is in all cases greatest in the first piece,

but the decrease is not gradual, since the last piece, except in experiment 34, shows a greater nitrogen increase than the last but one. Experiment 34 was carried out with longer leaf pieces of 60 mm., from which 7 small pieces of 8 mm. were cut; the last 3 small pieces showed no increase of nitrogen. This indicates that the accumulation in the other experiments in the last piece is a marginal effect. By means of a modification in the method we succeeded in eliminating this marginal effect at any rate partially. In this first series of experiments the last piece of the leaf had its edge entirely surrounded by the agar, so that the intercellular tubes, which had been opened by the cutting, were closed. Leaves of this kind showed some tendency to infiltration of the intercellulars and slighter turgescence of the parenchym. The phenomenon was especially strong when both ends were altogether in agar. In the later experiments (experiment 36 et seq.) we therefore always left the edge of the leaf pieces projecting somewhat from the agar. (cf. fig. 2). The leaves then retained their turgescence better, and the marginal effect was less noticeable. In so far as this marginal effect was due to injury it was impossible to eliminate it, unless the experiment was postponed until the effect of the injury had disappeared.

The transport experiments with caffeine had a somewhat different course. Table II gives a summary of these tests. Tests 15 A and B are

TABLE II. Transport of caffeine 0.05 mol. at 25° C. in the dark. Amount of nitrogen taken up in γ.

Pre-treatment	15—20 min. water-filter paper		$2^1/_2$ h.	4 h.
Number of experiment	15 B	21 B	20 B	23 B
Time of transport in hours	22 h.	22 h.	18 h.	$19^1/_2$ h.
First piece of 8 mm.	112	138	84	175
Second piece of 8 mm.	49	52	34	71
Third piece of 8 mm.	9	8	—	—
Fourth piece of 8 mm.	—	18	—	—
Total amount	170	216	118	246

parallel tests. It is a striking fact that caffeine is well absorbed, but less strongly transported; experiments 21 A and B also give a similar result, but here the diffusion of caffeine was somewhat stronger, so that here, too, the marginal effect occurs.

The result of the above tests is therefore that caffeine and asparagine are both absorbed by the Vallisneria leaf from agar, but that the transport of asparagine is somewhat different from that of caffeine, being less pronounced in the case of caffeine.

In this publication we will further occupy ourselves exclusively with the transport of asparagine. The influence of the pretreatment of the

leaf pieces on the subsequent transport was investigated, whilst a few orientation tests were made on the effect of temperature at which transport takes place. It was further determined to what extent the transport is polar. In all the experiments, with the exception of test 27, the direction of transport was from the top to the base of the leaf.

§ 4. Influence of the pretreatment on the transport of asparagine.

The tests summarized in table I agree well with one another. As it happened, however, the time that the leaf pieces remained in trade distilled water before the setting up of the experiment was considerably lengthened in the case of one experiment. In this case a much slighter transport was found. It was this which led us to make a more detailed investigation of the influence of the previous treatment. Table III contains

TABLE III. Transport of asparagine 0.05 mol. at 25° C. in the dark. Amount of nitrogen taken up in γ.

Pre-treatment hours in	Dist. water with filter paper						
	2 h.	2 h.	$2^1/_2$ h.	3 h.	4 h.	4 h.	4 h.
Number of experiment	33 A	40 A	20 A	31	23 A	32	33 B
Time of transport in hours	21 h.	$21^1/_2$ h.	18 h.	19 h.	$19^1/_2$ h.	19 h.	21 h.
First piece of 8 mm.	49	68	38	136	37	155	87
Second piece of 8 mm.	25	38	10	74	15	39	39
Third piece of 8 mm.	—	20	—	74	—	11	25
Fourth piece of 8 mm.	—	12	—	84	—	7	19
Total amount	74	138	48	368	52	212	170

the results of tests in which the leaf pieces remained for from 2 to 4 hours in trade distilled water on filter paper before being used for the transport tests. The results of these tests show a deviation from those of table I. With a short pretreatment the transport is less strong. With about 4 hours in water the transport in the case of test 23 was slight, with the others, however, it was strong, even stronger than with the tests described in table I.

In order to enable a closer study of this phenomenon to be made, the leaf pieces were put during the pretreatment into tap water distilled over glass, and came into contact with filter paper only when being dried. The duration of this pretreatment was from 20 minutes to 19 hours. Table IV contains the results of these tests. The results are much more regular than those of table III; the longer the pretreatment lasts, the stronger is the transport. The marginal effect mentioned in the previous § has here practically disappeared, owing to the modification of method, except in the case of the tests with short treatment in water, tests 41 and 39 A and B. Test 44 (table IV) was carried out with somewhat longer leaf pieces, from which 5 small pieces were cut.

Test 42 A shows a very strong absorption and transport in table V. In this case leaf pieces of 60 mm. were divided into 7 pieces of 8 mm.

TABLE IV. Transport of asparagine 0.05 mol. at 25° C. in the dark. Amount of nitrogen taken up in γ.

Pre-treatment hours in pure water	20 min.	1 h.	2 h.	2 h.	4 h.	4 h.	12 h.	19 h.
Number of experiment	41 C	39 A	39 B	40 B	39 C	41 A	41 B	44 A
Time of transport in hours	24 h.	21 h.	21 h.	21½ h.	21 h.	24 h.	24 h.	24 h.
First piece of 8 mm.	61	90	118	92	136	99	133	107
Second piece of 8 mm.	19	38	74	56	66	61	83	71
Third piece of 8 mm.	19	16	24	24	42	39	39	37
Fourth piece of 8 mm.	23	20	34	20	34	29	33	29
Fifth piece of 8 mm.								19
Total amount	122	164	250	192	278	228	288	263

Altogether 556 γ nitrogen was absorbed. A remarkable feature is the very gradual decrease in the transport from the point of absorption.

In connection with FITTING's investigations of chemodinesis in Vallisneria the influence of a pretreatment of 45 minutes and of one of 4 hours in a 0,00001 % asparagine solution was determined. With the first test a total of 170 γ nitrogen was absorbed, distributed over 4 leaf pieces 90, 34, 14, 32; with the second test 184 γ nitrogen was taken up, distributed as follows: 87, 35, 27, 35. With both tests in the leaf pieces pre-treated with asparagine 0.00001 % the same amount or somewhat less was absorbed than in the checks, which were pretreated with distilled tap-water.

§ 5. Influence of temperature on transport.

Only a few orientation tests can here be reported; they are summarized

TABLE V. Influence of temperature on transport of asparagine 0.05 mol. Amount of nitrogen taken up in γ.

Temperature of transport	5° C.	5° C.	25° C.	35° C.
Number of experiment	44 B	43	42 A	42 B
Pre-treatment hours in pure water	19 h.	18 h.	18 h.	18 h.
First piece of 8 mm.	49	37	178	196
Second piece of 8 mm.	19	—	106	127
Third piece of 8 mm.	13	5	70	106
Fourth piece of 8 mm.	—	—	58	100
Fifth piece of 8 mm.	—	8	49	77
Sixth piece of 8 mm.			49	64
Seventh piece of 8 mm.			46	55
Total amount	81	50	556	725

in table V. At 5° C. there is a distinct absorption in the first leaf piece, but the transport is slight; at 35° C. the transport is still greater than at 25° C. in the parallel test just discussed. The total amount here accumulated by a leaf surface of 2688 mm². in 18 hours is 725 γ nitrogen i.e. 3.4 mgr. asparagine. Of this ¾ was transported to other parts of the leaf, whilst the absorption took place through $^1/_7$ of the total leaf area, that is 384 mm².

§ 6. Influence of polarity on transport.

From test 27 described below it is seen that the transport is not polar. Leaf pieces 60 mm. long were placed with the middle zone between two agar strips 8 mm. broad, containing asparagine. Transport of the asparagine taken up in the middle of the leaf piece took place towards both ends. The pre-treatment of this test was from 15 to 20 minutes in trade distilled water with filter paper. The transport lasted 22 hours. After this time there were 84 γ nitrogen in the middle zone of the leaf, in the zones towards one end 60, 51, and 54 γ, and in the one towards the other extremity 69, 42, and 63 γ.

§ 7. Discussion of the results.

It has been shown in the above that a transport may take place in the cut-off leaf pieces of a Vallisneria leaf, and that this can be determined quantitatively with the aid of the Micro-Kjeldahl analysis method. The transport is not polar and dependent on the temperature during the transport and on the previous treatment. The question as to whether the asparagine absorbed is transported unchanged in the leaf, or whether it is transformed, is left out of discussion here.

With the preliminary experiments it was found that asparagine is transported better than caffeine, and that the accumulation in the leaf from the point of absorption decreases much more gradually with the asparagine experiments than with the caffeine experiments. Consequently asparagine is better transported than caffeine.

With longer leaf pieces a stronger absorption was found than with shorter ones. This points to the absorption being increased by stronger transport.

The principal question to be discussed here is which channels the transport takes place through. With the experiments of A. KOK the possibility was discussed that substances might be transported through the numerous air-channels of the leaf. She was able to show by experiments that no liquid can be pressed through the air-channels even under pressure. (KOK p. 70). SOLEREDER states that diaphragms consisting of one cell-layer occur in the channels with small intercellulars. It is extremely improbable that transport is possible through these partitions. Nor are the results of our experiments in agreement with the hypothesis of a transport through the air-channels. The transport is best precisely when

the air-channels are filled with air. The strong influence of the temperature, the difference in behaviour of caffeine and asparagine point to the cooperation of living cells. We also consider a transport along surface layers outside the living cells to be out of the question.

To what extent the transport occurs through particular tissue-elements cannot, however, be elucidated on the strength of the above experiments. The bundles of the Vallisneria leaves are but slightly differentiated. There are no water conducting vessels whatever.

The question as to whether there is any connection between protoplasmic streaming and the transport found cannot be discussed here either. It is remarkable in this connection that the pre-treatment has a great influence, but a previous treatment with substances which cause a rotation of protoplasm, such as filter paper, trade distilled water which may contain copper, and asparagine yields no appreciable result. The longer the leaves have remained in pure water during the pre-treatment, the more strongly they transport. This will partly depend on the recovery of the leaf after the injury. FITTING also found that the streaming of protoplasm excited in the cut-off leaf pieces gradually comes to a standstill, but it is certain that the state of turgescence also has something to do with it. After a long pre-treatment in pure water the leaf pieces were found to remain turgescent better during the transport tests than after a short pre-treatment. The infiltration of the air-channels too was slighter in that case. It is remarkable that BOTH also found in his transport tests in simple systems in the case of Impatiens Marianae, that the transporting leaf has to be turgescent in order to be able to transport substances to the bundles.

With transport tests with the tentacles of Drosera we also found a difference in behaviour between asparagine and caffeine. Asparagine is there probably transported by the protoplasm, whilst caffeine penetrates into the vacuole. The data hitherto obtained with regard to the transport in the case of Vallisneria may perhaps indicate that the transport of asparagine with these plants also is an accelerated transport in protoplasm.

Summary.

With the aid of the Micro-Kjeldahl analysis method the transport of asparagine and caffeine was investigated in the case of Vallisneria spiralis. A method was worked out to make the individual differences of the leaf pieces in nitrogen content so small that transport of nitrogenous substances containing more than 6 γ nitrogen ($\frac{1}{2}$ γ per small piece) could be demonstrated. In successive pieces of 8 mm. in length the nitrogen uptake decreases gradually from the point of absorption; for asparagine the transport is stronger than for caffeine.

It was possible to eliminate a marginal effect.

The following factors were found to have an influence on the transport of asparagine. 1°. the temperature during the transport, 2°. the pre-treatment of the leaf pieces before the transport begins; the injury by cutting the leaf, impure water and the length of time for which the leaf pieces remain in pure water are of influence, 3°. the length of the leaf pieces in which the transport takes place.

It is probable that the transport found takes place in living cells and is comparable to the transport demonstrated by us in the tentacles of Drosera.

Groningen, April 1937. *Laboratory for Plant Physiology.*

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Palaeontology. — *Sur une espèce nouvelle du genre Sabinia (Caprininés).* Par L. A. H. BOUWMAN. (Communicated by Prof. L. RUTTEN.)

(Communicated at the meeting of April 24, 1937).

Au bout de l'année 1935 M. H. KUGLER, géologue à Trinidad, envoya à l'Institut Géologique de l'Université d'Utrecht une collection de fossiles, provenant de Point à Pierre (Trinidad).

Ces fossiles ont été recueillis d'un bloc isolé d'un conglomérat calcaire. La collection se compose de Rudistides pour la plupart très fragmentaires et, en outre, de quelques fragments de coraux, de *Nerinea* et d'une (?) *Ostrea,* pas encore indentifiés.