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On the metabolic aspects of the transpiration of leaves

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INTRODUCTION

In the paper „The changes in distribution of water in plants as a result of the absorbtion of either nitrates or ammonium salts" (Buczek 1963) has been found that in poorly aerated media plants that absorb nitrates show an increased absorbtion of water by the roots and a more intensive transpiration, than the control plants, while in plants grown only on ammonium salts the blockade of both processes has been observed.

In accordance with the earlier papers of Arnon (1937), Gumiński and col. (1957), Stabrowska (1959) and Poskuta (1961) who have shown that in bad aerobic conditions nitrates are able to compensate the lack of oxygen in roots in some plants, the author has constructed a following hypothesis: The mentioned above differences in the distribution of water, result from the ability of NO_3 ions to compensate the lack of oxygen; thus in bad aerobic conditions the plant may obtain some energy that can be used up for processes of the absorbtion and transport of water.

On the basis of the results obtained in previous experiments a question rises, namely, whether the process of transpiration in leaves, provided that the root pressure is excluded, depends on the form of nitrogen source, and whether this possible relation has a metabolic aspect.

The experiments have been carried out on the cut off leaves by measuring the transpiration with the method applied by Gessner and Scumann-Peterson (1948). This method is a modification of the measurements of the transpiration widely applied by Arland and his school (1959). The above method has been chosen in order to exclude the possible influence of root pressure on the process of transpiration in leaves.

THE DESCRIPTION OF EXPERIMENTS

EXPERIMENT I

The effect of either nitrates or ammonium salts on the transpiration.

Methods

The experiments have been carried out on the leaves of *Tradescantia virginiana* grown up in flowerpots in a green house, and on the leaves

of *Malus purpurea hybr.* taken from Botanical garden in Wrocław. The leaves of both plants are hypostomatic and therefore are very convenient as well for the investigations of stomata transpiration (lower part of the leaf), as for investigations of cuticular transpiration (upper part of the leaf).

Tradescantia: Stems of the same age with at least 12 leaves have been taken for the experiment. Two youngest leaves were neglected and the remaining ones were cut off under water by leaving a small part of stem to each leaf. The leaves were put into suitable solutions for 24 hours, so that only ends of stems were immersed. This infiltration was carried out in day light in a chamber with constant humidity. In the next day the remaining parts of stem were removed and the leaves were placed on plates with investigated solutions in such a way that only a small part of each leaf (proximal to the stem) was in contact with the liquid. The plates with leaves were pat under a glass bell for 2 hours in order to assure the maximal saturation with water. Afterwards the leaves were weighted on a torsion balance with an accuracy up to 0.5 mg and after the initial fresh mass was determined the leaves were hanged on fine wire in a chamber with constant humidity and intensity of the light. The weighings took place in one hour intervals, and from the difference between the initial and successive measurements the amount of transpired water was computed. For the measurements of either stomata or cuticular transpirations the upper or lower parts of leaves, were covered with vaseline respectively. Further measurements were carried out by method used to determine the total transpiration.

Malus: The leaves were cut off from the twigs under the water. The leaves chosen so that their surfaces be possibly equal, were put by petioles into the suitable solutions. Their transpiration was determined by the same method as in experiment with *Tradescantia.*

Measurements of transpiration for both kinds of plants were carried out in a chamber, with constant relative humidity equal to 60—70 %, constant intensity of light — 7000 lx (for the lighting the usual electric bulbs were applied. The intensity of light was measured with a standard luxometer made by Carl Zeiss, Jena). The results are the mean values obtained from 10 replications made for each combination, and calculated per 100 mg of fresh mass per 1 hour.

Computed from the following formula:

$$s = \pm \sqrt{\frac{\Sigma (f)^2}{n}}$$

Before the measurements the leaves were kept in the following media:

a) medium without nitrogen: KH_2PO_4 — 0.127 g, $MgSO_4$ — 0.284 g, KCl — 0.490 g, $CaCO_3$ — 0.412 g, $FeCl_3$ — 0.112 g;

b) medium with nitrates: $Na_2HPO_4 \cdot 12H_2O$ — 0.335 g, $MgSO_4$ — 0.284 g, KNO_3 — 0.759 g, $Ca(NO_3)_2$ — 0.711 g, $FeCl_3$ — 0.112 g;

c) medium with ammonium salts: $(NH_4)_2SO_4$ — 0.943 g, $(NH_4)_2HPO_4$ — 0.124 g, $MgSO_4$ — 0.284 g, K_2SO_4 — 0.653 g, $CaCO_3$ — 0.412 g, $FeCl_3$ — 0.112 g.

To each medium microelements from A—Z Hoagland's solution have been added. The possible oscillation of pH have been compensated by 0.1 n KOH and 0.1 n H_3PO_4 up to pH = 6.8.

Discussion of the results of experiment I

The results of experiments carried out on the leaves of *Tradescantia* are presented on the diagram 1. Diagram 2 shows the results obtained for *Malus*.

Results from the described experiments may be formulated in following items:

1. The saturation of leaves with the solution containing nitrates had increased the transpiration in both investigated plants, when

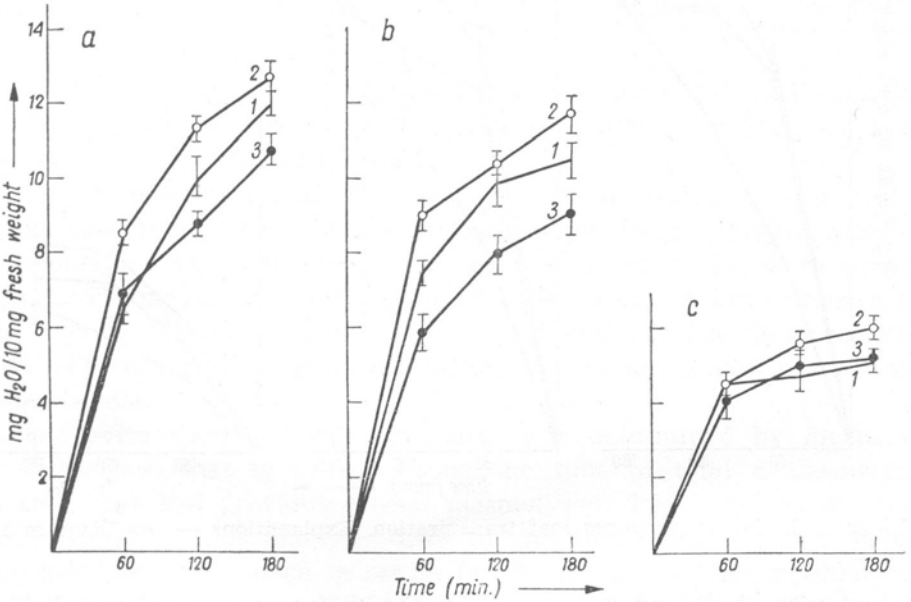

Diagram 1. *Tradescantia* leaf transpiration

a — total transpiration; *b* — transpiration of lower surface of leaf; *c* — transpiration of upper surface of leaf; *1* — medium without nitrogen; *2* — nitrate medium; *3* — ammonium medium.

compared with the control. This increased loss of water was observed in the total transpiration as well as in the transpirations of the upper and lower part of the leaf blade.

2. Ammonium salts have inhibited all the types of transpiration in *Malus*. However, in *Tradescantia* leaves, only total transpiration and

that of the lower part of the leaf have been inhibited, while the cuticular transpiration (the one of upper part of the leaf) have not been affected.

The above observations show a significant difference in transpiration of the tested leaves, if these leaves were infiltrated with media containing various forms of nitrogen. The saturation of leaves with nitrates has markedly increased the loss of water in the process of transpiration, while ammonium ions present in leaves have distinctly blocked it.

The observed differences in the process of transpiration affected by various form of nitrogen may be explained either by the opposite effect of nitrates and ammonium salts on permeability of the cytoplasm

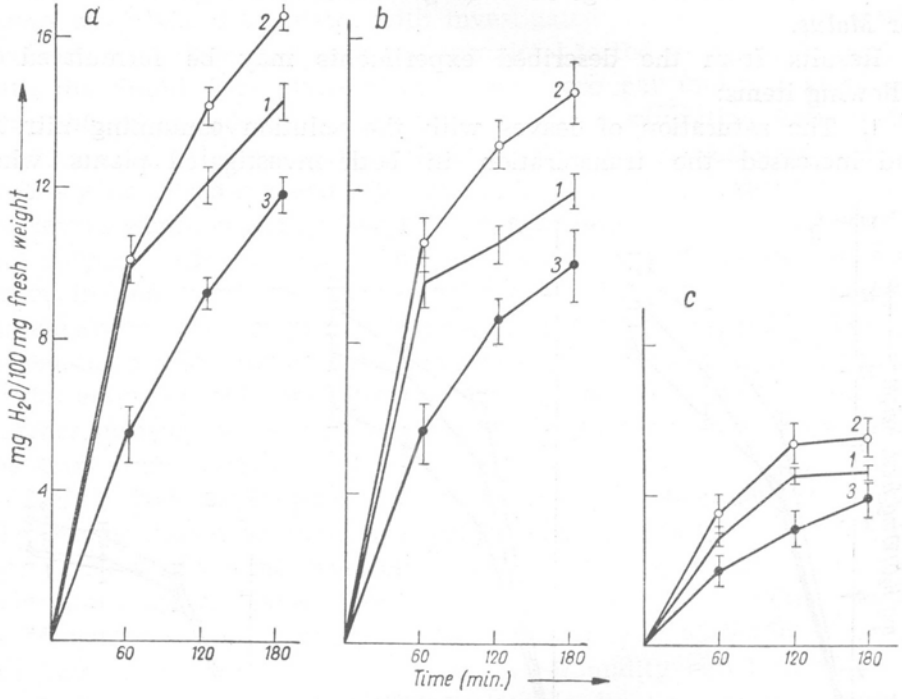

Diagram 2. *Malus purpurea* leaf transpiration. Explanations — see Diagram 1

of leaf mesophyll and cuticle, or by the different type of metabolism affected by either oxidised or reduced form of nitrogen present in the cells. There is, however, third possible explanation of this phenomenon. The increased loss of water in the process of transpiration affected by NO_3 — ions may be due either to the better water passage through the xylem of leaves or to the more intensive moistening of cellulose cell walls. They both might follow from the differences in viscosity of the applied media. As this three possible factors may influence the distribution of water in transpiring leaves, the further experiments were

carried out in order to solve this problem. In the case of stomata transpiration, however, one should also take under consideration the movements of stomata.

EXPERIMENT II

The measurements of the viscosity and permeability of cell cytoplasm by deplasmolysis method

The viscosity of the investigated solutions was measured by means of Osvald's viscosimeter in 20.2°C. The viscosity of the following media with regard to the distilled water have been measured:

Viscosity in 20,2°C	H_2O distilled	Solutions of media		
		without nitrogen	with NO_3-ions	with NH_4-ions
	0.010000	0.010042	0.009965	0.010051

From the given data it follows that the solutions of media applied in our experiments have almost the same coefficient of viscosity close to the value for distilled water.

The measurements of the velocity of infiltration of the tested media have been carried out on chromatographic paper Whatman No. 1. The ends of the paper strips of equal length and width were dipped into media and covered with glass bell. The time of infiltration from the starting till the terminal points was measured. The measurements have not shown any significant differences in infiltration for all the tested media.

The permeability of cell cytoplasm was determined by means of deplasmolysis, that is by measuring the time of total deplasmolysis in cells that had previously been plasmolysed. The experiments were carried out on 5 mm diameter discs cut out from leaves by cork borer. The discs were immersed in media for 24 hours, and then plasmolysed in 0.8 M solution of mannit during 1 hour. Afterwards the discs were transferred to distilled water and the time of total deplasmolysis was determined. The measurements of deplasmolysis were carried out under microscope. All cells seen under the low power microscope magnification were taken under consideration. The experiment was carried out on slices made from: *Tradescantia zebrina, Tradescantia viridis, Tradescantia atropurpurea* and on epidermis slices of *Alium cepa.*

On neither of the tested objects any significant difference in deplasmolysis time have been found.

As an example the results for *Allium cepa* slices are given below:

Type of medium used for immersion	Media		
	without nitrogen	with NO_3-ions	with NH_4-ions
The average time of deplasmolysis in minutes	2.36.0	2.26.4	2.25.7

In order determine the possible influences of media (with nitrates, with ammonium salts and without nitrogen) on the stomatal movements in the tested leaves, some direct observations (under microscope) of stomata have been carried out in course of experiment, and the rate of stomata width has been found by infiltrating the leaves with benzene, and castor oil with terpentine in ratio 1 : 2. Both, direct observations, and infiltration methods have not shown any significant differences in the width of stomata. It is evident that the width of stomata was always maximal in first hour of measurements and in course of time the size of stomata was gradually dimishing, but the rate of these changes was the same in all tested combinations.

The above observations have excluded the possibility of indirect effect of various forms of nitrogen on the transpiration through out the influence on stomata.

In the former paper (Buczek 1963) the author has proved, that the plants grown on media with nitrates transpired much more water than the same plants growing on media with ammonium salts. These differences were observed in poorly or non aerated media. This phenomenon, becomes clear if we assume that when molecular oxygen is locking the roots of plants can utilize oxygen contained in NO_3-ion. As it follows from the experiments the differences in transpiration in isolated leaves due to nitrates or ammonium salts are the same as in whole plants. In experiment with isolated leaves, however, the lack of oxygen has not been taken into consideration. In order to find whether the transpiration depends on the respiration of leaves, the following experiment with respiratory inhibitors was carried out.

EXPERIMENT III

Effects of respiratory inhibitors and stimulators on processes of transpiration in *Tradescantia* and *Malus purpurea* leaves

Methods

Leaves of *Tradescantia* and *Malus purpurea* prepared in the way described in experiment I were put for 24 hours into the media under investigation, then the measurements were carried out. The measure-

ments of transpiration were carried out in the same way as in the former experiment. The results are mean values from ten replications made for each combination and calculated per 100 g of fresh mass. The following solutions prepared with tap water were tested:

Solution of respiratory inhibitors:

a) KCN 10^{-3} M/l,

b) DIECA 10^{-3} M/l,

c) 2,4-DNP 10^{-4} M/l.

The solution of respiratory stimulator:

a) d-1, tyrozine 10^{-2} M/l.

The concentration of inhibitors and that of tyrosine have been chosen after several experiments, diluted solutions did not show any change in transpiration of tested leaves.

Discusion of the results of experiment III

Diagrams 3 and 4 show the results obtained for the leaves of *Tradescantia*. The data given in the table show that both cuticular and stomata transpirations are inhibited by KCN 10^{-3} M/l and DIECA 10^{-3} M/l. The inhibition of cuticular transpiration is very distinct and

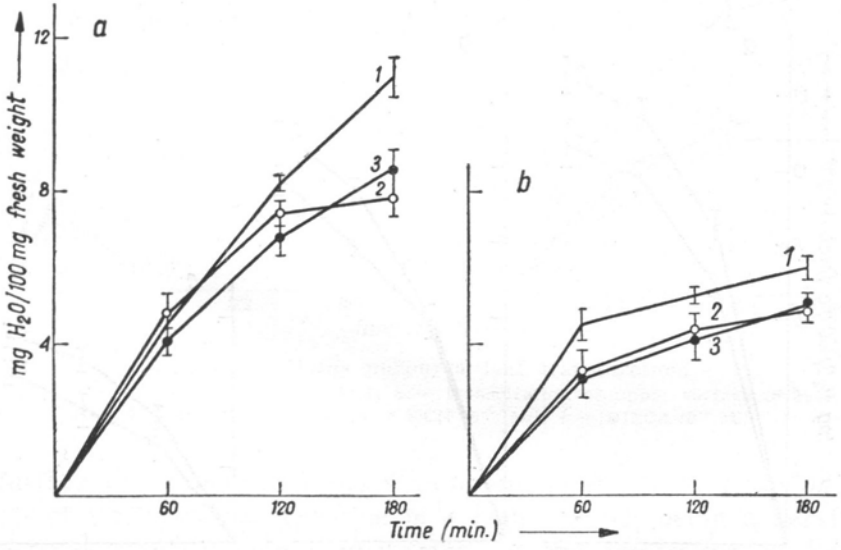

Diagram 3. *Tradescantia* leaf transpiration

a — transpiraiton of lower surface of leaf; b — transpiration of upper surface of leaf; 1 — H_2O; 2 — KCN 10^{-3} M/l; 3 — DIECA 10^{-3} M/l.

is seen even in the first hour of measurements. On the other hand, the inhibition of stomata transpiration is manifested later, namely when the leaves have lost some amount of water. DNP has also inhibited the cuticular and stomatal transpirations.

As a respiratory stimulator 10^{-2} M/l tyrosine has been used. Results

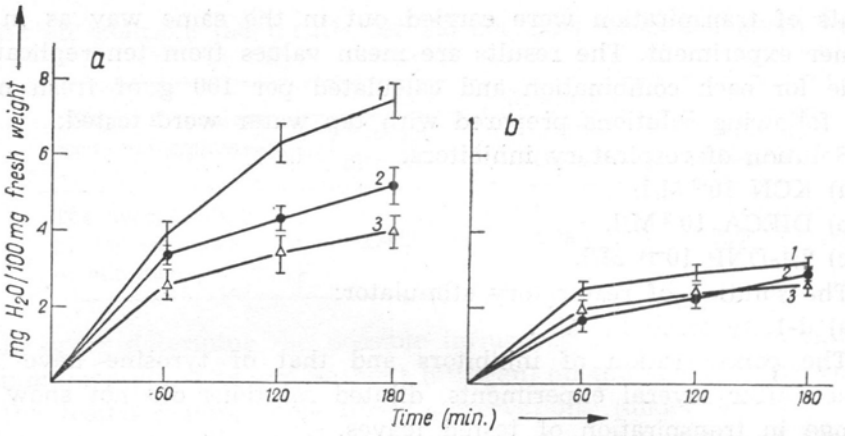

Diagram 4. *Tradescantia* leaf transpiration

a — transpiration of lower surface of leaf; *b* — transpiration of upper surface of leaf; *1* — H_2O; *2* — DIECA 10^{-3} M/l; *3* — 2,4-DNP 10^{-4} M/l.

obtained for transpiration of *Tradescantia* leaves are given in diagram 5. From these data it follows that tyrosine markedly stimulates the process of transpiration.

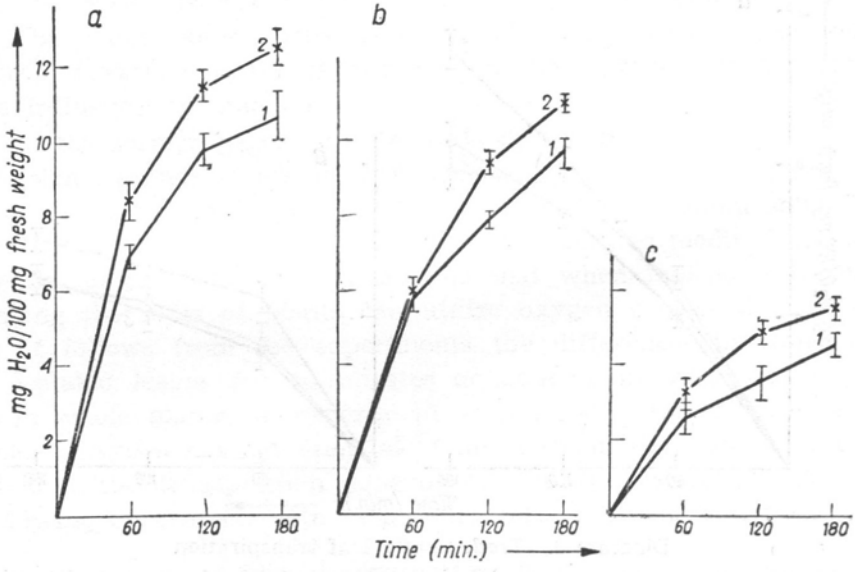

Diagram 5. *Tradescantia* leaf transpiration

a — total transpiration, *b* — transpiration of lower surface of leaf; *c* — transpiration of upper surface of leaf; *1* — H_2O; *2* — Tyrosine 10^{-2} M/l.

The effects of KCN, DIECA, 2,4-DNP and tyrosine on the transpiration of *Malus purpurea* leaves are in general the same as in *Tradescantia*. Results given in diagrams 6, 7 and 8 are a good illustration of these processes. The blockade of transpiration is affected by

the applied inhibitors. Tyrosine has the same stimulating effect on all the types of transpiration as it was found in *Tradescantia* leaves.

The stated dependence of the transpiration on the effect of typical respiratory inhibitors such as KCN, and DIECA sugests the possible relation between respiration and the loss of water in the tested leaves in the process of transpiration. This assumption has been testified by

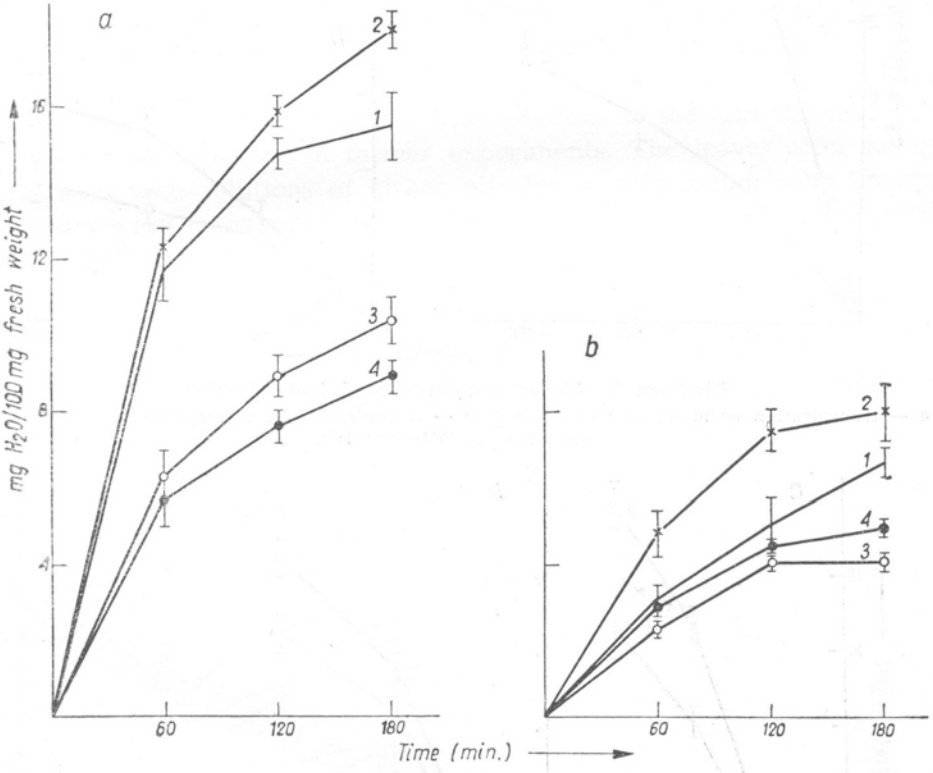

Diagram 6. *Malus purpurea* leaf transpiration
a — transpiration of lower surface of leaf; *b* — transpiration of upper surface of leaf; *1* — H_2O; *2* — Tyrosine 10^{-2} M/l; *3* — KCN 10^{-2} M/l; *4* — DIECA 10^{-3} M/l.

the further experiments on the stimulating effect of tyrosine on the process of transpiration. Tyrosine is a factor which being a substrate of phenolic enzymes may increase the rate of respiration in tissues of leaves. The inhibition of transpiration due to 2,4-DNP shows a relation between the transpiration and energetic processes.

In course of experiments on the effects of respiratory inhibitors and of tyrosine on the leaves transpiration, the measurements of stomata width have been carried out. The observations on the width of stomata have been made either directly, i. e. under microscope or by infiltration (the solutions were the same as used in former experiments).

Neither direct observations nor infiltration method have shown

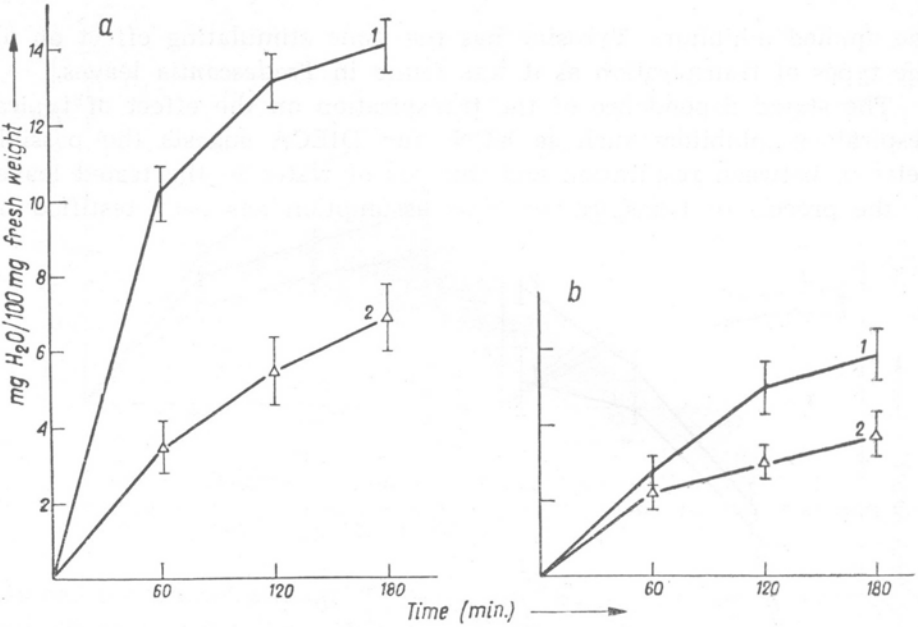

Diagram 7. *Malus purpurea* leaf transpiration

a — transpiration of lower surface of leaf; *b* — transpiration of upper surface of leaf; *1* — H_2O; *2* — 2,4-DNP 10^{-4} M/l.

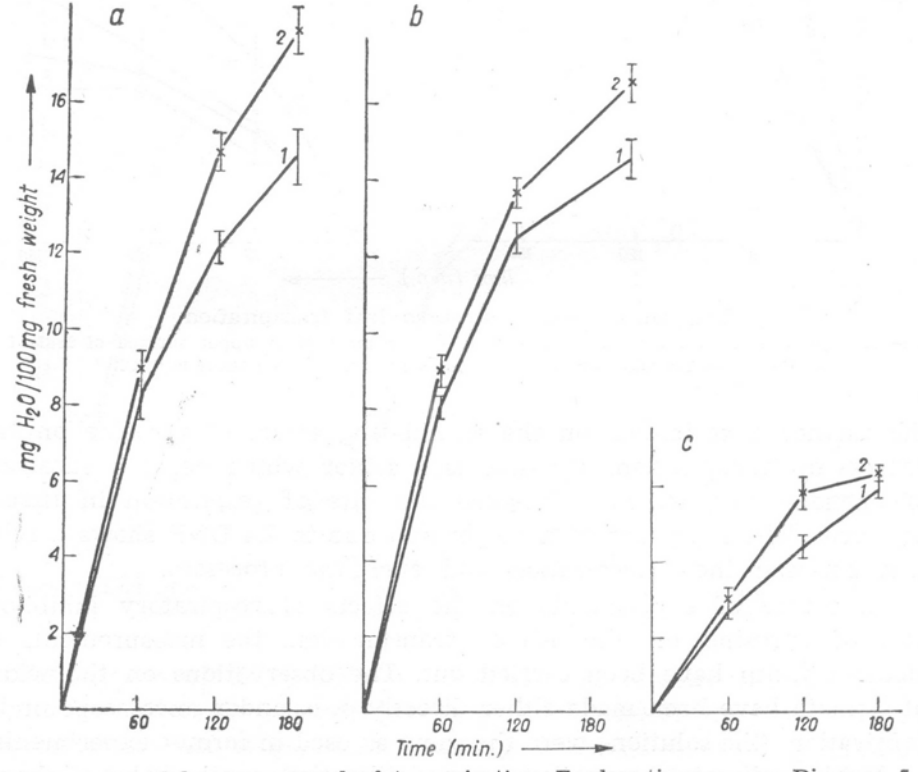

Diagram 8. *Malus purpurea* leaf transpiration. Explanations — see Diagram 5

any significant difference in width of stomata (due to the effect of either tyrosine or respiratory inhibitors). During the whole experiment the stomata were open, or at most half closed when the plant was loosing water in process of transpiration.

EXPERIMENT IV

The transpiration of leaves due to the effect of nitrates or ammonium salts taken separately or in combination with 2,4-DNP

The conditions of experiment: The solutions tested and the methods have been the same as in former experiments. The leaves after being infiltrated with solutions of either nitrates or ammonium salts during 24 hours were transferred into corresponding solutions completed with 10^{-4} M 2,4-DNP for 10—20 hours.

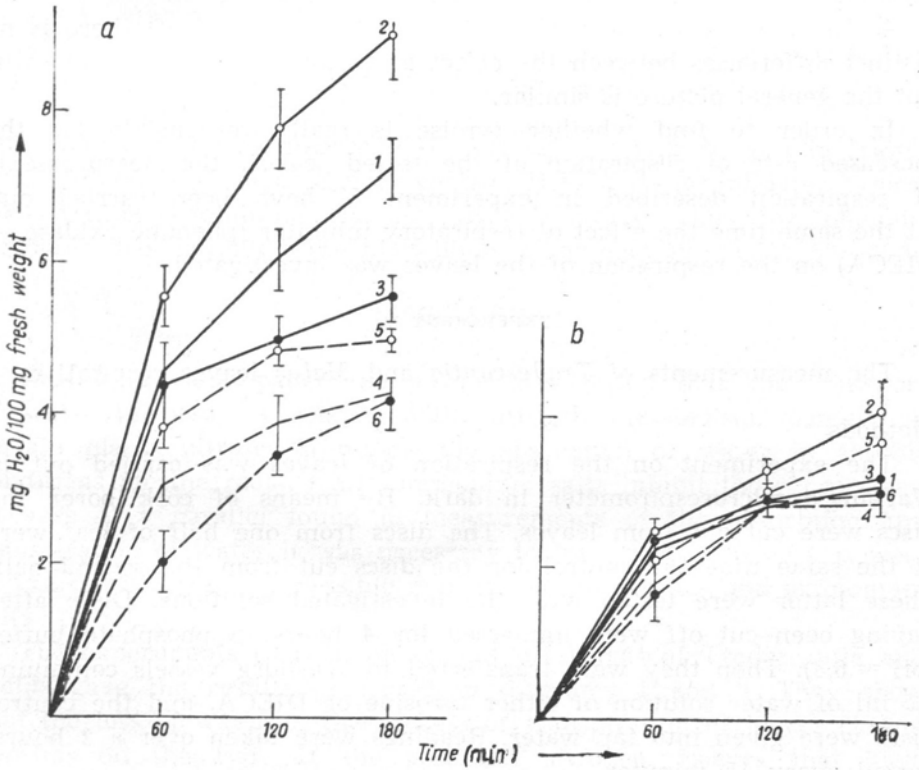

Diagram 9. *Tradescantia* leaf transpiration

a — transpiration of lower surface of leaf; *b* — transpiration of upper surface of leaf. *1* — medium without nitrogen; *2* — medium with nitrates; *3* — medium with ammonium salts; *4* — medium without nitrogen + 2,4-DNP 10^{-4} M/l. *5* — medium with nitrates + 2,4-DNP 10^{-4} M/l; *6* — medium with ammonium salts + 2,4-DNP 10^{-4} M/l.

Discusion of the results of the experiment IV

Results of the measurements of transpiration in *Tradescantia* in solutions containing 2,4-DNP are given in diagram 9.

From this diagram it follows that:

1. Nitrate ions stimulate the process of transpiration of the lower surface of leaf, while this process is inhibited by ammonium salts. Results obtained from the experiments carried out on the cut off leaves are in accordance with the data obtained in experiment carried out on the whole plants (Buczek 1963), and show that NO_3 ions affect the transpiration throughout the physiology of leaves.

2. 2,4-DNP inhibits the transpiration of the lower surface of leaf, being less efficient than the transpiration found for the control and even for ammonium salts.

3. If for the control we take a medium without nitrogen but with 2,4-DNP, then we observe the stimulation of transpiration by the solution of nitrates + 2,4-DNP and the inhibition of this process by the solution of ammonium salts + 2,4-DNP.

4. In cuticular transpiration (upper surface of the leaf) there is no distinct differenses between the effect of various combinations of salts, but the general picture is similar.

In order to find whether tyroise is really responsible for the increased rate of respiration of the tested leaves, the measurements of respiration described in experiment V have been carried out. At the same time the effect of respiratory inhibitor (phenolic oxidase — DIECA) on the respiration of the leaves was investigated.

EXPERIMENT V

The measurements of *Tradescantia* and *Malus* leaves respiration

Methods

The experiment on the respiration of leaves was carried out in Warburg's microrespirometer in dark. By means of cork borer the discs were cut out from leaves. The discs from one half of leaf were at the same time the control for the discs cut from the second half. These latter were tested with the investigated solutions. Discs after having been cut off were immersed for 4 hours in phosphate buffer (pH = 6,8). Then they were transferred to Warburg vessels containing 2,5 ml of water solution of either tyrosine or DIECA, and the control discs were given into tap water. Readings were taken over a 2 hours' period every 15 minutes.

Discusion of the results of experiment V

The results are given in Table 1. Leaves of both species show an increased absorbtion of oxygen due to tyrosine. In *Tradescantia* the

Table 1

Respiration of *Tradescantia* and *Malus purpurea* leaves

No. of experiment	Tyrosin 10^{-2}M/l µl O_2/100 mg dry weight per hour			DIECA 10^{-2}M/l µl O_2/100 mg dry weight per hour		
	Control	Tyrosin	%	Control	DIECA	%
Tradescantia						
1	225.68	253.00	112.5	214.82	88.10	40.9
2	184.02	212.01	115.2	196.20	80.90	41.3
3	181.50	235.80	129.8	170.10	74.40	43.6
4	192.10	206.10	107.5	174.51	61.00	34.9
Malus purpurea						
1	187.96	227.62	120.80	254.25	157.50	61.9
2	175.92	215.85	122.20	281.19	116.90	41.6
3	183.96	224.40	119.50	280.40	143.40	49.4
4	158.80	195.20	123.00	205.77	101.25	49.3
5	138.90	173.90	125.30	202.25	91.40	45.3

rate of respiration has increased in average by 16,25%, and in *Malus*, by 22,16% when compared with control. DIECA — an inhibitor typical for enzymes containing Cu as prostatic group, thus for phenolic oxydases, has inhibited the absorbtion of oxygen by about 50—60%.

DISCUSSION

In the previous paper the author has stated that the transport of water in plant is related with nitrogen metabolism, namely in certain plants nitrates stimulate the absorbtion of water in aerobic conditions for the roots, while ammonium salts inhibit those processes. Because of a parallel found in measurements of the absorbtion and transpiration of water it was necessary to investigate the very process of transpiration and its possible dependence on nitrates and ammonium salts.

The experiments carried out on cut off leaves of *Tradescantia* and *Malus* gave the results presented in diagrams 1 and 2. They show that the loss of water in the process of transpiration of leaves closely depends on the type of the absorbed nitrogen. Leaves that have absorbed nitrates show an increased transpiration (total, lower surface of leaves, and upper surface of leaves), while in leaves absorbing ammonium salts the rate of transpiration is lowered and even inhibited.

If we compare the above results obtained for the transpiration

of cut off leaves, with the observations received in former experiments on the transport of water in whole plants as an effect of either nitrates or ammonium salts in anaerobic conditions for the roots, then we see that the results are similar. In both cases nitrates stimulate the transpiration and transport of water, while ammonium salts have a negative effect on both processes. These experiments support the hypothesis that the process of transpiration which in general plays an important part in transport of water, is actively controlled by metabolic processes that take place in tissues of leaves. The infiltration of leaves with various forms of nitrogen induces specific changes in metabolism, which in turn affect the excretion of water by cells of leaf in the proces of transpiration. The permeability of cell cytoplasm for the water as well as the viscosity of solutions flowing through vessels and the so-called AFS (apparent free spaces) are very important factors in transport of water and its passage through the tissues from the root up to intracellular spaces.

Thus, the observed differences in loss of water in process of transpiration, due to various forms of nitrogen could be explained by changes either in permeability or in viscosity of the tested solutions or by these both factors. However, the measurements of viscosity and of the velocity of infiltration of the tested media, as well as the measurements of the permeability of leaf mesophyll in *Tradescantia* have not testified the above assumption. The observed differences in viscosity of the tested solutions are not significant and are confined to water viscosity. Similar results have been obtained from the measurements of permeability with deplasmolysis method.

The marked blockade of transpiration due to 2,4-DNP and the compensative effect of NO_3 ions (when compared with NH_4 ions, Experiment IV) suggest that the loss of water in leaves depends to some extent on metabolism of energy in tissues of leaves. These experiments suggest, moreover, that the nitrates may be used in some way in exoenergetic process of transpiration (the loss of water by transpiring leaves requires energy). From theoretical point of view such a situation is possible. The experiments carried out by Warburg and Negelein (1920) on *Chlorella pyrenoidosa* had shown that totally utilised nitrate oxygen yields about 162 000 calories. The computation is, however, quite theoretical, as the process of the reduction of NO_3 ions is correlated with the oxidation of sugars, and the reaction itself is endoenergetic.

What may be thus the causes of the observed changes in transpiration? According to author's opinion, there are two chief problems: One of them is the possible relation between the transpiration and the processes of protein, metabolism in cut off leaves, dependent to some

extent on the absorbed forms of nitrogen salts; the second problem is the dependence of transpiration on the general metabolism of energy.

As is well known, in leaves cut off from the parental plant the hydrolysis of proteins occurs very rapidly. This hydrolysis takes place in dark and in light just as well, though in dark the process of decomposition of proteins is more intensive.

(Cf.: Borodin (1878), Schulze and Kisser (1889) Chibnall (1952), Monthes (1926), Yemm (1935, 1937, 1950), Vickery and coll. (1937), McKee (1950), Moyse (1950).

With the decomposition of proteins the accumulation of the soluble nitrogen may be observed: first amino acids, and then amids, mostly asparagine, and glutamine. During the hydrolysis of proteins the carbohydrates are also rapidly metabolised. Immediately after the leaves are cut off, carbohydrates are the respiratory substrate, but gradually they are substituted by amino acids and amids. Respiratory coefficient, previously equal to 1.0 is decreased after 24—40 hours to 0.8.

In our experiments the leaves after having been cut off from the parental plant were immersed into tested solutions for 24 hours, being kept in light during the day. The measurements of transpiration have been taken in the next day noon in continuous electric light (7000 Lux). The above conditions were favourable for the reduction of nitrates, as the light stimulates this process (Delwich 1951, Mendel and Vissner 1951). The above mentioned authors carried out the experiments on the cut off leaves of tobacco and tomato, using labelled nitrogen in nitrates. They have shown that the reduction of nitrates occurs both in dark and in light, the process being more efficient in light. These results are in accordance with the experiments of Vishniac and Ochoa (1951), Tolmach (1951), Arnon (1951) and Evans and Nason (1953). These authors have shown that the reduced nucleotides TPN and DPN take part in the reduction of nitrates. As TPN and DPN are easily reduced in light (with cooperation of chloroplasts) then the indirect effect of light in reduction of the nitrates becomes clear. In view of this fact we should not expect the accumulation of NO_3 ions in the tested leaves, since the conditions of the experiment were favorable for the reduction of nitrates, nitrogen being used in the general metabolism in the cut off leaves.

It has been shown by many authors that the nitrates are relatively easily metabolised, and the intensity of this process depends on the light. Eisenmenger (1933) working on assimilation of nitrates in tabacco leaves has stated that the nitrates were metabolised with higher intensity in light than in darkness. Similar situation has been observed by McKee (1950) in barley leaves. Petrov (1917) has found that ammonium salts are better assimilated by plants in dark

than in light, nitrates however, are better assimilated in light. Andrejewa (1951) has observed the reduction of nitrates in beans leaves both in darkness and light, but the light has stimulated this process.

The above results can be summarised as follows:

1. The hydrolysis of proteins in cut off leaves of tested plants has a various intensity.

2. The decomposition is slower in light than in darkness.

3. Carbohydrates are respiratory substractes immediately after the leaves had been cut off from the parental plants, 24—40 hours later this function is at first partially and then entirely taken by the products of decomposition of proteins, a low value of RQ (less than 1) being a proof.

4. Amino acids, glutamine and asparagine are accumulated in leaves during the process of protein decomposition.

5. The decreased value of RQ does not result from the lack of carbohydrates (such situation occurs also in light), but is due to the contribution of products of protein decomposition into respiratory processes.

6. The reduction of nitrates is more intensive in the light than in darkness, while ammonium salts are better assimilated by plants in darkness.

Hence we could suppose that the similar changes had occurred in the tested leaves of *Tradescantia* and *Malus,* complicated however by the presence of high concentrations of NO_3 ions. These salts have probably been used in protein metabolism. May be the differences in assimilation of nitrates and ammonium salts in light (Delwich, Petrowa) were responsible for the observed differences in the process of transpiration. However the experiments on the combined effect of either nitrates or ammonium salts with 2,4-DNP (Diagram 9), with respiratory inhibitors and tyrosine allow us to look at the process of transpiration from different point of view, and to understand better the role of nitrogen compounds (nitrates and ammonium salts).

The experiments carried out with 2,4-DNP show that this factor restricts remarkably the process of transpiration. From these experiments it follows that the loss of water in the process of transpiration is closely related with general metabolism of energy, and that the processes of phosphorylation affect to some extent the loss of water by cells.

The differences in transpiration due to combined action of either nitrates or ammonium salts with 2,4-DNP may show the important role of nitrates: The infiltration of leaves with the solution of nitrates followed by 2,4-DNP has weakend the effect of nitrates, as compared with control without nitrogen and 2,4-DNP (Diagram 9), but when

compared with control without nitrogen and with 2,4-DNP an increased transpiration of water has been observed.

The experiments carried out with inhibitors prove our assumptions, that the loss of water in the process of transpiration is to some extent related with the process of respiration. The transpiration was much less intensive when the respiratory enzymes were blocked by either KCN or DIECA. The experiment with tyrosine distinctly shows the relation of both processes. This amino acid being a substrate of phenolic oxidases (Nelson and Dawson 1944, Robinson and Nelson 1944) can intensity the respiratory processes. Our experiments on the respiration of *Tradescantia* and *Malus* leaves infiltrated with solution of tyrosine (Table 1) had testified these assumptions, and the experiment on transpiration in the presence of tyrosine, carried at the same time, have shown the increased loss of water in both kinds of leaves.

It our hypothesis is true then, doubtless „the energy used up in process of transpiration" is an important factor in water excretion from the cells into intercellular spaces or into cuticle. It is possible that this energy is up to come over the resistances shown by cytoplasm in process of water excretion.

As we know the experiments of Gäumann and Jag (1947, 1950), Gäumann (1951) on the effect of some glucosanes on the leaf tissue, the current of transpiration flows through cytoplasm and cellulose membranes of celles. When the intramicellar spaces are chocked with large molecules of glucosanes, the transpiration becomes less efficient. Mereover some glucosanes (e. g. lycomarasmine) may be toxic for the cytoplasm and in consequence may block the transpiration. Hence we can assume that in process of transpiration an „active" excretion of water is related, of course, with the metabolic processes in the leaves. This hypothesis is supported by our experiments.

Finally the role of oxidized or reduced forms of nitrogen in the process of transpiration should be explained. In our experiments with whole plants the nitrogen compounds were very important factors in absorbtion of water in poorly or non aerated media, particularly the NO_3 ions, due to their ability for the compensation of oxygen deficency in roots. The observed relation between the absorbtion and transpiration of water in whole plants might show that the antagonistic activity of NO_3 and NH_4 ions is an important factor in the passage of water through the tissues of the root. A similar situation might be observed during the transpiration of the isolated leaves. These salts distinctly influenced the loss of water in the process of transpiration. Thus there is a strong parallel between the transport of water from the root cells to the vessels (root pressure) and its transport

from mesophyll to the intracellular spaces, and the both processes are controlled by the same factors.

This problem is known with regard to the process of guttation, which occurs through hydathodes of epidermis. Those hydathodes may be of passive or active type. The force that results in the excretion of water by means of passive hydathodes is the root pressure, yet in active hydathodes the force yielding in excreation of water is located in hydathodes themselves. It is possible that the guttation is a component element of transpiration, and then all the factors that increase the root pressure are at the same time increasing the guttation of passive hydathodes.

Since the activity of NO_3 ions in poorly aerated media should be included to those factors, then their role in the increased transpiration becomes clear. In experiments with cut off leaves it is quite possible that NO_3 ions are able in some way to increase the activity of forces controlling the active guttation of the leaf, and consequently to increase the transpiration.

CONCLUSIONS

1. The infiltration of the leaves with nitrates increases intensity of transpiration, while the action of ammonium salts is opposite.
2. Respiration inhibitors KCN 10^{-3} M/l, DIECA 10^{-3} M/l and phosphorylation inhibitor 2,4-DNP 10^{-4} M/l are markedly blocking the loss of water in process of transpiration.
3. Tyrosine which is a respiratory stimulator increases the excretion of water.
4. A hypothesis was discussed according to which the process of transpiration is related with energy released during the respiration of leaves and used in the process of water excretion to intracellular spaces or cuticle.

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