N° 4—10 *B* I AVRIL—DÉCEMBRE 1951

BULLETIN INTERNATIONAL DE L'ACADÉMIE POLONAISE DES SCIENCES ET DES LETTRES

CLASSE DES SCIENCES MATHÉMATIQUES ET NATURELLES

SÉRIE *B*: SCIENCES NATURELLES (I)

SUBVENTIONNÉ PAR LE MINISTÈRE DE L'ENSEIGNEMENT SUPÉRIEUR

CRACOVIE
ACADÉMIE POLONAISE DES SCIENCES ET DES LETTRES
1952

Publié par l'Académie Polonaise des Sciences et des Lettres, sous la direction de M. Z. Grodziński, Secrétaire de la Classe des Sciences Mathématiques et Naturelles (Cracovie, Institut d'Anatomie comparée, rue St. Anny 6).

POLSKA AKADEMIA UMIEJĘTNOŚCI — KRAKÓW 1952.
Nakład 800+50 egz. Papier druk. sat. b/drzewny 70×100 cm 80 g. ark. druku 13 i 10 tabl.
Krakowska Drukarnia Naukowa — Kraków, Czapskich 4. Zam. 290, 13.V. 1952
Druk ukończono w styczniu 1953

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Badania nad zdolnością przystosowania się Kropidlaka czarnego Aspergillus niger do miedzi. — Investigations on the adaptability of Aspergillus niger to copper.

Mémoire

de M^lle^ *H. JURKOWSKA*

présenté le 7 Mai 1951, par M. T. Lityński m. c. et M^lle^ A. Kozłowska m. c.

(Plate 8—9)

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Introduction

Although the literature concerning the influence of copper on *Aspergillus* is fairly rich, the problem of the adaptation of this organism to copper was not elaborated in detail. In 1902 Pulst published the results of his work about resistance and accommodation of different moulds to copper. His experiments concerned first of all *Penicillium glaucum*; this mould proved to be the most resistant and showed the greatest adaptability. The author based, the results of the investigations, first of all, on the capacity of spores of germination in the presence of different concentrations of copper salts. The investigations of this kind were not carried out by other authors.

The adaptability of *Aspergillus niger* to large concentrations of copper salts was the subject of investigation of this work which was to answer the following problems: 1) whether *Aspergillus* by being successively transplanted on nutrient solutions containing a relatively large dose of copper becomes accustomed to this dose; 2) whether the adapted form will be different from the non-adapted one in its morphology, physiology and biochemistry; 3) whether this adaptation will be stable.

The investigations were begun in September 1949 and finished in February 1951.

Methods

The investigated *Aspergillus* was a pure culture of *Aspergillus niger* obtained from Switzerland and given to us by the Department of Agricultural Microbiology of the Jagiellonian University.

The spores were taken from a culture growing on slant agar.

The investigations were carried out in the conditions described below. If any changes occurred they will be mentioned in the description of the particular experiments.

As a liquid nutrient a water solution with the following ingredients was used: 10% saccharose, 0·5% NH_4NO_3, 0·5% KH_2PO_4, 0·25% $MgSO_4,.5H_2O$ and a trace of $Fe^{\cdots}$ (in the form of $FeCl_3$). The saccharose used was the beet sugar which was on the market. The mineral salts were reagents of Merck «pro analysi». The water was distilled once in a distiller. The nutrient solution was prepared in the following way: all its ingredients except $FeCl_3$ were dissolved

in distilled water, then a few drops of 1% solution of $FeCl_3$ and the necessary amount of distilled water was added. The nutrient solutions were not purified from the admixtures of heavy metals so as not to take away the necessary trace elements from the fungus. Because of this, nutritive ingredients or stimulants needed by *Aspergillus* in very small doses were not added to the nutrient solution. As the ingredients of the solution were the same in all experiments, the presence of trace elements did not change the conditions of vegetation.

The admixtures of copper which were probably present in the solution had no great importance as a great concentration of this ingredient was used. More in this work the author was not interested in comparing the growth and metabolism of the mould growing on a nutrient solution lacking copper with a solution to which this ingredient was added, but only in investigating the influence of a great concentration of copper salt on a normal nutrient solution. We should remember that «the nutrient solutions not containig copper» or «normal nutrient solutions» mean solutions to which copper sulphate has not been added, to distinguish them from the «nutrient solutions containing copper» which have received this salt additionally.

The prepared solution was divided into 50 cm^3 doses (in some experiments 100 cm^3) and poured into Erlenmeyer flasks of 300 cm^3 capacity and these flasks were stopped with wads of cotton wool. Then the flasks with the nuntrient solution were sterilized three times during three successive days, for 1/2 hour at a temp. of $+100^0$ C. The pH of the solutions thus prepared was about 4·00. The solution of copper sulphate was prepared separately ($CuSO_4.5H_2O$ Merck «pro analysi») and it was also sterilized. Into the flasks whose nutrient solution was to contain copper 20 cm^3 (and with 100 cm^3— 40 cm^3) of this solution were introduced with a sterilized pipette. The concentration of copper in this nutrient solution amounted to 0·1143% which corresponds to 0·449% $CuSO_4.5H_2O$. The pH of nutrient solutions containing copper amounted to about 3·70. The above mentioned concentration of copper salt was used because it was found in the preliminary investigations that copper in this concentration had a great toxical influence hindering growth to a great degree, but not depriving the organism of the capacity

of sporulation. The sporulation although weak and delayed, still took place so that it made possible further transplanting.

Before sterilization 20 cm^3 or 40 cm^3 of distilled water were added to flasks to which copper was not introduced, to give the same conditions to the mould (the capacity of the liquid, its surface, the concentration of nutritive ingredients) in the flasks with and without copper. Other copper salts were introduced in the same way as the sulphate. Their quantities were so arranged that the concentration of copper was always the same — 0·1143%. When great concentrations of copper sulphate were used, this salt was added to the flasks in crystalline form. If other salts were used, such as nickel sulphate, zinc sulphate, and manganese sulphate, the author proceeded in the same way i. e. the solutions of these salts were prepared and added with a pipette to the sterilized nutrient solution. The quantities of these ingredients were so dosed that the concentration of the metal in the nutrient solution amounted to 0·1143%, that is, it corresponded to the concentration of copper. These salts were used individually so that the nutrient solution containing one of the above mentioned metals did not contain any others. In the preparation of nutrient solutions with a lower pH, the nutrient solutions were acidified by 1n H_2SO_4 to a pH 1·4 before sterilization. These solutions did not contain copper. In using nutrient solutions with a great concentration of sugar, 30 g of sugar were added to flasks containing the normal solution, before sterilization. Therefore in 50 cm^3 there were 35 g of sugar.

Cultures on solid medium were bred on slant agar.

The cultures were bred in Erlemeyer flasks with a capacity of 300 cm^3 made of Jena glass (Schottogen Jena 20). The other glass used was of Polish origin. After careful washing the glass was washed several times with distilled water.

The transplantations were made with the help of an ignited platinum needle. The author tried to get as large a quantity of spores as possible. The transplantation of the suspension of spores was not possible because the spores taken for each experiment had to come from different mycelia.

The vegetation was carried out in an electric thermostat in darkness at a temp. of + 30° C. The period of vegetation varied in particular experiments. It depended upon the quickness of the deve-

lopment of the mould and on the time needed for the production of the spores.

During the investigation of the metabolism each series was made in 5 repetitions, for determining only the dry weight of the mycelium 2—4 repetitions. All numbers given in the figures and tables are arithmetical means of the obtained results.

To make photographs *Aspergillus* was bred on liquid nutrient solutions in special glass vessels with a lid and on solid medium on agar on Petri dishes. The conditions of development were kept as above.

After finished vegetation the mycelium was separated from the solution by filtering off. Then the mycelium was washed several times with distilled water which was collected together with the filtrate (except the nutrient solution in which pH and the total acidity was determined). The distilled water was added to the filtrate up to 500 cm^3 and it was then chemically analysed. The drained and washed mycelium was weighed to determine the fresh mass. The obtained weight illustrates only approximately the true values because a part of the water from washing could have remained on the mycelium in spite of efforts to eliminate it. Moreover, during weighing the mycelium could lose weight because of evaporation. The error caused by the first of these two possibilities was probably the greater so that the given weight of fresh mycelium is rather too big than too small. Then the mycelium was dried in an electric oven at a temp. of $+60^0$ C during 24 hours after which the temp. was raised to $+100^0$ C and the drying continued till constant weight was obtained, in order to get the dry weight of mycelium.

The reaction of the solutions after vegetation was investigated in samples not diluted by water. The pH was determined by the Kalkdienst potentiometre with the use of a calomel electrode as the comparative one and chinhydrone electrode as the working one.

The total acidity was also determined in samples not diluted by water. In order to draw the buffer curves the method of electrometric titration was used. The total acidity given in the tables is expressed in cm^3 of 1/10 n NaOH and relates to 30 cm^3 of the nutrient solution.

The quantities of sugar used by the mould were computed from the difference between the contents of sugar in the nutrient

solution before and after vegetation. The sugar was determined in samples of solution diluted by distilled water by Bertrand's (1910) method having first inverted saccharose with hydrochloric acid.

The contents of nitrogen in the mycelium were determined by Förster's (1923) method. To investigate the form of the nitrogen taken from the solution, ammonium nitrogen and nitrate nitrogen were determined in the samples of solution diluted with distilled water by the colorimetric method with the help of a Lange-Riehm colorimetre, and then the results were subtracted from the quantities of these ingredients contained in the solution before the vegetation.

The contents of ash in the mycelium were determined by reducing it to ashes in quartz crucibles on a gas-burner.

The quantity of the absorbed copper was computed from the difference between the initial contents of copper in the solution and its contents after vegetation. Copper was determined in the samples of the solutions diluted with water distilled by the iodometric method of Haën-Low (1930).

Investigations on the adaptability of *Aspergillus niger*

In order to find out whether *Aspergillus* can adapt itself to an environment containing 0·449% of copper sulphate it was transplanted several times on nutrient solutions containing this concentration of copper salt.

The spores taken from mycelium grown on a slant agar (and called A_0) were transplanted in two series on liquid nutrient solutions, one containing 0·449% of copper sulphate and the other one copperless. The differences based on the change of osmotic pressure with this concentration of copper sulphate are so small that they were not taken into account. On copper nutrient solution the development of the mycelium was very slow, since after 17 days of vegetation its dry weight amounted to only about 0·2 g. As *Aspergillus* produced spores, although very weakly, a new transplantation was made with spores taken from the mycelium on a fresh nutrient solution with copper. At the same time the spores from the mycelium grown on copperless nutrient solution were transplanted on a fresh copperless solution. Such transplanting was repeated 25 times every 17 days. The form developing in the envi-

ronment containing copper was called form B, the successive number of the generation growing on copper being marked with an Arabic numeral, and the control form growing on the copperless solution was called form A. And so «form B_{10}» means that this culture was the tenth generation growing on nutrient solution with copper, and «form A_{25}» the 25 generation on copperless nutrient solution, etc. The scheme of successive transplantation is shown in Fig. 1.

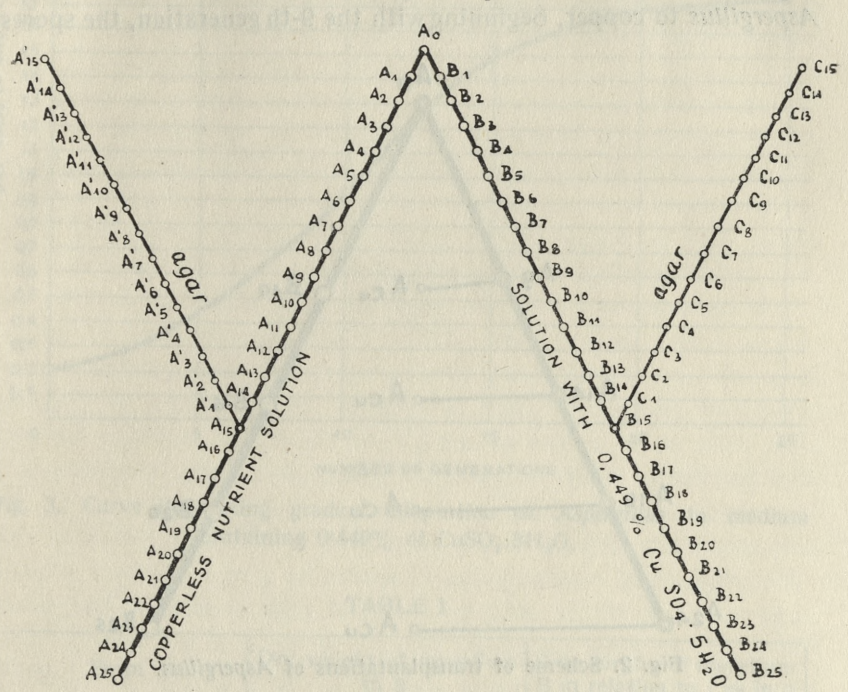

Fig. 1. Scheme of successive transplantations of *Aspergillus*.

Aspergillus gradually grew accustomed to the environment containing copper during successive transplantations. The germination of the spores occurred earlier, the mycelium developed more and more strongly. The sporulation took place sometimes a little earlier and it was ampler. It was also a characteristic phenomenon that many of the spores fell to the bottom soon after germination. Perhaps a certain selection of spores, leading to the preservation of the life of those which were from unknown causes more resistant to the toxic action of copper sulphate occurred here. This selection

could be one, undoubtedly not the only cause of the adaptation of *Aspergillus* to the new environment. It is not impossible that dead fragments of mycelium absorbed copper to a certain degree, making easier the development of spores germinating later. It is possible that during decomposition some substances which weakened the toxicity of copper were produced.

In order to determine the degree and course of adaptation of *Aspergillus* to copper, beginning with the 9-th generation, the spores

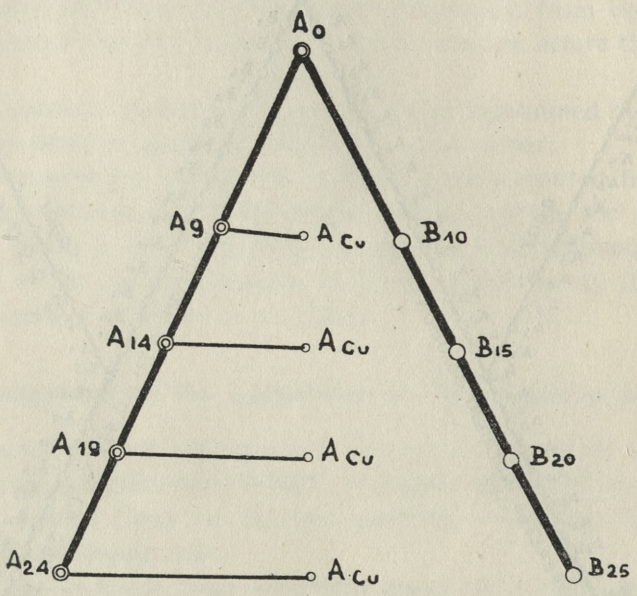

Fig. 2. Scheme of transplantations of *Aspergillus*.

of every fifth generation of form «A» were transplanted on copper solution and after 17 days the dry weight of the mycelium was determined as well as that of the mycelium of the corresponding «B» generation. In cases where form A was transplanted for comparison on copper solution it was marked (in this and in the following experiments) by adding the symbol Cu to A (A_{Cu}). The scheme of these transplantations is illlustrated on Fig. 2 which corresponds to the scheme shown in Fig. 1, but only the forms taken for comparing the dry weight of mycelium A and B on copper solution were taken into account. The results of the determination of the dry weight of the adapted and non adapted mycelium growing on

a solution with copper, and the crop surplus of mycelium B in relation to A are presented in Table I. Fig. 3 presents the curve illustrating the course of adaptation of *Aspergillus* to the media containing 0·449% of copper sulphate.

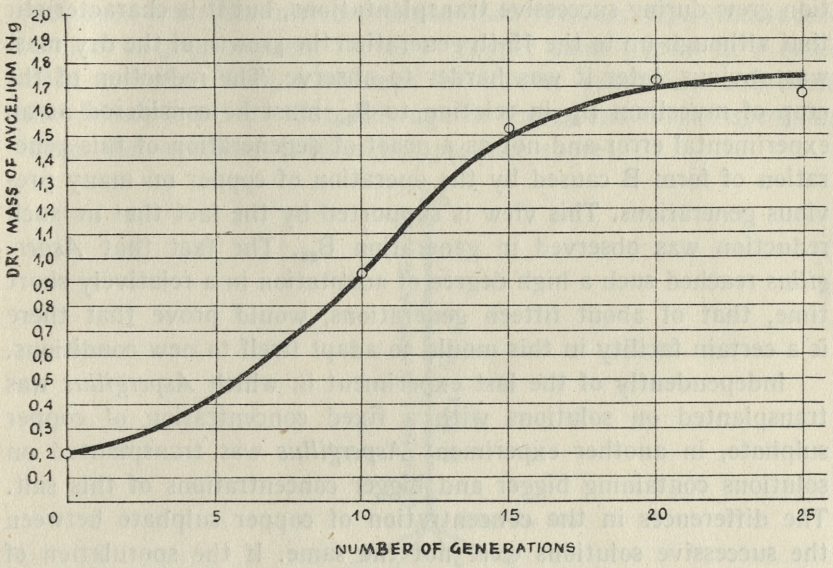

Fig. 3. Curve illustrating gradual adaptation of *Aspergillus* to medium containing 0·449% of $CuSO_4.5H_2O$.

TABLE I

Form	Dry weight of mycelium in g	Crop surplus of mycelium B in relation to A_{Cu} in g
A_9Cu	0·1942	0 7906
B_{10}	0·9848	
$A_{14}Cu$	0·2092	1·3301
B_{15}	1·5393	
$A_{19}Cu$	0·2200	1·5000
B_{20}	1·7200	
$A_{24}Cu$	0·2184	1·4656
B_{25}	1·6840	

As we see from these data, the successive transplanting of *Aspergillus* on nutrient solution with a fixed, relatively high concentration of copper produced a certain adaptation of this mould, which was expressed in the weaker toxic operation of copper. This adaptation grew during successive transplantations, but it is characteristic that although up to the 15-th generation the growth of the dry mass was obvious, later it was harder to observe. The reduction of the crop of mycelium B_{25} in relation to B_{20} must be considered as an experimental error and not as a proof of degeneration of this generation of form B caused by the operation of copper on many previous generations. This view is supported by the fact that no such reduction was observed in generation B_{26}. The fact that *Aspergillus* reached such a high degree of adaptation in a relatively short time, that of about fifteen generations, would prove that there is a certain facility in this mould to adapt itself to new conditions.

Independently of the last experiment in which *Aspergillus* was transplanted on solutions with a fixed concentration of copper sulphate, in another experiment *Aspergillus* was transplanted on solutions containing bigger and bigger concentrations of this salt. The differences in the concentration of copper sulphate between the successive solutions were not the same. If the sporulation of the mycelium grown on one nutrient solution was very weak, the concentration of copper was only slightly raised in the next one. The taking and transplanting of spores on a new nutrient solution was not done at the same intervals of time because it had to depend on the time necessary for the forming of the spores. After several transplantations and after reaching a concentration of 5% the spores from this culture were taken and put into nutrient solutions containing 5, 10 and 20% of copper sulphate and a saturated solution, where at the bottom of the flask there were undissolved crystals of this salt. The scheme of these transplantations is given on Fig. 4.

The spores germinated in all the three flasks although with some delay, and an ample white mycelium grew. The crystals of copper sulphate in the flasks with saturated solutions began to dissolve during the vegetation. The nutrient solution changed colour from intense blue to dark green. The mycelium sporulated in all the flasks, but it sporulated later if the concentration of copper was larger. And so the mycelium on the saturated copper solution

sporulated, very weakly, only after 18 days from the day of inoculation. In connection with this i. e. the importance of the time factor should be stressed in this kind of investigation, i. e. in the investigation on toxicity of a substance. With a three days' vegetation one could draw the false conclusion that even an *Aspergillus* accustomed to great concentrations of copper cannot grow in a saturated solution. With a 17 days' or shorter vegetation one could also falsely conclude that although in these conditions *Aspergillus* forms the mycelium it does not produce spores.

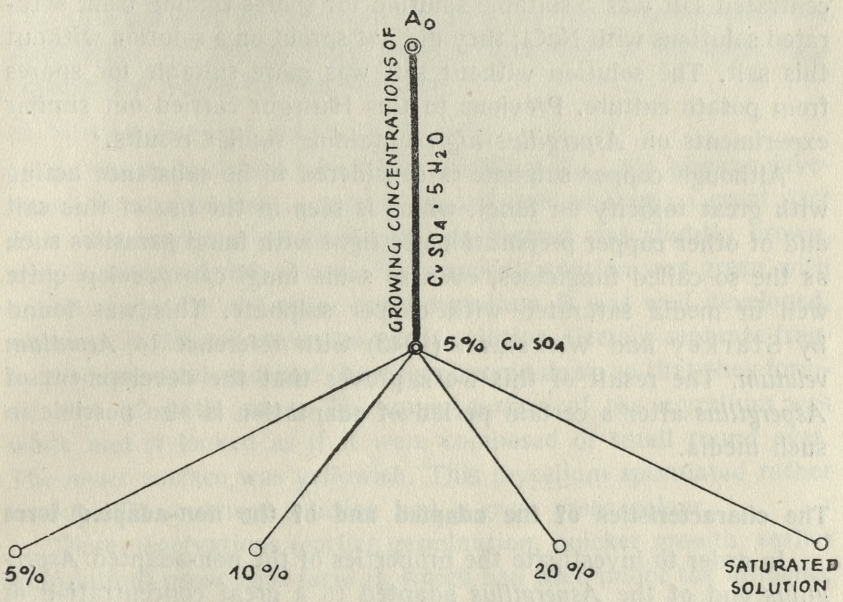

Fig 4. Scheme of transplantations of *Aspergillus*.

This experiment proves also that *Aspergillus* can adapt itself to the greatest concentrations of copper sulphate, as it develops in their presence, producing a rather abundant mycelium and it does not lose its capacity to sporulate. *Aspergillus* non-adapted to copper ceases sporulating under the influence of much smaller doses of copper (Roberg 1928, Fischer 1941).

Pulst (1902) obtained similar results while investigating the adaptability of fungi *(Mucor mucedo, Botrytis cinerea, Penicillium glaucum, Aspergillus niger)* to copper sulphate, but with much lower concentrations. Among the fungi investigated by him *Peni-*

cillium proved to be the most resistant to the action of copper sulphate and showed the greatest adaptability.

Investigations on the influence on the substratum on the successive generations of *Aspergillus glaucus* were carried out by Raciborski (1906). He says that the molecular concentrations of the liquid on which *Aspergillus* grows influences the produced spores in such a way that they sprout or they sprout better and grow better on a liquid with a concentration to which the maternal organism has become accustomed. The nutrient solution with concentrated salt was a suitable solution for spores coming from saturated solutions with NaCl; they did not sprout on a solution without this salt. The solution without salt was more suitable for spores from potato culture. Previous to this Hunger carried out similar experiments on *Aspergillus niger* obtaining similar results.

Although copper sulphate is considered to be substance acting with great toxicity on fungi, which is seen in the use of this salt and of other copper preparations to fight with fungi parasites such as the so called fungicides, even so some fungi can develop quite well in media saturated with copper sulphate. This was found by Starkey and Waksman (1943) with reference to *Acontium velatum*. The result of this work proves that the development of *Aspergillus* after a certain period of adaptation is also possible in such media.

The characteristics of the adapted and of the non-adapted form

In order to investigate the properties of the non-adapted *Aspergillus* and of the *Aspergillus* adapted to a great concentration of copper sulphate the following experiments were carried out.

A. VEGETATION ON A NUTRIENT SOLUTION CONTAINING 0·449% $CuSO_4.5H_2O$

One series of flasks with 100 cm^3 of solution was inoculated with spores coming from culture A_{15}, the second series with spores from culture B_{15}. The 15-th generation of *Aspergillus* was chosen because its adaptation to the toxic substratum was distinct and it could therefore be supposed that it had already caused certain changes parallel to the forming of a new equilibrium of the organism. The vegetation was allowed to proceed for 18 days, in which time the mycelium developed sufficiently to be analysed. As the

conditions of both experiments were exactly the same, all the differences between forms A and B should be attributed to the fact that form B in its previous generations developed in an environment containing 0·449% of copper sulphate.

a) The course of vegetation

The development of mycelium A_{Cu} could be seen only on the 5-th day after the inoculation. This mycelium developed very slowly, producing small lumps joining one another here and there as they went on growing. Sporulation began on the 12-th day of vegetation. The development of mycelium B began on the 3-rd day after the inoculation, its course being quicker than that of the form A_{Cu}. Sporulation began on the 9-th day.

b) Morphological characteristics

At the time it was taken the mycelium A_{Cu} was weakly developed; it covered only a part of the nutrient solution; a great part of it was immersed in the liquid, its bottom was slightly brown, and it produced few spores. The nutrient solution was green with a blue tinge. On the other hand mycelium B was well developed, although it did not cover the whole solution. Certain separate fragments were oval and had the edges curved down so that they formed sort of little caps. The upper surface of the mycelium was white and it looked as if it were composed of small round eyes. The lower surface was yellowish. This mycelium sporulated rather weakly. The nutrient solution had a grass green colour.

These observations (earlier germination, quicker growth, earlier sporulation) prove that form B, which had been under the influence of copper sulphate for several former generations, has a certain resistance to this salt.

In comparison with *Aspergillus* A growing on a normal solution, both the A_{Cu} and B forms on solution with copper begin to grow later more slowly and sporulate weakly and late. The hindering influence of larger doses of copper on the growth of *Aspergillus* was observed by Tamiya (1928), Fischer (1941), Waterman (1913) and others. Gollmick (1935/6) says that even stimulating doses whose presence gave a crop surplus, delayed the germination. Fischer (1941) and Roberg (1928) call attention to the weakening of the capacity of producing spores under the influence of bigger doses of copper.

Microscopic investigation showed the deformation of the cells of both forms, and it was more pronounced in the form B where the cells were often strongly shortened, and swollen, and the hyphae were twisted, divided into branches, and irregular. The microscopic image of mycelium B is given in photograph 10.

The changes in the shape of cells, the tendency to produce circular forms, the shortening and widening can be a certain adaptation of the mould to the toxic environment because these changes cause the shrinking of the surface of cells in relation to their capacity, owing to which their contact with the environment is smaller. Iwanow (1904) and Yoshimura (1934) observed the change of form of the cells of *Aspergillus* under the influence of copper. Raciborski (1905) was probably the first author to describe the chemomorphosis of *Aspergillus niger*. He obtained, under the influence of iodine, a mycelium whose cells were much bigger than the normal ones. Frey (1927) gives the conditions of the forming of the giant cells and a detailed description. He found that these cells formed in highly acid media. He sees the cause in the physiological lack of $K^{\cdot}$ ions and he thinks that the $H^{\cdot}$ ions hinder the adsorbing of $K^{\cdot}$ ions. The phenomenon of the forming of giant cells in media containing small quantities of potassium salts is a confirmation of this observation.

c) The crop of the mycelium and the metabolism

TABLE II

Analyses of crop of mycelium	form $A_{15}Cu$	form B_{15}
fresh mass of mycelium in g	14·84	37·78
dry mass of mycelium in g	1·0510	2·1517
% of dry mass	7	6
quantity of sugar in g used to produce 1 g of dry mass of mycelium	4·4	3·1
% of N in dry mass of mycelium	9	6
% of protein in dry mass of mycelium (N×6·25)	56·25	43·13
quantity of N in mg taken for 1 mg of used sugar	21	22
surplus of $N—NH_4$ on $N—NO_3$ taken by the mould in mg	39	13
% of ash in dry mass of mycelium	3·34	4·43
pH of nutrient sol. (3·70 at the beginning)	1·90	1·79
total acidity of 30 cm^3 of nutrient sol. in cm 1/10 n NaOH (12·0 at the beginning)	27·00	31·70
% of copper in dry mass of mycelium	1·1	3·2

Fig. 5 presents the values relating to form B in relation to the value of form A_{Cu} taken as a 100.

As we see from these data, the crop of mycelium B was twice as big as that of mycelium A. It proves also that this form is adapted to a high concentration of copper salt. We should still explain the phenomenon which seemingly could appear contradictory to the previous results, as the crop of mycelium on a solution with copper was bigger in this experiment than the crops obtained in other experiments on the same kind of solution. The intentional introduction of greater quantities of spores into these flasks to obtain a big mass of mycelium needed for chemical analyses was the basic cause of this. Moreover, the larger quantity of solution and a somewhat longer period of vegetation can have a certain influence.

The percentage of dry mass of mycelium B was lower than that of A_{Cu}.

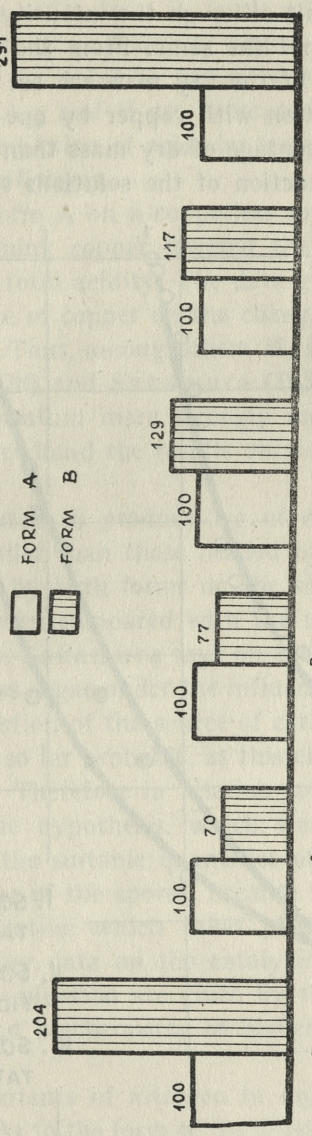

Fig. 5. Results of experiment carried out with form A_{15} and B_{15} on the solution with copper. 18 days vegetation. Values for form B in percentage of values for form A_{Cu}. 1. Dry mass of mycelium. 2. Quantity of sugar used to produce 1 g dry mass of mycelium. 3. % N in mycelium. 4. % ash in mycelium. 5. Total acidity of solution after vegetation. 6. % Cu in mycelium.

The comparison of the mycelium produced on the solution with copper both by forms A_{Cu} and B with form A from the normal solution shows that in spite of a much longer vegetation both forms on the solution with copper produced a smaller mass of mycelium. It proves distinctly the toxic operation of the used concentration

of copper salt although the intensity of this operation on the two forms was not the same. From the comparison of the percentage of contents of the dry mass we see that the mycelium produced on the solution with copper by one form or the other contained a lower percentage of dry mass than mycelium A on normal solution. The reaction of the solutions shows certain differences. The

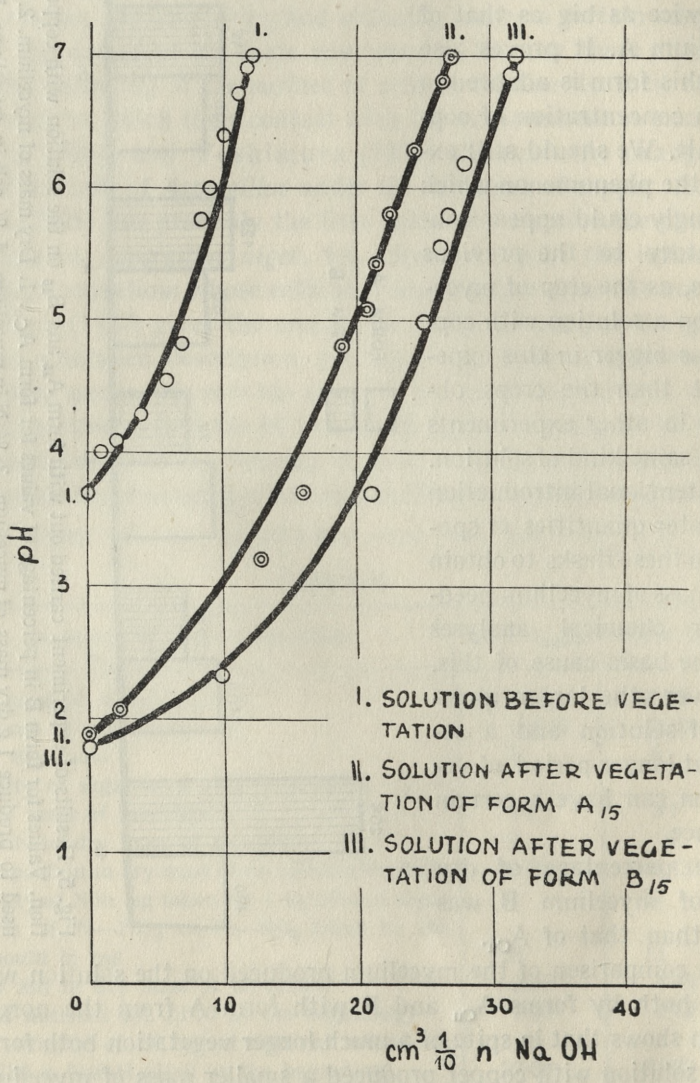

Fig. 6. Curves of electrometric titration of nurient solutions containing copper.

pH of the nutrient solution on which form B developed was lower, and the total acidity of solution B was slightly higher. The curves of electrometric titration of the solution before vegetation and after vegetation of the form A_{Cu} and B are presented on Fig. 6. These data show a greater production of acids by form B, in spite of the relatively smaller use of sugar.

Also in comparison with form A on a copperless solution, both forms on the solution containing copper lowered the pH more strongly and caused a rise in total acidity. The data given in literature relative to the influence of copper on the change of the pH of the solution do not agree. Thus, among others Mulder (1939), Fischer (1941), Roberg (1928) and Sakamura (1934) say that *Aspergillus* acidifies the substratum more strongly under the influence of copper. On the other hand the results obtained by Ono (1900) are opposite.

The quantities of sugar used to produce 1 g of dry mass of mycelium by form B are smaller than those needed by form A_{Cu}.

However the use of sugar by both forms on the solution with copper is relatively greater when compared with the use of sugar by form A on normal solution. Sakamura says on the other hand that *Aspergillus oryzae* took less sugar under the influence of copper and iron. The stronger combustion of the source of carbon as a result of the action of copper is so far probable, as this element catalyzes the oxidizing processes. Therefore in relation to *Aspergillus* Bortels (1927) put forth the hypothesis, which was confirmed by Gollmick (1935/6), that the suitable quantities of copper are a condition of the black colour of the spores, because this element catalyzes the process of oxidation which takes place when the black colour is produced. Many data on the catalyzing operation of copper in the processes of oxidation are given by the literature relating to the physiology and biochemistry of higher plants and animals.

The percentage of the contents of nitrogen in mycelium B is lower than in mycelium A_{Cu}. As to the form of the intaken nitrogen both forms are ammonophilic, and this characteristic is more pronounced in form A_{Cu}. The quantity of the intaken nitrogen for 1 g of used sugar is higher in form B.

In comparison with form A growing on a normal solution, both forms on a solution with copper contain a higher percentage of

nitrogen. Also the quantity of the nitrogen intaken for 1 g of used sugar is bigger on a solution with copper. Contrary to this, Mc Hargue and Calfee (1931) found that copper lowers the content of nitrogen in *Aspergillus flavus*.

The percentage of ash in the mycelium of form B is higher than that of mycelium A_{Cu}. In comparison with form A growing on a normal solution, both forms on the solution with copper contain a lower percentage of ash. McHargue and Calfee say that *Aspergillus flavus* contains more ash in the presence of copper.

The contents of copper in the dry mass of form B is three times greater than that in form A_{Cu}. The percentage of contents of this ingredient is very high, amounting to nearly 3% in comparison with quantities found for fungi by Ramage (1930), 0·002—0·02%. But this author investigated organisms developing in a normal environment and not on a substratum containing great quantities of this element. The amount of a given trace element in the substratum has a great influence on the contents of this element in the plant. Therefore the fact of a high percentage of copper in the contents of mycelium grown on a solution with a high concentration of this element is quite easy to understand. But the phenomenon of a greater accumulation of copper by the adapted form is rather unexpected. As this fungus gained resistance against copper, one could suppose that this resistance is based on the forming of a mechanism which lessens the penetration of the toxic metal into the cells. Perhaps, however, this element does not accumulate in the plasma, but only in the cell membrane. It is also possible that the mycelium produces some substances which precipitate copper on its surface immersed in the solution, in the form of insoluble compounds which are not toxic at all or only slightly toxic. The fact observed in many cultures i. e. the blue colour of certain fragments of the mycelium, would be an argument for this. The cultures showing a greater intensity of this phenomenon grew especially well. The forming of this substance, eliminating copper to a certain degree from the substratum, could be the cause, or one of the causes, of the adaptation of *Aspergillus* to the more difficult conditions of growth. As the percentage of copper in the mycelium was computed from the difference between its initial contents in the solution and its contents after the vegetation, it is hard to say whether this element was accumulated mostly in

the plasma, in the cell membrane, or on the surface of the mycelium as a precipitate. It would be interesting to investigate the localisation of copper in the particular elements of the cell by chemical microanalysis. Oxalic acid, among other things, could be the substance precipitating copper, the produced copper oxalate could settle on the surface of the mycelium or in the cell membrane. The greater production of acids and the appearance of oxalic acid in the adapted form confirm this supposition to a certain degree. This phenomenon agrees with the observations of Pulst (1902) and Wehmer (1902) who observed the precipitation of copper under the influence of oxalic acid, which was a product of the metabolism of *Aspergillus*.

B. THE GROWTH OF THE MYCELIUM ON SOLUTION CONTAINING DIFFERENT COPPER SALTS

In order to investigate the behaviour of both forms of *Aspergillus* on solutions containing other copper salts, the author prepared solutions containing copper sulphate, copper chloride and copper nitrate. The concentration of copper in all these solutions amounted to 0·1143%; it was the concentration to which *Aspergillus* B was adapted. Spores A_{25} were transplanted on the solutions and spores B_{25} on similar ones. The development of form B on all solutions independently of the anions was better than of the form A_{Cu}. The results of the experiment are given in Table III.

TABLE III

used copper salt	dry mass of mycelium in g	
	form $A_{25}Cu$	form B_{25}
copper sulphate	0·3355	1·5400
copper chloride	0·6098	2·0430
copper nitrate	0·4672	1·0950

These data show that *Aspergillus* adapted to a high concentration of copper sulphate, grows also much better in a high concentration of copper nitrate or chloride. Among the used salts copper chloride proved to be the least toxic. Similarly in the experiment of Pirschle (1936) the toxic action of copper chloride was slightly weaker than that of copper sulphate.

C. THE GROWTH ON SOLUTION CONTAINING THE SALTS OF OTHER METALS

In order to investigate the reaction of both forms to other trace elements acting toxically in high concentrations, solutions containing different minor elements were inoculated with spores of forms A_{25} and B_{25}. Each series of solutions contained a different metal in the concentration 0·1143% in the form of sulphate. The growth of both forms was visible on the second day on the solution containing zinc sulphate. Form B developed much more abundantly than A. At the time of collection mycelium B formed a white, abundant coat full of small strong folds. Form A on the contrary produced a small mass of mycelium growing in tiny tufts. Neither mycelium sporulated. Also on the mycelium containing manganese sulphates both forms began to grow on the second day. Their growth was quick and in both cases a large mass of mycelium was obtained. The mycelium of form A was compact, uniform, leather-like with a slightly flesh-coloured bottom and a white surface covered with a few brown spores. On the other hand mycelium B was all white, full of small, very strong folds, covered with few, but black spores. On the solution containing nickel sulphate form A did not develop at all. Form B began to sprout with great delay, only on the 4-th day. It developed so slowly that its mycelium was collected only after 15 days of vegetation. The mycelium formed a shred which did not cover the whole surface of the solution; it was partly immersed and partly protruding above the level of the liquid to a height of 3 cm. The sporulation was very abundant, the spores were black. The results of the experiment are given on Table IV.

TABLE IV

salt used	form A_{25}		form B_{25}	
	d. m. in g	pH	d. m. in g	pH
zinc sulphate — 6 days	0 4895	2·45	2·3460	1·75
manganese sulphate — 6 days	1 7958	3 52	2·1865	2·23
nickel sulphate — 15 days	0		1·1638	2·08

As we see from these data, the crop of the dry mass of mycelium is bigger for form B than for form A. In the case of nickel form A

did not give any crop, while the crop of form B was relatively large. Of all the salts used nickel proved to be the most toxic, zinc less toxic, manganese toxic to a very small degree because even form A developed well in its presence. These observations agree with many other works relative to the toxic operation of metals. Thus it was found that *Aspergillus* can grow, though slowly, in 40% solution of manganese salts.

The fact that the form of *Aspergillus* adapted to 0·1143% of copper seems to be also adapted to the same concentration of other metals is very interesting. It is proved distinctly by the surplus of crops in relation to the non-adapted form, by making growth possible in the presence of nickel, which when used in a large dose proved deadly to the non-adapted form. This phenomenon, investigated in detail, could serve to explain the nature of adaptation to copper. One might suppose that the form adapted to living in a medium containing a relatively high concentration of copper sulphate, was able to produce (in defence against its toxic action) a certain mechanism lessening the porosity of the plasmatic membrane, in consequence of which the penetration of copper was hindered. If this supposition was correct the contents of copper in the mycelium of the *Aspergillus* resistant to copper should be smaller than in the mycelium of the non-resistant *Aspergillus*. But the investigation of the contents of copper in the mycelium of both forms showed that form B contained a much higher percentage of this metal. This fact would not contradict our supposition if we assumed that the absorbed copper had been retained in the cell membrane, and that it had not penetrated into the plasma. The lessening of porosity could explain the way in which *Aspergillus* became resistant to copper, and, moreover, it is probable that this phenomenon can also concern other metals, owing to which the form adapted to copper becomes resistant also to them. One could look for another explanation in the change of the shape of the cells of the adapted form, as a result of which the contact of the cell with the surrounding medium is decreased. The supposition that an operation of long duration of such a harmful element as copper has produced a form more resistant to all conditions unfavourable to its growth does not seem very likely, but the two following experiments were devoted to the explanation of this.

D. THE GROWTH IN A STRONGLY ACIDIFIED MEDIUM

Because all the experiments showed that form B acidifies the substratum more strongly than form A, the growth of both these forms on a solution acidified with sulphuric acid to pH 1·4 was investigated. The vegetation proceeded for 9 days. The growth of both forms which was slow and gave a rather small mass of mycelium, began on the third day after the transplantation. Form A produced a very thin, white mycelium which did not cover the surface of the whole solution; no spores being produced. Form B gave a slightly thicker mycelium with an uneven surface of a slightly yellow hue, spores were not produced. The results of the experiment are given in Table V.

TABLE V

	form A_{25}	form B_{25}
dry mass of mycelium in g	1·0221	1·5507
pH of the nutrient solution (1·4 at beg.)	1·28	1·20

From these data we see that the adapted form, which acidifies the substratum more strongly, growing on a solution with pH equal to 1·4 gave a larger crop of dry mass of mycelium. Neither forms produced spores which is probably connected with the extremely acid pH of the substratum (Frey 1927). The crop surplus of mycelium B in relation to A could be caused by the fact that form B supports better the great acidity, because in producing itself greater quantities of acids it also grew accustomed to a lower pH. Another supposition is that this form had become more resistant to all unfavourable conditions owing to the previous, numerous transplantations on solutions containing copper in a toxic dose.

E. THE GROWTH IN A MEDIUM WITH A HIGH CONCENTRATION OF SUGAR

To see what would be the course of growth of the two forms on a solution with a high concentration of sugar, they were both transplanted on solutions containing 35 g of sugar in 50 cm^3 of the solution. The vegetation lasted 9 days. On the 9-th day on the solution with form B there appeared only loose fragments, not resembling mycelium in their appearance. The crop was so small

that it was impossible to determine the dry mass. Form A produced a weak, slimy coat which did not cover the whole surface of the solution. There was no sporulation. The results of the experiment are given in Table VI.

TABLE VI

	form A_{25}	form B_{25}
dry mass of mycelium in g	0·7877	not determ.
pH of nutrient solution (initially 4·00)	2·28	2·20

The results of this experiment prove that the form adapted to copper did not become resistant to all factors hindering the growth of *Aspergillus*. We see that on the contrary it does not bear a great concentration of sugar so well as the non-adapted form which is shown by the much weaker growth. A closer investigation of this phenomenon would lead perhaps to the knowledge of other differences between the two forms not taken into account in this work.

It is interesting that the pH of the solutions after the vegetation of both forms is very similar in spite of the weak growth of form B. It may prove that this form really became capable of lowering the pH to a greater degree. As in the previous experiments the acidity of the substratum was parallel to the better growth of the mycelium it could lead us to suppose that these two phenomena, are related, so that lower pH was caused by a greater growth of fungi, a greater living mass producing acids.

Investigations on the degree of fixation of adaptability of *Aspergillus niger* and the characteristics of de-adapted form

A. VEGETATION OF ADAPTED AND NON-ADAPTED FORMS ON A COPPERLESS SOLUTION

In order to find whether the changes caused by numerous transplantations of *Aspergillus* on solutions containing copper are retained when this form adapted to copper is transplanted on to a normal solution, the following experiment was conducted. One series of copperless solutions was inoculated with spores coming

from mycelium of the A_{15} and the second series from B_{15}. The vegetation proceeded for 8 days.

a) The course of vegetation

The growth began on the 2-nd day after inoculation in both mycelia, but it was not identical. Form A produced at first a thin coat of mycelium extending over the whole surface of the solution. During growth this coat became thicker and thicker and slightly folded. Sporulation began on the 5-th day. However, form B formed at first a mycelium in the shape of little colonies resembling triangles and trapezia in their outline. These colonies grew and join together so that in the end the whole surface of the solution was covered with a coat of mycelium. This mycelium, however, did not sporulate. The differences in the growth of the two forms are shown on photograph 11 made on the 4-th day of the vegetation.

b) Morphologic characteristics

When it was collected, the mycelium of form A formed an abundant white coat covered with rather few spores. Form B also gave an ample white mycelium which differed from the previous one in that it was full of small deep folds and it was not covered with spores. These differences are shown in photographs 12 and 13 made after the collection of mycelium.

These observations show that the basic difference in the appearance of mycelia A and B was the lack of spores of mycelium B. Because of this it is possible to suppose that the influence of copper on the previous generations had deprived *Aspergillus* of the capacity of sporulation. That this is not true we know from the previous series of experiments, where *Aspergillus* was bred on a solution with a concentration of 0·449% of copper sulphate for 25 generations, and each of these generations formed spores which rendered transplantations possible. One might suppose that the fungus which had grown accustomed during several generations to a large concentration of copper salt, and which was deprived of this salt in this generation would not be able to sporulate. We know from literature (Mulder 1939) that a complete lack of copper in the medium prevents the forming of spores. In this experiment the solution contained traces of this element in the form of impurities, but these small quantities could be unsufficient for a form accusto-

med to large quantities. This supposition is not correct either since it was proved that if such non-sporulating cultures are left for a long time they begin finally to sporulate. Perhaps the action of copper on the previous generations caused a certain delay in sporulation in an organism growing already on the normal solution. This phenomenon can also be explained in another way. As the reaction of the nutrient solutions of form B was very acid, the mould could not sporulate just because of this. (Frey 1927). After a certain time when the acids were burnt, in the lack of sugar, which could be already used up, and when the pH rose, *Aspergillus* began to sporulate. The lack of sugar and a higher pH in the nutrient solutions of old, already sporulating cultures would be a confirmation of this supposition, but investigations to ascertain this were not carried out.

In order to investigate the structure of the mycelium both forms were examined under the microscope. Form A presented a structure of mycelium characteristic for *Aspergillus*. Again, form B differed in that its cells were shorter and wider, circular, sometimes of irregular shape. These differences are illustrated in photographs 14 and 15.

c) The crop of mycelium and the metabolism

TABLE VII

Analysis of crop of mycelium	form A_{15}	form B_{15}
fresh mass of mycelium in g	20·97	31·32
dry mass of mycelium in g	3·2010	3·4746
% of dry mass	15	11
quantity of sugar in mg used to produce 1 g of dry mass of mycelium	3·0	2·6
% of N in dry mass of mycelium	4·8	3·8
% of protein in dry mass of mycelium (N×6·25)	30·0	23·75
quantity of N in mg taken for 1 mg of used sugar	17	14
surplus of N—NH_4 on N—NO_3 intaken by the mould in mg	17	35
% of ash in dry mass of mycelium	4·61	6·30
pH of nutrient sol. (4·10 initially)	2·22	1·60
total acidity of 30 cm^3 of nutrient sol. in cm^3 1/10 n NaOH (7·90 initially)	21·50	63·40

Fig. 7 presents values relative to form B in relation to the values of form A taken as 100.

The data given in this table and on Fig. 7 show that the crop of the dry mass of mycelium B was 0·27 g larger than the crop of mycelium A. This proves that the toxic influences acting on the previous generations did not weaken the capacity of the mould for growth. A certain slight excess of the crop of mycelium B may be caused by the fact that the spores of this form, produced on a substratum containing a large quantity of copper, contained a certain amount of this element. Steinberg (1935) calls our atten-

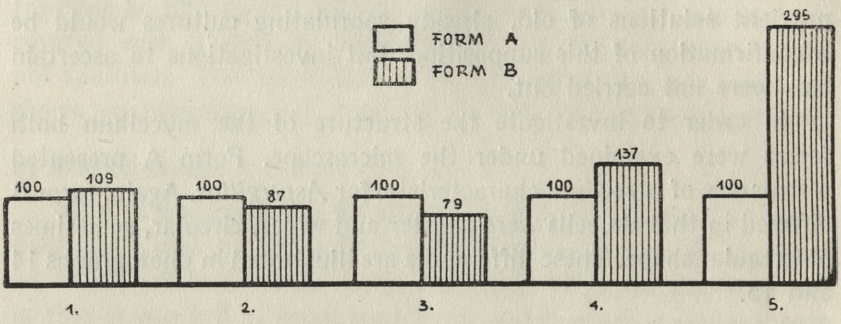

Fig. 7. Results of experiment carried out with form A_{15} and B_{15} on copperless solution. 8 days vegetation. Values for form B in percentage of values for form A. 1. Dry mass of mycelium. 2. Quantity of sugar used to produce 1 g dry mass of mycelium. 3. % N in mycelium. 4. % ash in mycelium. 5. Total acidity of solution after vegetation.

tion to the presence of copper in the spores. The copper which was in the spores might stimulate the growth of the mould and increase its crop. Perhaps the traces of copper present in the solution as impurities were lower than the optimal ones, so that the introduction of this trace element with the spores gave a surplus of the produced mycelium. It is not impossible that this form, poisoned for several generations, fighting with the environment, produces as a result of this a stronger organism, which grows better already in normal conditions.

As to the content of the dry mass, it is lower in the mycelium of form B.

Great differences are shown in the two forms as to the presence of acids in the nutrient solution. Form B acidifies the substratum

more, this being shown by the lower pH. The total acidity of this nutrient solution is much higher, almost three times so, than that

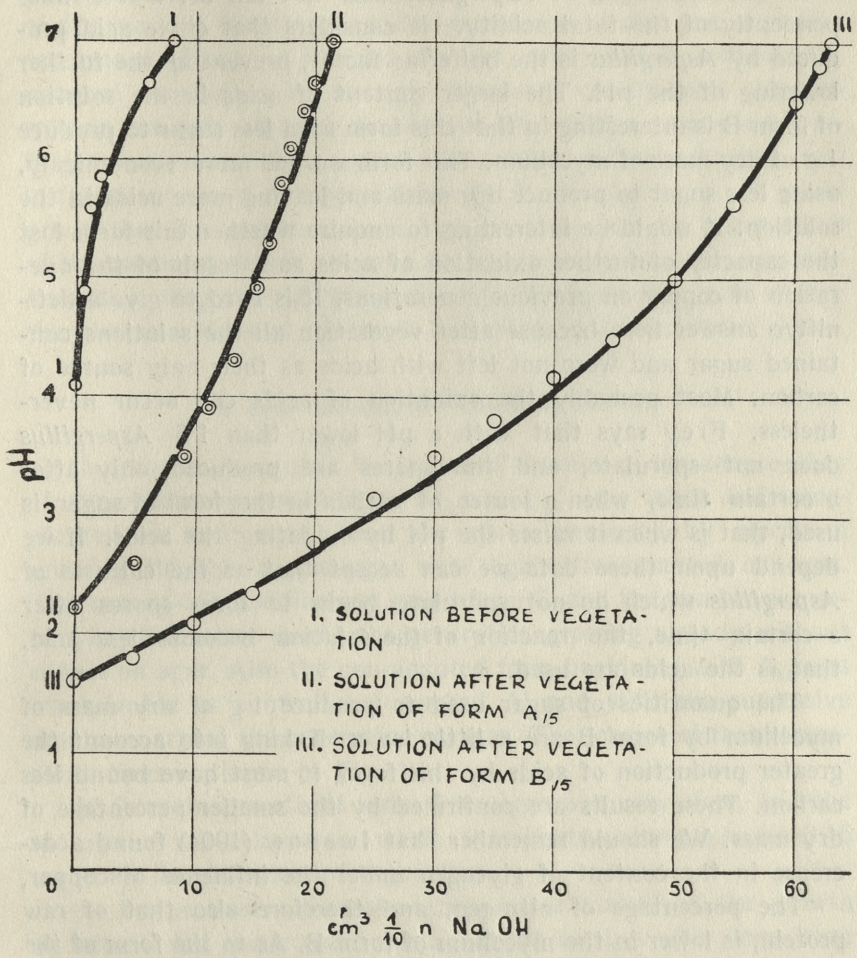

Fig. 8. Curves of electrometric titration of copperless nutrient solutions.

of the nutrient solution of form A. Fig. 8 shows the curves of electrometric titration of 30 cm of the nutrient solutions before and after the vegetation of forms A and B.

That differences of total acidity are so great in relation to the differences of pH is a striking fact. We have to take it that the solution of form B was much more strongly buffered. A certain

product (acids) of the metabolism of the mould must have been the buffering factor. Frey (1927) says that the pH of the solutions after the vegetation of *Aspergillus* does not fall below 1·4 independently of the total acidity. He considers that citric acid produced by *Aspergillus* is the buffering factor, preventing the further lowering of the pH. The larger content of acids in the solution of form B is interesting in that this form used less sugar to produce 1 g of dry mass of mycelium. This form worked more economicaly, using less sugar to produce dry mass and leaving more acids in the solution. It would be interesting to enquire whether this form lost the capacity of further oxidation of acids as a result of the operation of copper on previous generations. It is hard to give a definitive answer here because after vegetation all the solutions contained sugar and were not left with acids as their only source of carbon. Most probably the oxidation of acids can occur nevertheless. Frey says that with a pH lower than 1·6 *Aspergillus* does not sporulate, and the spores are produced only after a certain time, when a source of carbon in the form of sugar is used, that is when it raises the pH by oxidating the acids. If we depend upon these data we can accept that as the cultures of *Aspergillus* which do not sporulate begin to form spores after a certain time, the reaction of the solution becomes less acid, that is the acids are used.

The quantities of sugar used to produce 1 g of dry mass of mycelium by form B are a little lower. Taking into account the greater production of acids by this form, it must have bound less carbon. These results are confirmed by the smaller percentage of dry mass. We should remember that Iwanow (1904) found a decrease in the content of glycogen under the influence of copper.

The percentage of nitrogen, and therefore also that of raw protein, is lower in the mycelium of form B. As to the form of the intaken nitrogen, both forms proved to be ammonophilic, the difference between the amount of the intaken ammonium nitrogen and nitrate nitrogen was twice as great for form B as for form A.

The percentage of ash in the mycelium of form B was higher than in mycelium A.

Comparing the differences between the metabolism of forms B and A on a copperless solution with the differences of these two forms on a solution containing copper, we can observe that they

all go in the same direction, showing a certain correlation. In both cases form B gave a larger crop of fresh and of dry mass, a lower percentage of dry mass, it used less sugar to produce one gramme of dry mass, it contained a lower percentage of nitrogen, a greater percentage of ash, it lowered the pH more, and it buffered the nutrient solution more strongly. Contrary results were obtained only in the fact that the amount of the intaken ammonium nitrogen was greater than that of the intaken nitrate nitrogen, and in the quantities of nitrogen intaken for 1 g of used sugar. It would prove that the external conditions (in this case the composition of the medium) decide not only the characteristics of the organism on which they are operating directly but that they also change the characteristics of the next generation, obtained from spores.

B. THE COURSE OF DE-ADAPTATION

In order to investigate the degree of fixation of the adaptability of *Aspergillus*, spores of forms A_{15} and B_{15} were taken and transplanted on slant agar. Then the spores produced by mycelium growing already on the agar were again transplanted on agar. This new de-adapted line was called form C, and the number accompanying this letter marks the generation growing already without copper on agar. Also the non-adapted form beginning with A_{15} was transplanted as control and marked A' and with the successive number marking the generation on agar. The scheme of these transplantations is shown in Fig. 1.

At first the growth of form C on agar was very slow, and the sporulation was delayed and weak. It is illustrated in photograph 16 made on the 4-th day of vegetation.

As the successive transplantations were carried on, the growth of form C was more and more abundant so that after some time it equalled the growth of form A'. However the sporulation was still delayed and weaker.

From each generation of A and C spores were taken and a solution containing 0·449% of copper sulphate was inoculated with them. The vegetation proceeded for 17 days after which the dry mass of the produced mycelium was determined. The results are given in Table VIII, only form C being taken into account, the dry mass of form A'_{Cu} oscillated round 0·2 g.

TABLE VIII

generations	dry mass of mycelium in g
B_{15}	1·7793
C_1	1·7987
C_2	1·7229
C_3	1·5592
C_4	1·3547
C_5	1·3947
C_6	1·0000
C_7	0·8000
C_8	0·9630
C_9	0·6607
C_{10}	0·4820
C_{11}	0·6500
C_{12}	0·4002
C_{13}	0·3200
C_{14}	0·1901
C_{15}	0·3420

The curve showing the course of de-adaptation of *Aspergillus* to the previously used concentration of copper is shown in Fig. 9.

As we see from these data *Aspergillus* gradually becomes disaccustomed to the concentration of copper amounting to 0·449% of copper sulphate. The greater the number of generations which grew on a copperless substratum, the smaller was the crop of mycelium grown on a solution containing this element. We should note, however, that the oscillations in the obtained crop were relatively great, both for the adapted and for the non-adapted form. The computation table gives only arithmetical means, but we should take it that these data contain a relatively high experimental error in spite of preserving the same conditions of vegetation. Probably the quantity of spores introduced into the solutions has an influence on this. However, it was impossible to avoid this by transplanting the suspension of spores, because the spores used to inoculate the series which were to be compared came from different cultures. A similar decline on resistance to copper salt was found by Pulst (1902) in *Penicillium*. He conducted his experiment for five gene-

rations and his observations concerned only the capacity of spores for germination.

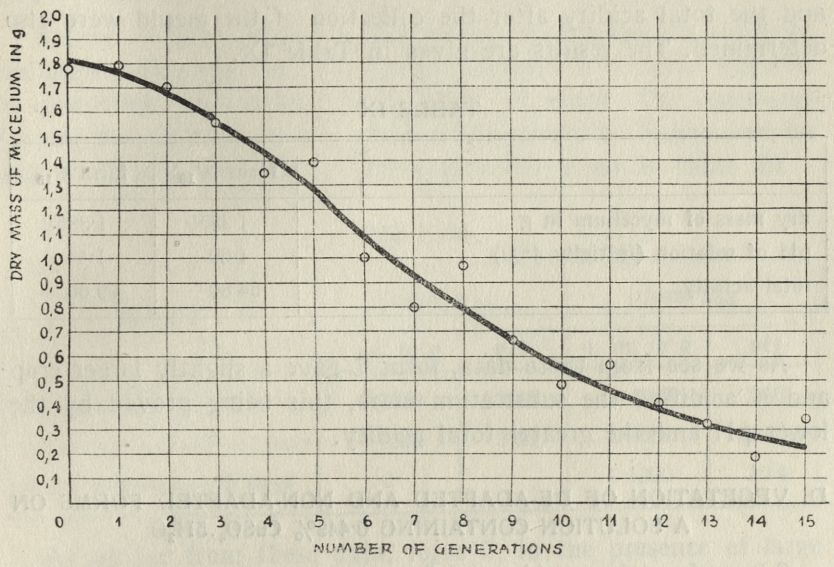

Fig. 9. Curve illustrating course of de-adaptation of *Aspergillus* from medium containing 0·449% $CuSO_4.5H_2O$.

To see whether with the decline of resistance to a great concentration of copper salt other changes in the growth, morphology and metabolism decline also and whether *Aspergillus* after a certain period of de-adaption returns to its starting point, the following experiments were conducted:

C. VEGETATION ON A COPPERLESS SOLUTION

Spores of forms A'_{10} and C_{10} were introduced into the copperless solution. The vegetation was carried on for 7 days. The growth of the mycelium began on the 2-nd day and its course was similar in both forms. After some days certain differences could be seen. Mycelium C had deeper folds, and it sporulated more weakly. It is illustrated by photograph 17 made after the collection of mycelium.

Differences could also be seen in the microscopic image. The cells of form C were less regular, the hyphae of the mycelium more branched. This is illustrated by photograph 18 made on the 2-nd day of vegetation.

Besides the investigations of the appearance and the morphological structure of the mycelium the dry mass of the crop, the pH and the total acidity after the collection of the mould were also determined. The results are given in Table IX.

TABLE IX

	form A'_{10}	form C_{10}
dry mass of mycelium in g	1·2855	1·3858
pH of solution (initially 4·00)	1·60	1·44
total acidity	64 80	99·00

As we see from these data, form C gave a slightly larger crop and it acidified the substratum more, this being proved by the lower pH and the greater total acidity.

D. VEGETATION OF DE-ADAPTED AND NON-ADAPTED FORMS ON A SOLUTION CONTAINING 0·449% $CuSO_4.5H_2O$

Spores from forms A'_{10} and C_{10} were transplanted on solutions with copper. The vegetation proceeded for 17 days. The growth of form C was slightly stronger. The results are given in Table X.

TABLE X

	form A'_{10Cu}	form C_{10Cu}
dry mass of mycelium in g	0·1748	0·3815
pH of solution (initially 4·5)	2·43	2·09
total acidity	24·3	27·9

As we see from these data, form C gave a slightly larger crop of the dry mass of mycelium and it acidified the substratum more strongly.

Similar experiments were carried out with forms C_5 and C_{15}. In comparison with A'_5 and A'_{15} they showed that both forms C acidified the media more strongly both on a solution with copper and on a copperless one. What is more, the capacity to acidify the solution did not diminish gradually during the de-adaptation to copper.

E. GROWTH OF FORMS A' AND C ON A SOLUTION CONTAINING SALTS OF OTHER METALS

Spores of forms C_{10} and A'_{10} were transplanted on solutions containing zinc sulphate, manganese sulphate and nickel sulphate in quantities corresponding to 0·1143% of metal. The vegetation for the first two metals was allowed to proceed for 6 days and for nickel sulphate for 15 days. The results are given in Table XI.

TABLE XI

used salt	form A'_{10}		form C_{10}	
	d. m. in g	pH	d. m. in g	pH
zinc sulphate — 6 days	0·2768	2·45	0·4360	2·00
manganese sulphate — 6 days	1·7658	3·50	1·8658	2·40
nickel sulphate — 15 days	0		0·1300	2·14

As we see from these data, form C, in the presence of large doses of toxic metals, gave a slightly larger mass of mycelium than the mass of mycelium of form A' and it acidified the substratum more strongly. In this experiment manganese sulphate proved to be the least toxic, zinc sulphate more toxic and nickel sulphate acted most toxically. In the presence of nickel form C did not sporulate although it produced weakly developed colonies of mycelium. Form A did not develop at all.

F. GROWTH OF FORMS A AND C IN A MEDIUM WITH A HIGH CONCENTRATION OF SUGAR

The spores of forms C_{10} and A'_{10} were transplanted on a solution containing 35 g of sugar (in 50 cm^3). The growth of form A' was more abundant, but form C also produced a thin coat of mycelium. There was no sporulation. The results are given in Table XII.

TABLE XII

	form A'_{10}	form C_{10}
dry mass of mycelium in g	0·7527	0·4722
pH of solution	2·20	1·95

These data show that form A' grew better on a solution containing a great concentration of sugar. Form C, however, acidified the substratum more strongly.

The above experiments prove that the form de-adapted to copper loses its resistance to the toxic operation of this metal but it does not return, at least for 10 or 15 generations, to its initial form. The appearance of the mycelium, the shape of the cells, the greater production of acids, greater resistance to the operation of other metals and the weaker growth in the concentrated solution of sugar make it similar to the form adapted to copper. It is difficult to compare the intensity of these characteristics, because the de-adapted form (C) is derived from the 15-th generation of the adapted form (B_{15}) and was de-adapted to copper only for ten generations, while most of the experiments concerning the characteristics of the adapted form, relate to the 25-th generation of the adapted form (B_{25}).

Summary of results

The results of the experiment based on A) successive transplantations of *Aspergillus* on solutions containing 0·449% of copper sulphate in order to adapt it to this salt, B) successive transplantations of the adapted form on copperless solutions in order to de-adapt it, and C) the investigation of the characteristics of these forms can be formulated in the following points:

1. The use of a concentration of copper sulphate of 0·449% acts very toxically on *Aspergillus*; we see this in the weak and delayed growth, weak and delayed sporulation and small crop of the produced mycelium.
2. *Aspergillus* bred for many generations in a medium containing the above concentration of copper sulphate gradually adapts itself to it. The tempo of adaptation is quicker at first, afterwards slowing down. *Aspergillus* successively transplanted on solutions with growing concentrations of copper sulphate, becomes so resistant to the toxic action of this salt that it develops rather well in a medium saturated with this salt, producing a white mycelium and sporulating.
3. The form adapted to 0·449% of copper sulphate is different from the non-adapted form growing on a solution with the same concentration of copper, in its morphology, physiology

and biochemistry. These differences seem to be the cause in certain cases of a greater resistance of the organism. The long operation of copper has perhaps shifted the metabolism of the organism, creating a balance between it and the medium surrounding it, securing better development.

4. The form adapted to copper becomes at the same time resistant to the action of other salts of copper such as copper nitrate and copper chloride.
5. This form also became resistant to the operation of other metals such as zinc, nickel and manganese. From these, nickel proved to be the most toxic, zinc was less toxic and manganese acted the most weakly in the used concentration.
6. The form adapted to copper sulphate bears better a great concentration of hydrogen ions, giving a larger mass of mycelium than the non-adapted form on a solution with pH amounting initially to 1·40.
7. The adapted form grows much more weakly than the non-adapted form on a solution containing a high concentration of sugar.
8. The adapted form transplanted on a copperless solution differs from the non-adapted form growing on a solution of the same kind, and these differences are mostly the same as the differences between the two forms on a solution containing copper.
9. As a result of successive transplantations on copperless solutions the form adapted to copper loses its resistance to the toxic operation of this element.
10. The form de-adapted for 15 generations does not return to its original form, it keeps certain morphological, physiological and biochemical characteristics.

The above work was carried out in the Department of Agricultural Chemistry of the Jagiellonian University. I am indebted to Professor T. Lityński for his kind help and guidance while I was carrying out experiments and writing the present paper.

I should also like to thank Dr J. Zurzycki for making the photographs.

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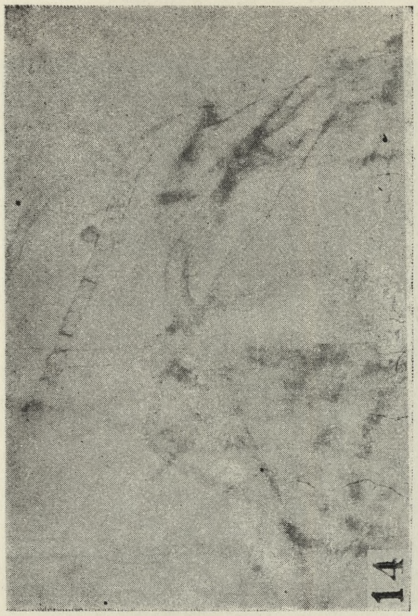

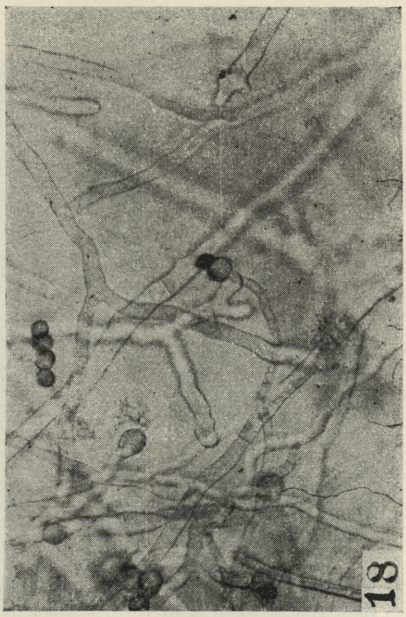

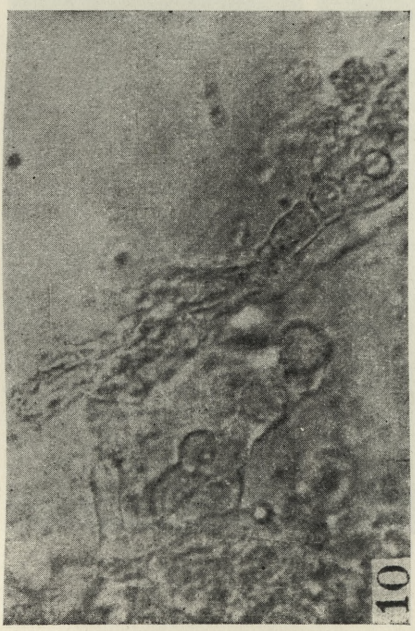

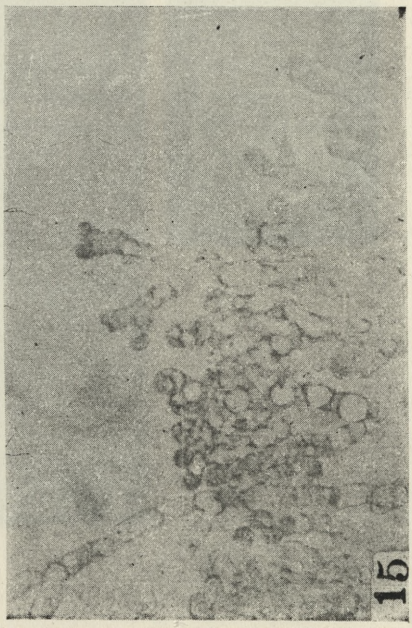

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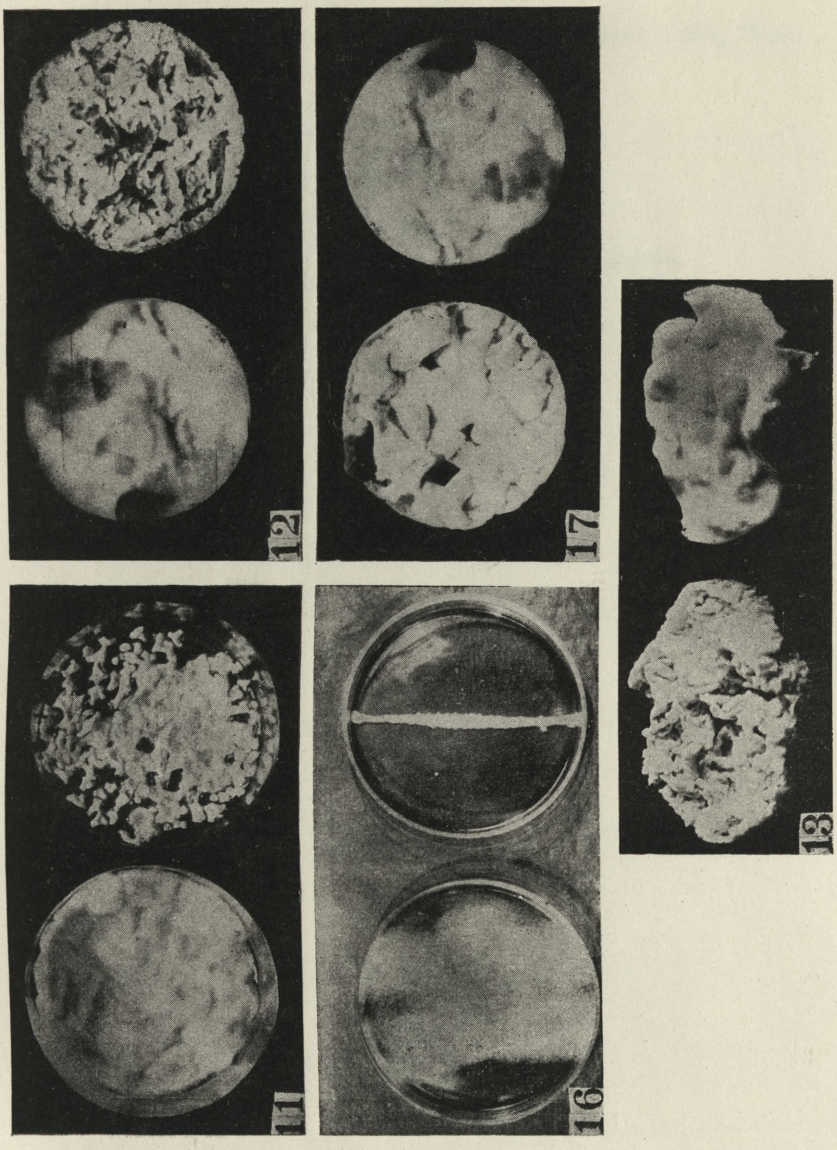

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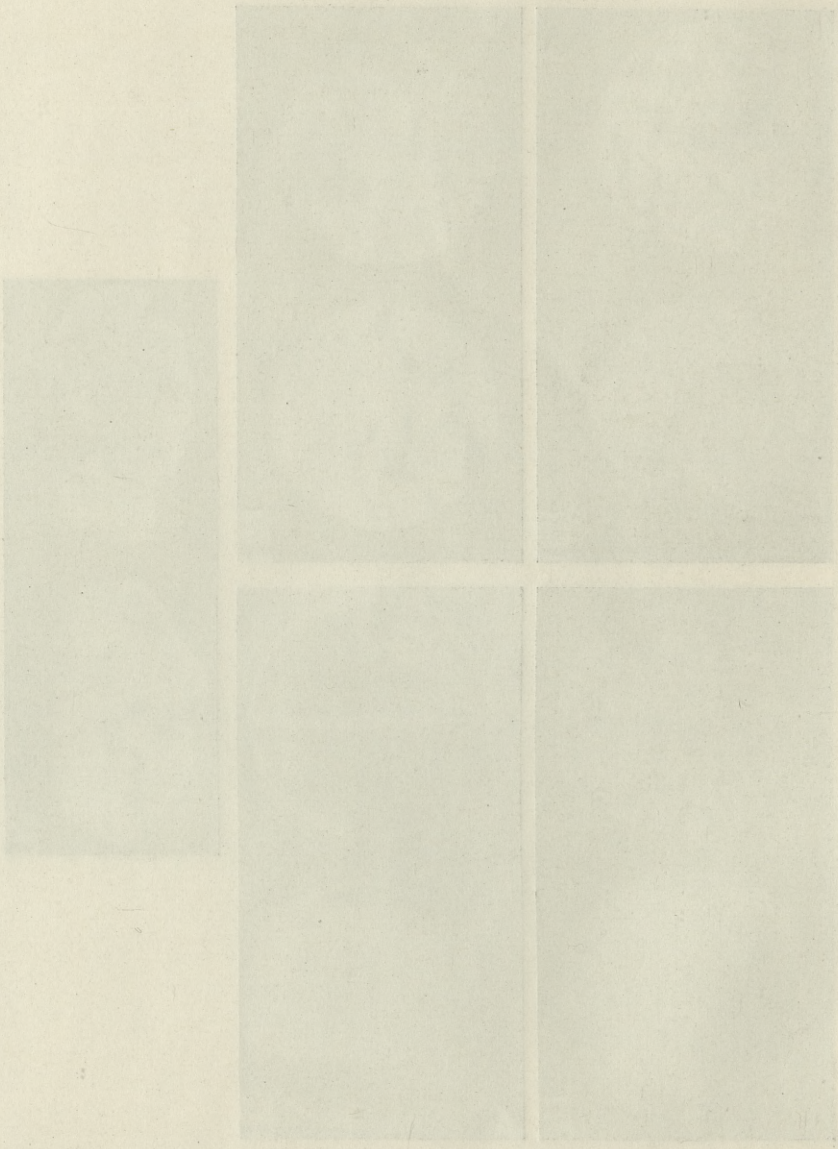


Explanation of plates

Fig. 10. Microscopic image of form B_{15} in solution with 0·449% $CuSO_4.5H_2O$.
Fig. 11. Forms A_{15} and B_{15} in copperless solution.
Fig. 12. Forms A_{15} and B_{15} in copperless solution.
Fig. 13. Forms B_{15} and A_{15} in copperless solution.
Fig. 14. Microscopic image of form A_{15}.
Fig. 15. Microscopic image of form B_{15}.
Fig. 16. Forms A' and C_1 on agar.
Fig. 17. Foms C_{10} and A'_{10} in copperless solution.
Fig. 18. Microscopic image of form C_{10} in copperless solution.

Z zagadnień istoty działania jonów molibdenu na przemianę materii w komórce roślinnej. — Problems concerning the activity of molybdenum on metabolism in plant cells.

Mémoire

de M[lle] **A. KOZŁOWSKA**

présenté le 7 Mai 1951 par Mlle A. Kozłowska m. c. et M. B. Pawłowski m. c.

(Plates 10—12)

Contents:

1. Absorption of Mo by plants cultivated in water cultures without nutrients

An attempt has been made to show the direct influence of metabolism in plants by means of water-cultures containing in their solutions one single salt. As a rule such solutions hindered the development of the plant, in many cases they acted toxically. Pirschle (1930) applied this method, using water-cultures which contained single 1/50 n solutions of metal salts arranged in the successive order of the periodical system of elements. He was to

a certain extent justified in this. The metals which belonged to the 3rd and 4th period, placed in the centre of the periodical system, displayed the weakest toxic activity of such indispensable elements as: Mg, K, Ca, Fe as well as of those which are often a ballast, and finally microelements. Molibdenum stands in a more distant position in the first range of the 5th period. Concerning the ranges in the periodical system Frey-Wyssling (1945) points out that all the elements necessary to plants stand along the line which runs across 8 ranges from Argon to Coal.

Solutions with only 2 kinds of ions belonging to one salt increase the permeability of the cytoplasm. Consequently, when the nutrient contains ions of a single metal, those ions are absorbed by the plant with greater intensivity than when those ions are present in the solution together with other ions (Osterhout 1930). According to the toxic activity of the added metal ions the death of the cytoplasm follows sooner or later, losing its half-permeability qualities. The reaction of the plant and the quantity of the metal ions singly absorbed by it indicate its direct toxic activity on the cell cytoplasm.

In order to state to what degree molybdenum ions act directly toxically on plants, I carried out several water cultures, which contained exclusively natrium molybdate in a concentration of 2 g and 1 g of natrium molybdate per litre. We used cucumbers for these experiments. The experiments were carried out in a hothouse in March and May.

The quantity of molybdenum in the plant in relation to the dry mass was determined by colorometric method, described by Thiele (1939).

I st experiment. On the 1st March the seeds of cucumbers were sown on muslin in a glass pot. On the 9th March the seedlings were placed in two litre glass pots filled with full water nutrient. On the 28th March the plants had two green cotyledons and the two first leaves developed to a length of 4 cm. On the 28-th March at 9 o'clock in the morning the plants were transferred to 2 litre glass pots, filled with water containing 2 g natrium molybdate per litre. The solution of pH=7 reached the stalk. After 8 hours the first series of plants was taken out of the pots and after 24 hours the second, in order to carry out an analysis of molybdenum in the plant tissues. The plants showed no toxic symptoms whatever.

Mo was separately determined in the roots as well as in the aerial parts. Taking the plants out of the pot after 8 hours for a chemical analysis I transferred at the same time a part of those plants into two different full nutrients, containing N in the form of nitrate and ammonium form.

TABLE I

28. III.	8 hours in Mo	48 hours in nutrient NO_3	48 hours in nutrient NH_4	24 hours in Mo
Root — Mo	0·15%	0·12%	0·12%	0·34%
Stalk, leaves — Mo	0·085%	0·09%	0·08%	0·15%

This table shows that young plants kept for 24 hours in water with Mo, contained in roots and stalks about twice as much molybdenum as those kept in the same conditions for 8 hours. No symptoms of poisoning could be observed. Plants kept for 8 hours in molybdate-water aud brought for 2 days into full nutrient, continued to develop normally. The percentage of Mo in the roots diminished a little, in the stalks and leaves it remained the same, whereby the whole mass of the plant increased.

IInd experiment. The plants were in a younger stage of development than the previous ones. One leaf developed to a length of 3 cm. Half the plants were kept for 24 hours in water with 1 g of natrium molybdate per litre. The other half in a full nutrient with 1 g of natrium molybdate per litre. One part of the plants of the first series was put after 24 hours for 3 days into a different full nutrient with nitrogen in the form of nitrate and ammonium. Table II shows the results of the analyses.

TABLE II

I. Water with natrium molybdate, 1 g per litre

15. V.	16. V. 24 hours in Mo	19. V. 3 days in nutrient NO_3	19. V. 3 days in nutrient NH_4
Root — Mo	0·69%	0·0336%	0·0348%
Stalk, leaves — Mo	0·045%	0·0912%	0·0976%

II. Full nutrient with 1 g natrium molybdate per litre

15. V.	16. V. 24 hours in Mo
Root — Mo	0·212%
Stalk, leaves — Mo	0·06%

The roots of the plants of the water culture which contained a single molybdenum salt, absorbed after 24 hours three times as much of this element, as when the water contained at the same time a full nutrient. The semi-permeability of the cytoplasm increased under the influence of the singly acting molybdate ions. The toxic influence was not great, since the plants began to develop normally when brought into the nutrient for three days; the molybdenum ions moved partly into the stalks and leaves. (Fig. 2).

III[rd] experiment. Plants cultivated in a full nutrient in a later stage of development than those of the I[st] and II[nd] experiment. They had 3 leaves 8 cm long. 24. V. half the plants were placed into a full nutrient (nitrogen in form of nitrate) with 1 g of natrium molybdate per litre. The second half was put into pure water containing 1 g natrium molybdate per litre. Two analyses were carried out: after 24 hours and after 4 days. The plants taken out after 24 hours were put into a normal nitrate nutrient. Plants kept in molybdate water for four days showed no symptoms what ever of poisoning, but the leaves became yellowish. Table III shows the results of the analyses.

TABLE III

I. Water with natrium molybdate 1 g per litre

24. V.	24 hours in Mo	3 days in nutrient NO_3 without Mo	4 days in Mo
Root — Mo	0·5%	0·15%	1·016%
Stalk, leaves — Mo	0·09%	0·03%	0·218%

II. Full nutrient — 1 g molybdate per litre

24. V.	24 hours
Root — Mo	0·3%
Stalk, leaves — Mo	0·04%

After 24 hours the plants in a later stage of development absorbed from the molybdate water only twice the amount of Mo ions as when the molybdate was in a full nutrient. Plants cultivated in molybdate water placed again in full nutrient developed normally, so that in comparison to the dry mass Mo showed a smaller percentage. As in the II-nd experiment the percentage of Mo diminished considerabely in the roots.

The chemical analyses of plants, which were kept for 4 days in molybdate water proved to be very interesting. Although the roots contained 1% Mo, the plant did not fade and developed normally.

All these experiments showed a strikingly weak toxic activity of Mo ions when those ions were absorbed by plants from a water culture without any other salts.

2. **Absorption of Mo from soil**

Molybdenum is an element which, unlike Zn and Pb, does not appear anywhere in soils in great quantities. Yet it appears in a minimal percentage in all soils. According to Winogradov (1948) the percentage of molybdenum in ZRSS soils oscillates between $1{\cdot}5.10^{-4}$ to $1{\cdot}2.10^{-30}\%$. It is absolutely indispensable to plants, because a lack of this element in the soil causes in plants different symptoms of disease, among them a disease of cauliflowers called «Whiptail» (Waring 1947, 1949), described in Australia. In spite of the minimal percentage of molybdenum in soils, Winogradowa (1943) demonstrated that the plant had the capacity to gather this element in its tissues. The medium percentage of molybdenum in seeds of grass amounts to $4{\cdot}6.10^{-5}\%$. With papilionaceus plants it is much higher, namely $5{\cdot}54.10^{-40}\%$. In the case of diseases caused by a lack of Mo in the soil the addition of a small amount of natrium molybdate or ammonium molybdate (Waring 1947,

Wilson a. Waring 1948) sufices to effect a normal development of the plant. Many water culture experiments confirm this fact (Arnon 1939, Warrington 1946). 10 γ of molybdate added to 1 litre of full nutrient are completely sufficient for a normal development of the plant. A lack of that minimum amount causes symptoms of disease.

Conversely, it proved that very great amounts of Mo added to the soil did not act toxically. Warrington (1937) used in pot culture experiments with *Solanaceae* 1 g of molybdate for 10 kg of soil. Yet the plants did not show any symptoms of disease. Bobko and Sawin (1940) quote in their experiments with peas that the plants developed normally in a soil with a large amount of molybdenum.

In my first experiments doses of 2 kg ammonium molybdate per 10 kg soil caused in the plants such first symptoms as the stopping of growth, earlier blossoming, change of leaf colouring, etc. Together with these symptoms we also observed changes in the proteins (Kozłowska 1947). The proteins extracted from plants watered with large doses of molybdate showed a different quality, above all a specific serological reaction with antiserum virus X. Experiments carried out by me year by year since 1946 in different soil conditions with plants watered with ammonium molybdate, showed that the proteins obtained from them do not always possess different obtained qualities and do not always give the same distinct specific serological reaction as those from plants grown without Mo. Sometimes they gave none at all. The most important factors in these experiments were: the kind of soil, temperature, wetness, light, etc. To understand these facts it was necessary first to discover the quantity of molybdenum absorbed by the plant in various soil conditions, to show if the percentage of Mo contained in the plant undergoes any changes and if the quantity of molybdenum is in direct relation to the specific serological reaction of the proteins. The amount of molybdenum absorbed by the plant can depend as well on the kind of fixation of molybdenum ions by the soil in non replaceable form, as on the capacity of absorption of cations and anions by root hairs. The following experiments illustrate those relations. They were carried out in pots in greenhouses.

I^{t} experiment. During the experiments we changed the quantity of water in the soil. The plant: potato, variety President

The pots contained 8 kg of earth (3 kg of garden humus, 3 kg of sand and 2 kg of loam).

4. V. 20 tubers germinated in light were put into the pots.

23. V. haulms were 20 cm high and had 19 leaves.

From 23. V. to 14. VI. the plants were kept in as dry a condition as possible. They were watered every day in a hot and dry atmosphere in the greenhouse with 50 cm of water. Every other day we dissolved 0·3 g of molybdate in the watering water.

14. VI. each pot received 3 g of molybdate. In comparison with the control the plant had stopped growing, the leaves were smaller, the haulms 40 cm high, the leaves 10 cm long. The percentage of Mo in the dry mass of leaves (average of the low, high, and medium situated leaves) = 0·06%.

14. VI, we watered the pots with a 4 times greater amount of water: 200 cm a pot, using always the same doses of molybdate.

23. VI each pot received altogether 4·2 g of molybdate. Since 14. VI the earth in the pots was completey satiated with water. Within 10 days the plants grew rapidly, showing no difference in comparison with the control plants: haulms 60—75 cm high, leaves 20 cm long. The percentage of Mo in dry substance of leaves = 0·027%.

Under the influence of the watering the plants became twice as large, whereas the average percentage of Mo fell by a half. In spite of a continued watering with molybdate the leaves did not absorb greater amounts of Mo ions.

From 23. VI to 28. VI a return to dry conditions, namely 50 cm water a day, took place.

28. VI each plant was given 5 g of molybdate. Plants again checked in their development. Haulms and leaves as on 23. VI.

Percentage of Mo unchanged = 0·027%.

From 28. VI to 13. VII watered without Mo. The plants developed again, percentage of Mo fell again, in dry substance of leaves = 0·01%.

13. VII the proteins were extracted from the plants. Their chemical and serological quality did not show any difference compared with the proteins of the control plants.

The leaves and stalks of the plants absorbed molybdenum ions only in the first phase of the experiments, when the earth in the pots was dry. From 14 VI, when the earth was satiated with water, the quantity of molybdenum in the plant did not change, in spite of continual watering with molybdate. The plant no longer absorb molybdenum, and in the second phase of the experiment showed no external symptoms of intoxication. The extracted protein did not show any changes. The satiation of the earth with water evidently caused the fixation of molybdenum by soil colloids in the non-replaceable form for the plant. A similar phenomenon has

been described by many scientists for potassium. Repetition of the wetting and the redrying of the soil augments the fixation of potassium in the non-replaceable form. (Hoagland a. Martin 1933, Hogland a. Broyer 1942).

IInd experiment. Two kinds of earth, loamy earth and garden humus earth. Plant: cucumber, *Cucumis sativus*.

Several pot experiments were carried out with loamy earth in the period 1940—44 (Kozłowska 1947). Loamy earth mixed with garden earth was used for the experiments. The influence of molybdenum was seen externally on plants already after a dose of 1 g ammonium molybdate added to 10 kg of earth. After two or three grams of molybdate we observed a stopping of growth, earlier blossoming, yellow colour of leaves. Table IV shows the amount of molybdenum in the dry mass of the plant (May 1943).

TABLE IV

Quantity of Mo per pot	May 1943	May 1948
	Loamy earth	Garden humus
1 g	0·057% Mo	0·042% Mo
2 g	0·0688% ,,	0·046% ,,
3 g	0·1% ,,	0·06% ,,

From the juice of these plants we extracted with $(NH_4)_2SO_4$ the lipoprotein which gave a very distinct complement fixation test with antivirus X serum. A serum obtained from this lipoprotein showed for a longer period of time a positive complement fixation test with virus X, with the same intensivity as the normal antivirus X serum.

In the years 1948—1950 we carried out several analogous experiments with garden humus earth. After adding 3 g of ammonium molybdate per pot no symptoms whatever were observed, although the percentage of Mo was in relation to the first experiment only imperceptibly smaller than in our first series of experiments (Table IV, May 1948).

The proteins isolated with difficulty from fresh juice did not contain a greater amount of lipoids. With antvirus X serum they gave a much weaker complement fixation test (Table V).

TABLE V

Complement fixation test, virus *x* antiserum

Dilution of antiserum	1/5	1/10	1/20	1/40	1/80	1/160	1/320	control
Cucumber, 3 g Mo Loamy earth	+++	+++	+++	++	+	+	+	—
Cucumber, 3 g Mo Garden humus	+++	+++	—	—	—	—	—	—
Virus *x* *Nicotiana*	+++	+++	+++	+++	++	+	+	—

The checking influence of humus earth on the specific action of molybdenum upon plants was confirmed in analogous experiments carried out with other species of plants.

Datura stramonium (summer 1948). 3 g of molybdate were added to 10 kg of strong garden humus earth. During the experiment the plant grew twice as high, showing no differences in comparison with the control plants. The quantity of Mo in the dry mass of the plants amounted to 0·11%. The protein extracted in small quantities from the green mass of the plant did not show any specific serological qualities (Table VI).

Datura stramonium (winter 1948/49) 3. XI. seedlings were planted in pots. 25. II. watering with ammonium molybdate began. In comparison with the summer experiments the plants were smaller by half. 30. XII. every pot was given 3·5 g of molybdate. In comparison with the controls the plants were several cm smaller. The quantity of Mo in the dry mass of the leaves amounted to 0·18%. The isolation of soluble protein substances was difficult. Specific complement fixation test weak.

TABLE VI

Complement fixation test, virus *x* antiserum

Dilution of antiserum	1/5	1/10	1/20	1/40	1/80	1/160	1/320	Control
Datura stram. humus summer	—	—	—	—	—	—	—	—
,, winter	+++	++	—	—	—	—	—	—

Similar results were obtained in analogous experiments with phlox, potatoes and primroses.

III[rd] experiment. In the summer of 1949 we made experiments with cucumber in glass pots containing 1·5 kg of different kinds of earth and sand cultures.

1) 20 pots: garden humus earth.
2) 20 pots: $^1/_3$ loam, $^1/_3$ sand, $^1/_3$ garden earth.
3) 20 pots: washed sand with full nutrient (nitrogen in form of nitrate).
4) 20 pots: washed sand with full nutrient (nitrogen in form of NH_4).

23. IV. the cucumbers were sown in pots in which earth and sand were 3/5 saturated with water. Then they were every day watered with 50 cm water.

27. V. the plants had 6—8 developed leaves. We began to water them with ammonium molybdate, dissolving every day 0·2 g in 50 cm^3 water.

7. VI. each glass pot received 1·4 g molybdate. Lowest leaves of the cucumbers of the fourth series grown on sand with ammonium nutrient, were yellow and dry. The youngest leaves were dark green. The cucumbers of the first three series showed no toxic symptoms at all. Table VII shows that the plants did not absorb molybdenum equally.

TABLE VII
Quantity of Mo in the dry mass of green leaves

1·4 g molybdate per pot			
Garden humus	Nitrate nutrient	Loam	Ammonium nutrient
0·064%	0·3%	0·12%	0·03%

Proteins extracted with $(NH_4)_2SO_4$ from the fourth cucumber series were easily isolated from the fresh sap. With them gave antiserum virus X a distinct complement fixation test.

8. VI. we continued to water with molybdate the cucumbers of the first three series.

28. V. each pot received 2·5 g molybdate. The chemical analyses of molybdenum in the dry plant mass showed the same quantity of Mo in cucumbers grown in humus earth as in those grown on nitrate nutrient. Cucumbers grown in loam absorbed less molybdenum in their stalks and leaves.

TABLE VIII

Quantity of Mo in the dry mass of green leaves

2·5 g molybdate per pot		
Humus earth	Nitrate nutrient	Loam
0·4%	0·4%	0·12%

In comparison to the control the plants of all three series had yellowish leaves. Proteins were isolated from the fresh juice of all three series with more difficulty than from the cucumbers grown on ammonium nutrient. Their serological reaction showed weaker specific properties.

3. Distribution of Mo in stalks and leaves

The fact that the lower leaves of the cucumber plant became yellowish under the influence of Mo and faded when grown on ammonium nutrient, induced me to investigate how the molybdenum is distributed in the stalk and the single leaves. I wanted to find out which leaves, the young developing ones, or those which had stopped growing, absorbed a greater amount of that element. These experiments were carried out on potatoes.

The potatoes were grown in sand pot-culture, containing full nutrient with nitrogen in the form of ammonium and nitrate. Lavishly watered, the potatoes were by the end of June 1·4 m high. The tops of the plants were cut down to 90 cm. In the corners of the leaves fresh sprouts quickly began to develop. 11. VII. we began to water with molybdate (0·2 g each 50 cm^3 of water). 28. VI. each pot received 2·4 g of molybdate. The percent of Mo was analysed in the old leaves (Fig. 1 c—d), in the young developing sprouts (Fig. 1 a—b) and in the middle part of the stalk (Fig. 1 e).

The above scheme shows, that the developing sprouts contained twice as much Mo as the ripe leaves, and the latter four times as much as the stalk.

The external symptoms of molybdenum activity are not in direct relation to the absorbed amount of molybdenum in the single organs of the plant. The lower leaves were the first to become yellowish whereas the developing leaves, which absorbed a greater amount of that element, did not show specific symptoms, only

Fig. 1. Distribution of Mo in leaves and stalk of the potato. a=0·148% Mo, b=0·143% Mo, c=0·078% Mo, d=0·07% Mo, e=0·018% Mo (in the dry mass of the plant).

becoming dark green. A different reaction of the plant in different soil conditions can be explained only in exactly determined conditions, which can only exist when plants are grown in water cultures.

4. Experiments in water cultures

Numerous further experiments were carried out in 2 or 3 litre glass pots. In the first period of development, when the nutrient reached the neck of the root, the cultures were aired every day. In the older stage the pots were only half filled with water. In the first stage of development the plants were kept in a nutrient containing nitrogen in the form of ammonium and nitrate. When the nutrient contained only nitrate the plants usually showed chlorosis. Cucumbers with branched roots and developed first leaves were transferred to two different nutrients: with nitrogen in the form of nitrate and in the form of ammonium.

a. NITRATE NUTRIENT

We used a nutrient of the following composition:

H_2O	— 1 litre
$Ca(NO_3)_2$	— 1 g
KNO_3	— 0·25 g
KH_2PO_4	— 0·25 g
$MgSO_4$	— 0·25 g
KCL	— 0·12 g

The cucumbers developed quite normally. Several experiments were carried out with plants in different stages of development adding 1 g or 1/2 g natrium molybdate to each litre of nutrient. The first experiment was carried out with a nutrient of pH=7·6. In the second experiment the nutrient was acidified with 10/n HCL the pH was then 6·2, in the third the pH was 4·8 (1/2 g natrium mol. per 1 litre). We examined the percentage of Mo in relation to the dry mass of plants, seperately in the roots and in the aerial parts. We took the average of the lower leaves, the upper ones and the stalk. Investigations were carried out one day after keeping the plant in a nutrient with molybdenum and then after 7 or 8 days.

Table IX shows the results.

We came to the following conclusions:

I. When the plants were grown either in an alkalic medium or in a weakly acid one we stated:

1. In the first 24 hours molybdenum collects in the roots and only a small percent reaches the stalks and the leaves. After 7 or 8 days the amount of molybdenum in the leaves slightly exceeds the amount contained in the roots.

2. In spite of a high percentage of Mo which amounted to 0·4%, the plants showed nothing but a yellowing of the leaves. No other symptoms were visible.

3. Very small amounts of protein isolated from the aerial parts showed no specific physico-chemical and serological qualities in comparison with the control plants.

II. In the case of the pH of the nutrient being below 5 (pH=4·8) the plants behaved differently:

1. After a few days the lower leaves withered.

2. Molybdenum collected in the roots, without rising into the leaves and stalks. The extracted protein showed a positive complement fixation test with anti-serum virus X. After 2 months the extracted protein losed the specific serological property.

According to Mulder (1948), minimal amounts of Mo are indispensable in the green plant tissue at the reduction of the NO_3 ions to NH_2. There exists the probability that molybdenum is a part of the enzymes which reduce the nitrate ions. Proteins can form in the plant tissue without molybdenum only upon the ammonium nutrient.

TABLE IX

Nutrient with nitrogen in form of nitrate

Date		Days	Leaves developed	pH of nutr.	Quantity of Mo in nutrient	% of Mo in plants		Symptoms	Complement fixation test, antiserum virus x					
from	to					Roots	Leaves		1/10	1/20	1/40	1/80	1/160	K
23.V.	24.V.	1	3	7·6	1 g natr. molybdate per litre	0·3%	0·04%	none	—	—	—	—	—	—
23.V.	31.V.	8	3	7·6	,,	0·3%	0·4%	leaves yellowish	—	—	—	—	—	—
15.V.	16.V.	1	1	6·2	,,	0·2	0·06	none						
26.VI.	27.VI.	1	2	6·2	,,	0·06	0·013	none						
26.VI.	3.VII.	7	2	6·2	,,	0·32	0·4	leaves yellowish	—	—	—	—	—	—
16.VI	23.VI.	7	3	4·8	1/2 g molybdate per litre	0·1%	0·027	lower leaves withered	+++	+++	+++	+	—	—

It has not yet been proved whether great amounts of molybdenum accelerate to a higher degree the reduction of nitrate.

The absorbtion of salt through the roots does not depend on the absorbed water. Transpiration is closely connected with respiration (Frey-Wyssling, 1945). It is a well known fact that a greater amount of ions gather in the upper parts of the root (Hoagland a. Broyer 1942, Steward etc. 1942). The metabolism of the plants, namely respiration and photosynthesis, influence the mounting of the ions from the roots to the stalks and leaves (Broyer and Hoagland 1943). A great amount of sugar in the cells increases the absorption and the rising of the ions. On the first day of our experiments with molybdenum the ions of that element gathered in the roots. In the next period at a ph=7·3 and 6·2 there followed a regular translocation of molybdenum into the stalk and leaves, whereby the cell cytoplasm was in no degree attacked. Toxical symptoms appeared only when the pH of the nutrient was below 5.

Lundegardh created the notion of anion respiration «Anionenatmung» (Burström a. Lundegardh 1933). When the nutrient contains NO_3 ions and at the same time a factor of its reduction namely molybdenum, we augment without doubt the amount of free oxygen within the cell. In consequence, respiration is heightened and a translocation of the ions absorbed by the plants takes place. It may be that more intensive metabolic processes are the cause of a considerable collection of molybdenum in the green leaves of cucumbers, grown on alkalic and weakly acid nitrate nutrients.

Except in the case of the nutrients, being strongly acid (pH below 5) it is probable that the reduction capacity of molybdenum augments within the cell to such a degree that it causes a toxic influence. It is a very interesting fact that cucumbers grown on mould, watered with molybdate, gathered in their tissues, as was evident in the former experiments, the same great amount of molybdenum as the cucumbers grown on nitrate nutrient in water cultures. This analogy is caused perhaps by the energetic processes of nitrification taking place in the mould.

The absorption of molybdenum in the aerial parts of the plant underwent a change when the amount of sulphates in weakly acid nitrate nutrient pH=5·8 was increased. We added 0·82 g $MgSO_4$ per litre of water instead of 0·25 g. We carried out 2 experiments

adding per litre of nutrient 1 and 2 g of natrium molybdate. After 6 days the results were as follows:

TABLE X
Nutrient with nitrogen in form of nitrate, 0·82 g $MgSO_4$ per litre

Date		Days	Stage of development	pH	Quantity of Mo in nutrient	% Mo root	% Mo stalk	Symptoms
from	to							
27. IV.	3. V.	6	3 leaves developed	5·8	1 g per litre	0·3%	0·03%	leaves yellowish
27. IV.	3. V.	6	,,	5·8	2 g per litre	0·24%	0·157%	,,

The absorption of molybdenum by the roots of the plant was the same as in the previous experiments. Yet the mounting of that element into the stalks and leaves did not take place. Even in the case of a double dose of molybdenum in the nutrient, the amount of molybdenum in the leaves was half as small as that in the roots. No toxic symptoms were visible.

b. NUTRIENT WITH NITROGEN IN THE FORM OF AMMONIUM

We carried out several experiments using 2 following kinds of the nutrients.

A. H_2O ..... 1 litre
$(NH_4)_2SO_4$ ..... 0·9 g
KCL ..... 0·3 g
KH_2PO_4 ..... 0·25 g
$MgSO_4$ ..... 0·25 g
$CaCl_2$ ..... 0·66 g
$FeCl_3$ ..... traces

B. NH_4Cl ..... 0·6 g
$NH_4H_2PO_4$ .... 0·22 g
$MgSO_4$ ..... 0·82 g
KCl ..... 0·43 g
$CaCl_2$ ..... 0·66 g
$FeCl_3$ ..... traces

During the whole vegetation period from spring to autumn we carried out with both nutrients several experiments with a different pH, from 7·3 to 4·2. The nutrients were alkalized by NaOH.

Table XI shows the results.

The first experiment with a weakly alkalized nutrient lasted 7 days. 8. VI. we added one gram of natrium molybdate per litre of a nutrient alkalised with NaOH to pH=7·5. The cucumbers had two developed leaves. We added day by day NaOH to the

TABLE XI

Nutrient with nitrogen in form of ammonium

Date		Days	Leaves developed	pH of nutrient	Quantity of Mo in nutrient	% of Mo in plants		Symptoms	Complement fixation test, antiserum virus *x*					
from	to					Roots	Leaves		1/10	1/20	1/40	1/80	1/160	K
8. V.	15. V.	7	2	7·3	1 g am. molybdate per litre	0·37%	0·07%	leaves yellowish	+++	+++	+++	++	+	
30. IX.	7. X.	8	5 chlorosis	6·6	,,	0·1%	0·13%	,,						
26. VI.	3.VII.	7	2	6	,,	0·26%	0·09%	Cotyledons and leaf withered						
19. IV.	25. IV.	6	3	5·8	,,	0·56%	0·028%	Cotyledons, leaf, stalk withered						
27. IV.	3. V.	6	2	5·5	,,	0·38%	0·06%	,,	+++	+++	+++	+	+	
27. IV.	3. V.	6	2	5·5	2 g am. molybdate per litre	0·43%	0·19%	,,	+++	+++	+++	++	+	
7.VII.	12.VII.	5	4 flouri-shing	5·3	1 g am. molybdate per litre	0·94%	0·03%	all leaves withered	+++	+++	++	+	+	
28. IX.	6. X.	8	3	4·2	,,	1·1%	0·16%	,,	+++	+++	+++	++	+	

nutrient so that the pH oscillated between 7·5 and 7·3. The plants then developed normally, only the leaves becoming yellowish. The protein isolated from them gave a positive complement fixation test with antiserum virus X (Fig. 7).

The change of pH below 7 to pH=6·6 caused in our further experiments no notable changes in the plant. The turning point was the acidifying of the nutrient up to pH=6. Already on the 4th and 5th day these appeared symptoms of poisoning: namely the lower leaves and cotyledons became dry. When the pH was 5·8 we observed in many experiments, repeatedly carried out, that the lower leaves and stalks always withered (Fig. 4a). Together with a further lowering of pH this phenomenon increased, so that on the fifth day at a pH=5 all leaves were completely withered. The extracted proteins of these plants gave with virus X antiserum prevention of hemolysis in complement fixation test.

Yet we must state that the specificity of serological reaction of the protein of the plants cultivated in water cultures was weaker than in the case when the plants watered with molybdate were cultivated in loam soil. In the very case the proteins inoculated into a rabbit gave a specific antiserum, while the proteins of plants grown in water cultures possessed that quality to a much lesser degree.

The percentage of Mo in water-cultures experiments (see Tbl. XI) oscillated in the leaves between 0·03% and 0·16%. The amount of Mo greatly increased in the roots together with a lowering of the nutrient acidity, amounting to 1%. Thus molybdenum strongly absorbed by the roots migrated even at pH from 5·8 to 4·2 to the leaves in a relatively small percentage.

The withering of the leaves and stalks as well as the necrosis of the tissues with a sour nutrient were symptoms of intoxication.

The above described phenomena appeared still more strongly in sand cultures. We added to pots filled with 3·5 kg of washed sand the following nutrient:

$NH_4H_2PO_4$	0·82 g
$MgSO_4$	0·25 g
KCL	0·43 g
$CaCO_3$	0·33 g

14. VII. we sowed cucumber seeds. 19. VII. the plants had 4 large developed leaves and buds. Every other day we watered the plants

with 0·2 g ammonium molybdate per pot. 23. VIII. ammonium molybdate amuonted to 0·6 g per pot the lower leaves withered, the plants blossomed and developed new leaves. 1. IX. the plant had received altogether 1 g ammonium molybdate per pot. Of 8 leaves 5—6 had faded and partly dried. Percentage of Mo was the following: roots 0·21%, dry leaves 0·04%, withered leaves 0·054%. Proteins extracted from the aerial parts of the plant gave a complete prevention of hemolysis in complement fixation test with virus X antiserum in a solution of 1:160.

Withering therefore takes place in plants when the living cells lose their turgidity. It occurs mostly when the water balance has been disturbed in consequence of increased transpiration. Yet there exists another withering which stands in no relation to transpiration, namely that withering which has been caused by a disturbance of the half permeability of the cytoplasm. In consequence there follows a lowering of the osmotic pressure in the cell. Gäumann and Joag (1947) describe a physiological withering caused by the activity of toxic substances which attack the cytoplasm. The water balance plays no part in that phenomenon. In our experiments we meet probably the same fact caused by a specific activity of molybdenum especially in a sour medium.

The cucumber is an exceptional example of a plant which has in its cells usually alkalic sap of pH=7·2 to 7·6. The plants most frequently met with have a but slightly sour sap. We wanted to find out whether those plants would react similarly to the cucumber. We carried out the same experiment with tomatoes which have in their cells sap of pH from 5·2 to 6·2.

c. EXPERIMENTS WITH TOMATOES

I[st] experiment. Tomatoes were cultivated in a full ammonium — nitrate nutrient.

4. X. one series was put into ammonium nutrient, the other into nitrate nutrient. 1 g natrium molybdate was added to both nutrients. In both nutrients the pH=5. After 12 days the tomatoes of both series showed no difference in their development. All of them were quite normal, no withering in ammonium nutrient was observed.

TA

Date		Days	Leaves developed	Nutrient pH	Quantity of Mo in nutrient
from	to				
6. X.	20. X.	12	8	NO_3 pH=6	1 g Na molybdate per litre
6. X.	20. X.	12	8	NH_4 pH=5·8	,,

Table XII shows that the distribution of molybdenum in the roots and aerial parts of the tomatoes is analogous to that in the cucumbers. The accumulation of molybdenum in the root in the case of an ammonium nutrient is striking. Although the tomatoes of both nutrients showed no difference in their exterior, yet the proteins extracted from them had principally different serological qualities. The protein of the plant grown on ammonium nutrient completely prevented hemolysis in complement fixation test with antivirus X serum, whereas the proteins of the tomatoes grown on nitrate nutrient showed no specific serological reaction.

We observed the interesting fact that the plant reacts upon molybdenum in such a way when it is a in young stage of development. When the plant is blossoming or fruit-bearing changes in the protein structure under the influence of molybdenum are slight.

5. Experiments with divided roots

At the beginning of this paper we described experiments concerning the direct activity of molybdenum upon plants in water cultures without nutrient and we stated that the plants stood large doses of that element. The plants transported from the molybdate water bath into the sour ammonium nutrient showed no toxic symptoms whatever. It is evident that toxic symptoms in plants appear only when molybdate is present together with the ammonium nutrient.

This fact became still more obvious in the following experiment. At the beginning of the experiment the cucumber plant cultivated in nitrate nutrient already had 3 leaves.

BLE XII

% of Mo in plants		Symptoms	Compl. fix. test antiserum virus x					
Roots	Leaves		1/10	1/20	1/40	1/80	1/160	K
0·3%	0·18%	lower leaves yellowish	—	—	—	—	—	—
1%	0·09%	,,	+++	+++	+++	+++	+++	—

I[st] experiment. On 19. IV. one part of those plants was transported into ammonium nutrient with 1 g ammonium molybdate per litre of pH=5·8. In the second case we placed half of the roots into a tube containing natrium molybdate of ph=5·8 and the other half in ammonium nutrient of ph=5·1 (Fig. 3). After 6 days, on the 25. IV., the plant with divided roots was quite normal, even developing during the experiment the 4[th] leaf. On the other hand it proved that the cucumber dipped in the molybdate nutrient did not develop during the experiment. The lower leaves were withered, the stalk had lost its turgidity and was bent, only the developing top leaves showed normal turgidity. The plants of both experiments showed almost the same percentage of Mo in leaves and stalks. 1) Mo=0·021%, 2) Mo=0·028%.

It therefore results that toxic symptoms appeared in cucumbers only when other ions contained in the ammonium nutrient were active together with molybdenum.

II[nd] experiment. We divided the roots of a cucumber plant growing in a normal nitrate nutrient. The smaller lateral roots, 15 cm long, were put into tubes with three different nutrients.

1. Natrium molybdate in a solution of 1 g per litre of ph = 3·3.
2. Natrium molybdate with $MgSO_4$ of ph = 5.
3. Ammonium molybdate with $MgSO_4$ of ph = 5.

The main roots were left in normal nitrate nutrient.

After 7 days the plants showed no symptoms of poisoning except a yellowing of the leaves (Fig. 6). In the second experiment the low acidity in the tube containing ions SO_4 and MoO_4 caused a complete destruction of the root. In consequence the analysis of molybdenum in the stalks and leaves showed 0·36% Mo, though no traces of withering were visible. In two other experiments the leaves of the cucumber contained such a percentage of Mo as was

characteristic for plants entirely immersed in ammonium nutrient with Mo (Fig. 6).

These experiments showed that the combination of ions SO_4, NH_4 and Mo_2O_7 or MoO_4 did not act toxically when great amounts of ions NO_3 were simultaneously added to the nutrient. Molybdate reduces NO_3 supplying the plant with oxygen. In the described experiments molybdate fulfils this role, without any other indirect action. Matters are quite different when there are no NO_3 ions in the nutrient. Ions NH_4 immediately take part in the building up of amino-acids. Molybdenum as a reducer, added simultaneously to the plant, begins to act indirectly, maybe upon other ions rich in oxygen which are in the nutrient. To verify this we carried out several experiments, paying attention, to the SO_4 ions.

6. Ammonium nutrient deprived of SO_4 ions

Cucumbers were placed in an ammonium nutrient deprived of sulphur and composed as follows:

H_2O	1000 g
NH_4Cl	0·6 g
$NH_4H_2PO_4$	0·22 g
$MgCl_2$	0·25 g
KCL	0·43 g
$CaCl_2$	0·66 g

The pH in these experiments oscillated between pH=7 and pH=4·2. We added 1 g of ammonium molybdate per litre. The results of our experiments are seen on Table XIII.

The accumulation and distribution of Mo in roots and leaves is similar to that in the experiments with full ammonium nutrient. Only in a neutral nutrient the percentage of Mo was more or less the same in the whole plant. Together with a rising acidity of the nutrient molybdenum accumulated above all in the roots, mounting to the stalks, but in a small percentage. The exterior of the plant showed a certain difference in comparison with the plant grown in full ammonium nutrient. Withering and drying of the leaves appeared as a rule later and rather more weakly, sometimes not appearing at all. A certain difference was observed in the serological qualities of proteins. Extracted proteins from the cucumber of a neutral nutrient of pH=7, as well as of the sour nutrient of

pH=4·2, gave as a rule no positive complement fixation reaction with antivirus X serum.

Yet we must stress the fact that the negative result of the complement fixation test was not quite distinct. Plants cultivated in a nutrient containing profuse Cl ions very often showed symptoms of intoxication when grown without Mo.

The same experiment carried out on a nutrient partly deprived of Cl ions, showed more distinctly that Mo did not influence in a specific way the proteins of living cells when the nutrient was deprived of SO_4 ions.

The second nutrient, pH=5·6, was the following:

$NH_4H_2PO_4$	0·8 g
$MgCl_2$	0·25 g
KH_2PO_4	0·39 g
$CaCO_3$	0·36 g

After 8 days the young cucumber plants showed no signs of withering at all. The extracted proteins had no specific serological qualities.

The reaction of the plant to the presence of SO_4 ions appeared for instance very clearly in the experiment with divided roots. The plant grew in a normal nitrate nutrient. 25. V. the main roots, 40 cm long, were placed in tubes containing two different nutrients: 1) 2 g of ammonium molybdate and $MgSO_4$ per litre, pH=5·2. 2) 2 g of ammonium molybdate per litre, pH=5·2. The 15 cm long side-roots remained in the normal nitrate nutrient. On the second day in the first experiment the cotyledons and the first leaves began to become dry. On the 5-th day the plant withered completely. In the second experiment no symptoms of poisoning were visible exept the yellowing of the leaves, even the cotyledons were a normal intensive green (Fig. 5). The amount of molybdenum in the leaves of both experiments differed but little: 1) Mo=0·124%, 2) Mo=0·085%.

7. Assimilation of SO_4 ions by the plants

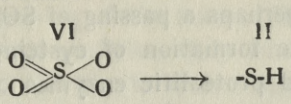
Sulphur can be assimilated by green plants only in the form of SO_4 ions. No other anorganic compounds of sulphur can be utilized by the plants and often they act toxically. The assimilation of SO_4 ions by the plant cells takes place as follows. In consequence of the reduction process the oxygen of SO_4 ions is substituted by

TABLE XIII

Ammonium nutrient deprived of SO_4 ions

Date		Days	Leaves developed	pH of nutrient	Quantity of molybdate in nutrient	% of Mo		Symptoms	Complement fixation test Antiserum virus *x*					
from	to					Roots	Leaves		1/10	1/20	1/40	1/80	1/160	K
17.VI.	24.VI.	7	6	7	1 g per litre	0·113%	0·12%	none	++	—	—	—	—	—
26.VI.	27.VI.	1	2	5·8	,,	0·15%	0·023%	,,	—	—	—	—	—	—
26.VI.	3.VII.	7	2	5·8	,,	0·64%	0·084%	lower leaves partly withered						
22.V.	30. V.	8	2	5·5	,,	1·28%	0·094%	partly withered	—	—	—	—	—	—
28. IX.	6. X.	8	5	4·2	,,	1·2%	0·1%	partly withered	—	—	—	—	—	—

hydrogen. 6 value sulphur changes into 2 value sulphur, the — SH group comes into existence.



This change, as well as the reduction of NO_3 ions can take place in the roots. In this process the energy of respiration is utilized. Next the SH group enters into the compound of aminoacid alanine, where it fulfils an analogous role as the alcohol group OH, forming the cysteine compound.

$COOH.CH\ NH_2.CH_3$ ..... Alanine
$COOH.CH\ NH_2.CH_2OH$ ..... Serine
$COOH.CH\ NH_2.SH$ ..... Cysteine

When hydrolising proteins we do not obtain cysteine but cystine which is an aminoacid deriving from two molecules of cysteine which have been deprived of hydrogen. Cystine also has the capacity to bind hydrogen and to separate into two molecules of cysteine.

$$COOH.CH\ NH_2.CH_2S—S\ CH_2.CH\ NH_2.COOH \underset{-H_2}{\overset{+H_2}{\rightleftharpoons}} 2\ COOH.CH\ NH_2.CH_2SH.$$

Cystine Cysteine

This reaction depends on the quantity of the reducing H in the cell. If there is enough hydrogen, cysteine is formed, but when there is not enough H, cysteine loses hydrogen, changing into cystine playing the role of reducer. Aminoacids containing sulphur regulate the reduction process in the cell.

Balls (1939) attributes an extraordinary role to cysteine in a living cell. According to his hypothesis cysteine activates proteolitic enzymes. In his case it was papaine, obtained in crystallic form, which fulfilled in the plant cell the role of trypsin. By introducing in our experiments molybdate into plant cells we introduced factors which acted reductively. When the pH of the nutrient was lower the reducing activity was stronger, this was visible in the cucumber which showed toxic symptoms. When the nutrient possessed NO_3 ions at a alkalic or weakly acid pH the reductive activity of molybdate accelerated, as mentioned before, the formation of NH_2 ions, which were introduced at once into the amino-acids

of the proteins. When the plant received exclusively NH_4 ions other processes had to take place. The reductive action of molybdate accelerated perhaps a passing of SO_4 ions into a tiolgroup SH which led to the formation of cysteine. A relation existing between cysteine and proteolitic enzymes could explain the fact observed by us that the proteins underwent structural changes particularly when the nutrient contained SO_4 ions.

8. Summary

1. Molybdenum added to a pure water culture which contained exclusively Na and MoO_4 ions in a strong concentration amounting to 2 g natrium molybdate per litre H_2O exercised no toxic influence on the plant. When the root contained even 1% Mo the plant was quite normal and did not wither.

2. In soil conditions the quantity of the absorbed Mo and its toxic activity in the plant depends on several factors:

a) The fixation of molybdenum by soil colloids in the non replaceable for the plant form. The wetting and the drying of the soil augments this fixation to a considerable degree.

b) Kind of soil. The plants grown on humus earth and absorbing great quantities of Mo show no or very slight symptoms of poisoning. Proteins extracted from these plants show no specific serological qualities. On the other hand, when the plants are grown on clay earth, poor in nitrate compounds, they react distinctly to large amounts of Mo in the substrate (stopping of growth, yellowing of leaves). Proteins extracted from them show a specific serological reaction, a positive complement fixation test with antivirus X.

3. Plants grown on sand cultures with a full nitrate nutrient react in the same way as those grown on humus earth. On the other hand, when the plants were grown on an ammonium nutrient without nitrogen in the form of nitrate, molybden acted toxically, and the protein showed a specific serological quality.

4. Molybdenum is not evenly distributed in all parts of the plant grown in sand cultures. In our experiments the young developing leaves contained twice as large a quantity of Mo as the ripe leaves, which again contained 4 times as much as the stalk.

5. Table IX shows the results of water culture experiments with cucumbers grown in a nutrient with nitrogen in the form

of nitrate. After 7 or 8 days the cucumbers contained in their leaves 0·4% of Mo in relation to the dry mass. They exhibited no toxic symptoms except a yellowing of the leaves. The extracted proteins had no specific serological qualities. Only when the pH of the nutrient was below 5 did the plants wither and display changes in the protein substances. In the first 24 hours molybdenum accumulates in the roots, and after 7 to 8 days the amount of Mo is the same in the leaves as in the roots. Introducing molybdate into the plant cells, we introduce a reduction factor, which increases the reduction of the NO_3 into NH_2 ions. In consequence the «Anionenatmung» increases the rising of Mo from the roots into the stalk. In humus earth rich in nitrates the plant behaves in relation to molybdenum in the same way as it behaved in a nutrient with nitrogen in the form of nitrate.

6. Table X indicates the results of experiments conducted with cucumbers grown in a nutrient with nitrogen in the form of ammonium. At a pH of 7·5 to 6·6 in the nutrient the plants showed no symptoms of intoxication. Only the extracted proteins had a specific serological quality. When the pH was under 6 (pH=5·8) the plants always withered. Molybdenum gathered in the roots a small percentage mounting into the leaves and stalk.

7. Experiments with nutrients deprived of SO_4 ions proved that considerable symptoms of intoxication were visible on the cucumber plant, when the plant received simultaneously SO_4, NH_3 and Mo_2O_7 ions. When there were no SO_4 ions in the nutrient, the extracted proteins displayed no specific serological qualities.

8. When NO_3 ions were led through the side roots to the plant immerged in an acid nutrient containing SO_4, NH_4 and M_2O_7 ions, no withering of the plant followed. When the nutrient is deprived of NO_3 ions it is probable that molybdenum introduced into the plant acts as a reducer on other ions, perhaps on SO_4 changing it into SH. Probably this change is connected with the forming of cysteine and cystine in the plant cells which in turn changing the oxydo-reduction potential influence the structure of the protein cells.

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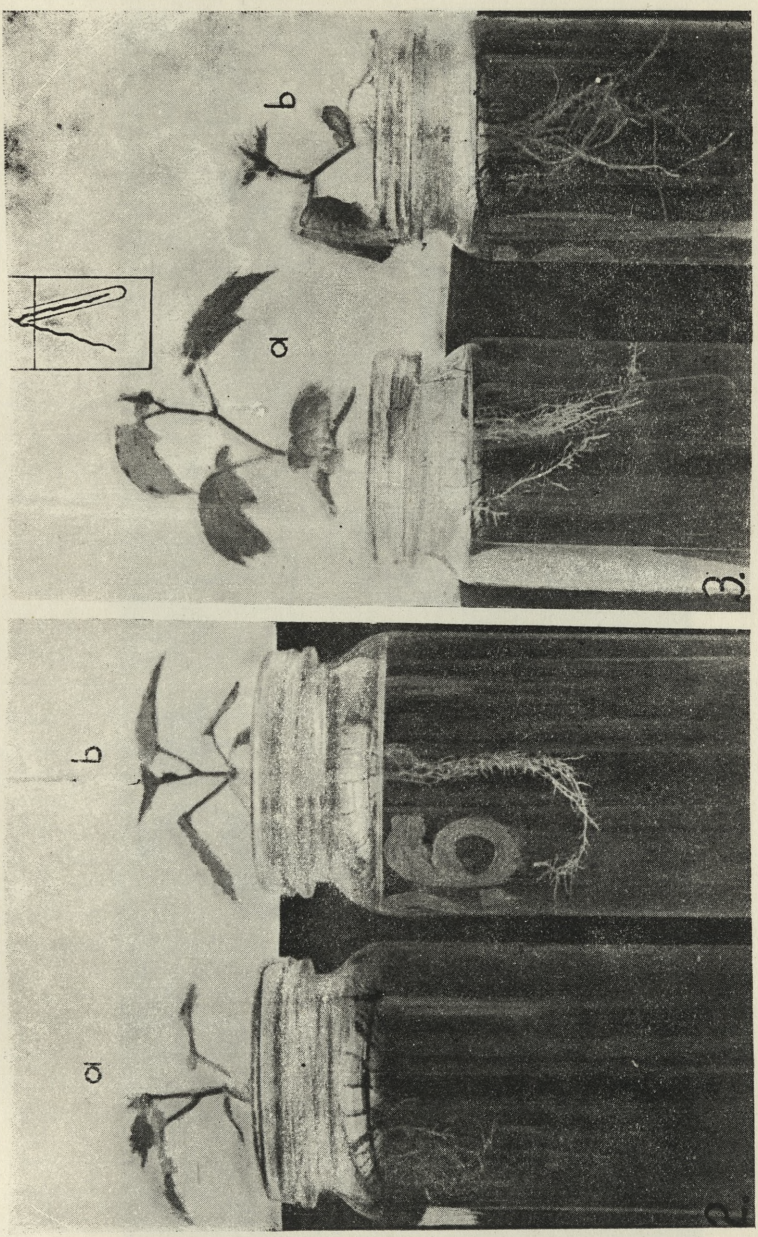

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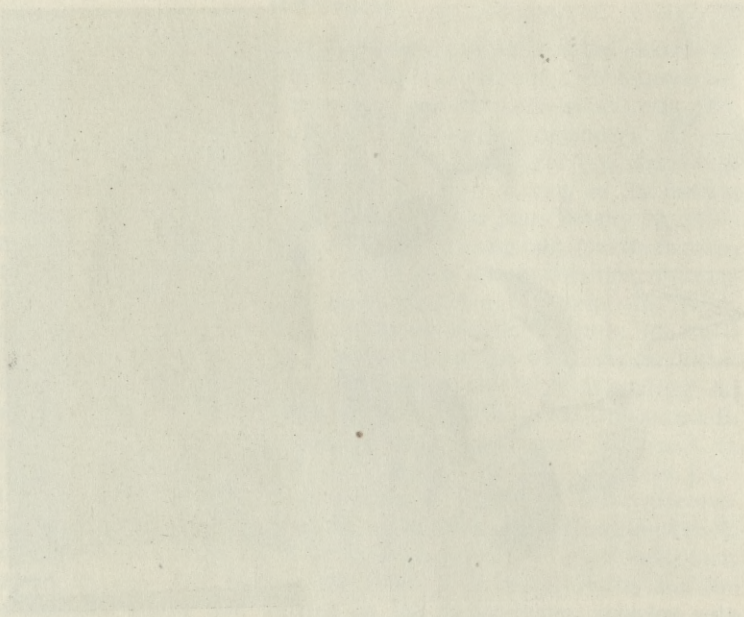

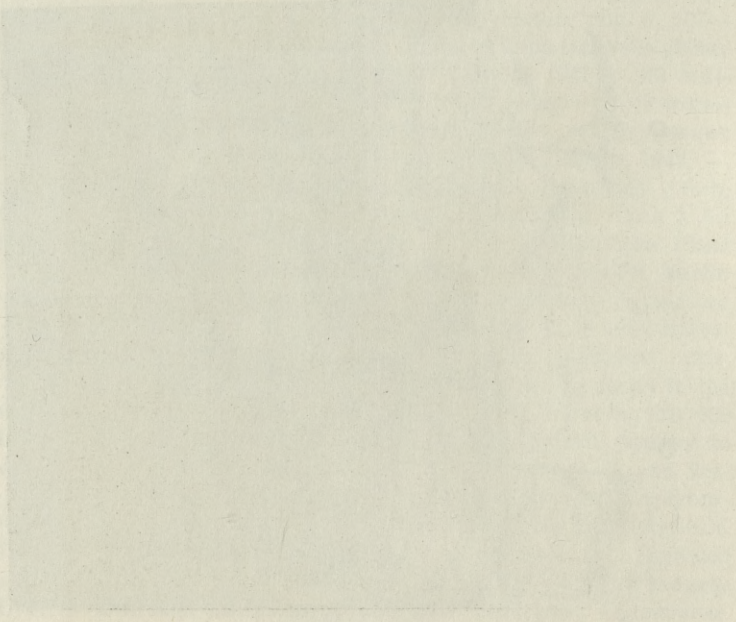

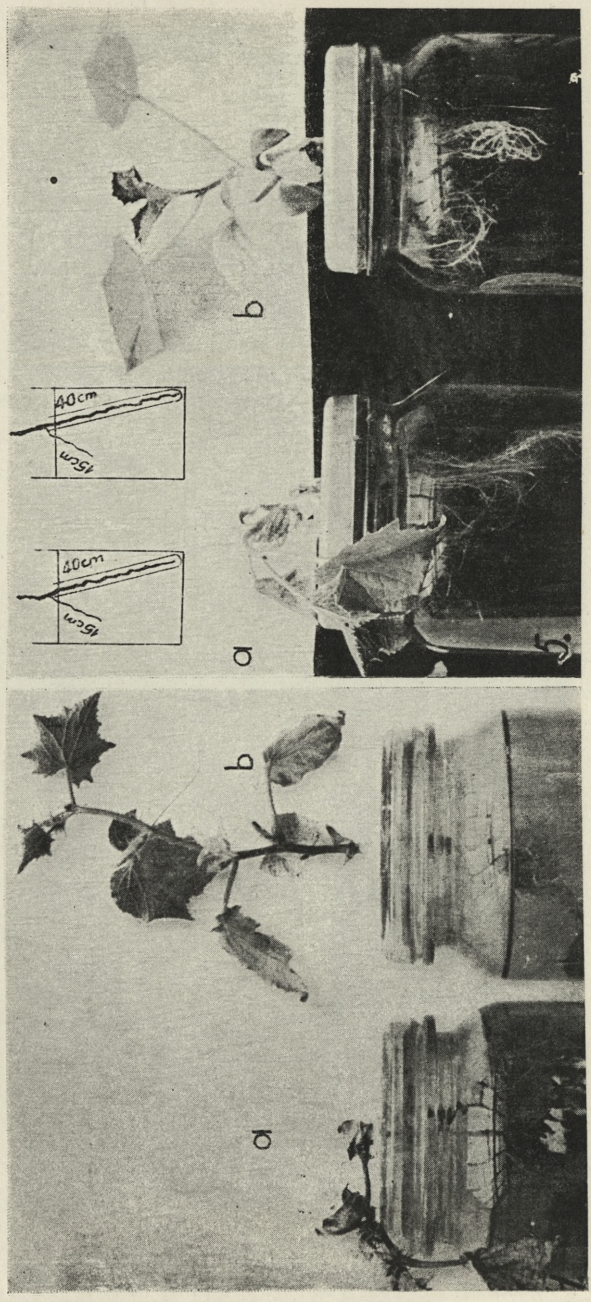

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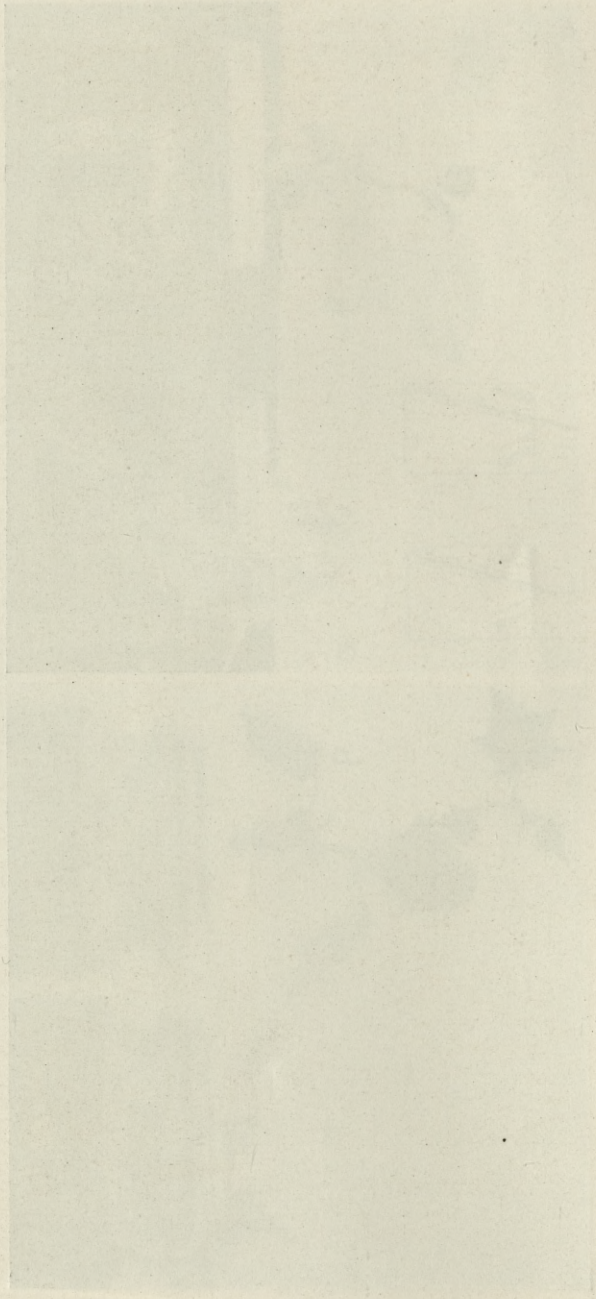

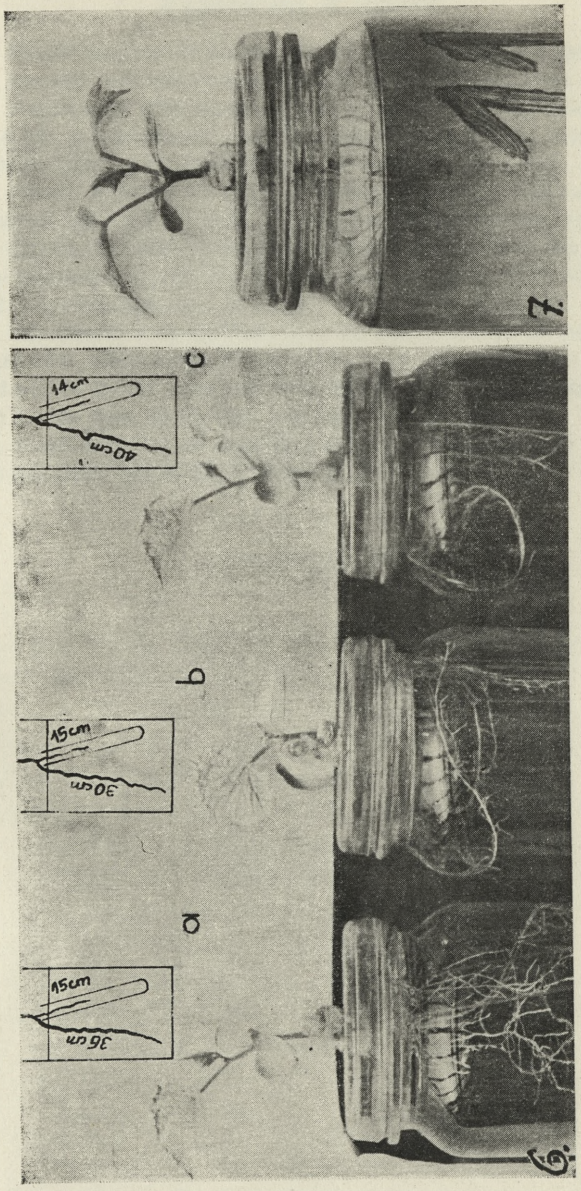

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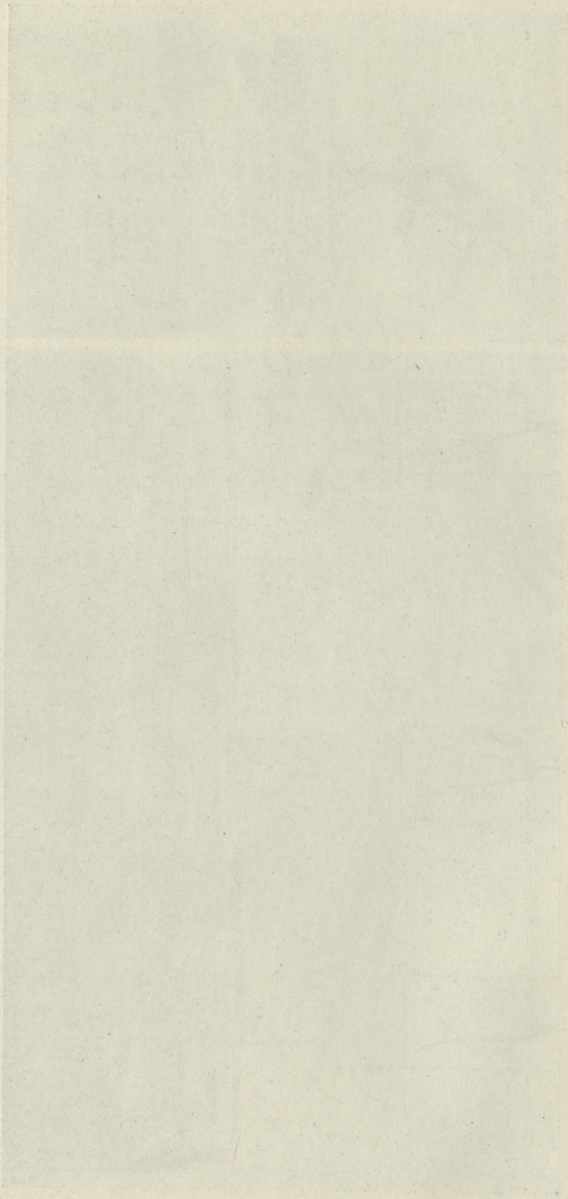

Explanation of figures

Fig. 2. Plants kept for 24 hours in water with 1 g natrium molybdate per litre; then brought into ammonium (a) and nitrate (b) nutrients of pH=5·8.

Fig. 3. Experiment with divided roots. a) In the tube natrium molybdate pH=5·8, in the pot ammonium nutrient pH=5·1. b) All the roots in ammonium nutrient with 1 g natrium molybdate per litre, pH=5·8.

Fig. 4. Plants kept for 11 days in ammonium nutrient pH=5·8 (a) and nitrate nutrient pH=5·7 (b) with 2 g natrium molybdate per litre.

Fig. 5. Experiment with divided roots. Small side roots in the pots in normal nitrate nutrient pH=6·5, the main roots in the tubes. a) In the tube NH_4, Mo_2O_7 and SO_4 ions, pH=5·2. b) In the tube Mo_2O_7, NH_4 ions.

Fig. 6. Experiment with divided roots, the main roots in the pots in nitrate nutrient pH=6·5, small roots in the tubes. a) In the tube ions: NH_4, SO_4, Mo_2O_7, pH=5. b) In the tube ions SO_4, MoO_4, pH=3·6, root completely destroyed. c) In the tube MoO_4 ions, pH=3·3.

Fig. 7. Plant kept for 11 days in ammonium nutrient with 1 g natrium molybdate per litre, pH of the nutrient=7·2.

Badania nad ruchami fototaktycznymi chloroplastów u Selaginella Martensii Spring. — Investigation onto phototactic movements of chloroplasts in Selaginella Martensii Spring.

Mémoire

de M. J. ***ZURZYCKI*** and M^me^ ***A. ZURZYCKA***

présenté le 1 Juin 1951 par Mme M. Skalińska m. c. et Mlle A. Kozłowska m. t.

(Plates 13—14)

I. Introduction

In the cells of the upper epidermis of leaves of *Selaginella Martensii* there are single cupshaped chloroplasts which are capable of phototactic movements. The phenomenon of sensitivity to light of chloroplasts of *Selaginella* has already been described several times (Prillieux 1874, Stahl 1880, Haberlandt 1905, Senn 1908, Suessenguth 1923, Schürhoff 1924). All these authors, however, dealt with the subject descriptively and from the qualitative point of view in its estimation. Moreover the various descriptions do not coincide e. g. some differences between Senn's (1908) and Suessenguth's (1923) works can be found.

Accurate quantitative investigations on the phototactic movements of chloroplasts were so far carried out only in a few instances and only on disc shaped chloroplasts which are characteristic for higher plants (Voerkel 1934, Zurzycka 1951, Zurzycka & Zurzycki 1950). For this reason and as chloroplasts of *Selaginella* are morphologicaly completely different from other chloroplasts it was thought profitable to work out a method of quantitative estimation of the phototactic movements of these chloroplasts and with this method to investigate the main types of reactions to the stimulus of light.

II. Methods and material

Investigations were made in the winter and spring of 1951, on leaves of *Selaginella Martensii* Spring. grown in a hothouse of the Botanical Garden of the Jagiellonian University.

The direct observation of the leaf cells was difficult because of the air filling the considerable intercellular spaces. To overcome this difficulty the air was pushed out by water infiltration in vacuum from the leaves cut away at the stem (Strugger 1935). The infiltration which was easy to obtain, began at the centre and

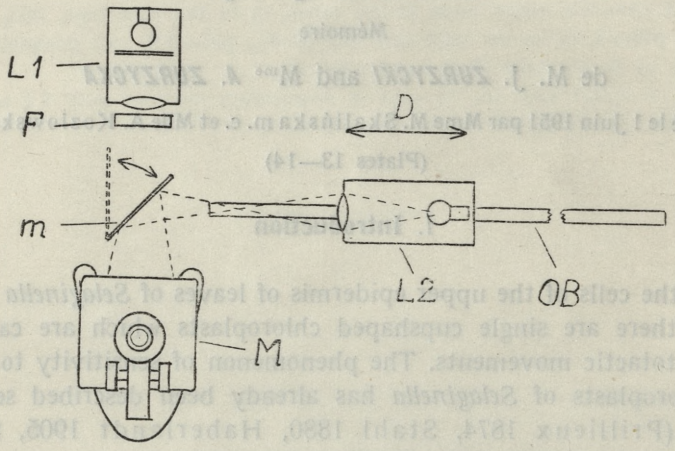

Fig. 1. Apparatus: M — microscope, m — adjustable mirror, L_1 — lamp for microphotographing, F — colour filter, L_2 — lamp for illuminating chloroplasts, OB — optical bench, D — direction in which L_2 was moved.

passed to the edges of the leaf. After 1—2 minutes all the intercellular spaces were filled with water, the leaf became transparent and observations could be made on living and uninjured cells.

During the experiments the leaves were kept on a microscope table heated to $+20^0$ C$\pm 1^0$ C with the upper face towards the objective. Observations of chloroplast movements were made in the cup-shaped cells of the upper-face epidermis, a Zeiss 40× objective and a 15× eyepiece being used. The arrangements of chloroplasts were drawn with Abbè's apparatus. The magnification of the drawing obtained was 1125×.

To induce the phototactic reactions the preparation was illuminated from below (after removing the condensor from the microscope) with a 12 V 35 W lamp and a convex lens. The intensity of illumination was changed by altering the distance of the lamp from the object. The intensity of light was greatest when the image of the source of light fell on the microscope table, this intensity in relative units being defined as 100. To determine the relative intensity of light use was made of the law of squares. For drawing or photographing the condensor was replaced and the preparate was illuminated with a supplementary lamp (Fig. 1).

The development of the reaction was recorded by drawing approximately 10 cells and marking on the drawings the position of the chloroplasts at the given moment. The same cells were drawn at intervals of from 30—60 minutes according to the rapidity of the reaction.

The development of the reaction was expressed as the ratio of the area of a chloroplast as seen from above to the area of the whole cell also as seen from above. These areas were measured on the obtained drawings with a Maho planimeter. Measurements obtained were expressed in square microns or as the percentage of the area of chloroplasts in relation to the area of the cell.

III. Description of chloroplast movements

The leaves of *Selaginella Martensii* Spring. have three layers of cells: the upper and lower-face epidermis and the interposed layer of parenchyma tissue. Chloroplasts can be found in the cells of all three layers, though it is only in the upper-face epidermis that a single chloroplast with the caracteristic cup-shape occupies each cell.

As seen from above the cells in the upper-face epidermis of the leaf have a rounded or polygon shape, and in their cross section they have the shape of a cup covered with a slightly convex membrane.

If before the experiment the plant was kept in diffused daylight then the chloroplasts lie at the bottom of the cells and adjust their shape to the cup-shape of the cell. As seen from above the chloroplasts occupy then the whole area of the cell. It can be assumed

that this position corresponds to the epistrophic position of chloroplasts in *Lemna trisulca* and *Funaria hygrometrica*.

If a leaf with the epistrophic arrangement of chloroplasts is illuminated with intensive white light, the first phototactic transpositions will be noticeable after 10—20 minutes. In one part of the cell membrane there appears a semicircular gap in the outline of the chloroplast, gradually the gap increases, though as a rule it remains semicircular in shape and owing to this the chloroplasts as seen from above are crescent-shaped. (Sometimes two gaps appear instead of one, and subsequently either one of them disappears or both of them develop simultanously, in which case a broad bridge is formed across the cell). The cross section of the leaf at this stage shows that the chloroplast has moved to a part of the side walls, leaving the bottom and the opposite walls of the cell uncovered. The points at which the chloroplasts detach themselves from the side walls depend on the individual state of each cell and are usually different in adjacent cells. It happens sometimes, however, that most of the displacements take place in one direction (Figs. 19 and 20).

The process of movement of the chloroplasts onto the side walls is usually accomplished after two or three hours. The chloroplasts will remain on the side walls if the illumination is not interrupted, this arrangement corresponding to the parastrophe in *Lemna trisulca* and *Funaria hygrometrica*. Sometimes the ends of the crescent-shaped chloroplast contract and shrink. Then the chloroplast as seen from above is round and placed totally on one of the side walls. The nucleus of the cell moves together with the chloroplast.

The displacement of chloroplasts of *Selaginella Martensii* from epistrophe to parastrophe as described above is illustrated by photographs: Figs. 8—23.

When the intensity of light is reduced the chloroplasts return from the parastrophic to the epistrophic position and slide back to the bottom and to the side walls of the cell. This movement as seen from above appears as the filling of the gap between the two crescent ends. (Sometimes the chloroplast slides simultanously to the bottom and to the side walls of the cell causing a jagged outline of the chloroplast). Usually the epistrophe obtained in this way is not quite so complete as the epistrophe of the starting position in a leaf which was not yet illuminated.

No change of position of chloroplasts is caused by keeping in darkness leaves with chloroplasts in epistrophe even for several days; the apostrophic arrangement cannot therefore be distinguished in *Selaginella Martensii*.

In order to check whether the transposition of chloroplasts as described above takes place also in leaves which were not infiltrated, leaves on undamaged stems were illuminated with the same intensity of light which causes the parastrophic arrangement of chloroplasts in infiltrated leaves. As continous observation is possible only of infiltrated leaves, only the effect at the end of a three hour illumination was investigated. The results obtained after the three hours did not differ in the least from the results obtained on infiltrated leaves. The chloroplasts are partly displaced to the side walls and as a rule are crescend-shaped. Only the shrinking of the area of chloroplasts in leaves which were not infiltrated is more considerable after illuminating with the same intensity of light. e. g. on an average the chloroplasts in a not infiltrated leaf occupy 81 pp, and in an infiltrated leaf 51 pp. of the cell area. This can be explained by a greater diffusion of light in the intercellular spaces filled with air.

IV. Development of phototactic movements of chloroplasts in relation to the intensity of light

The displacements of chloroplasts in relation to time and intensity of light were investigated only in two types of reactions: epistrophe to parastrophe and parastrophe to epistrophe. As it was impossible to obtain the apostrophe in the plant under investigation no other types of reactions were considered.

EPISTROPHE TO PARASTROPHE REACTION

The development of the epistrophe to parastrophe reaction during the three hours of the experiment and at a relative intensity of light = 100 is given in Table I. and Fig. 2. The data in the table are based on drawings of the same cells made at intervals of 30 minutes. From the table it is easily seen that each cell has it own individual characteristic. In some cells the reaction develops more or less evenly, fairly quickly at first, then gradually becoming slower (i. e. cells 2, 3, 6, 8). In other cells no noticeable shrinking

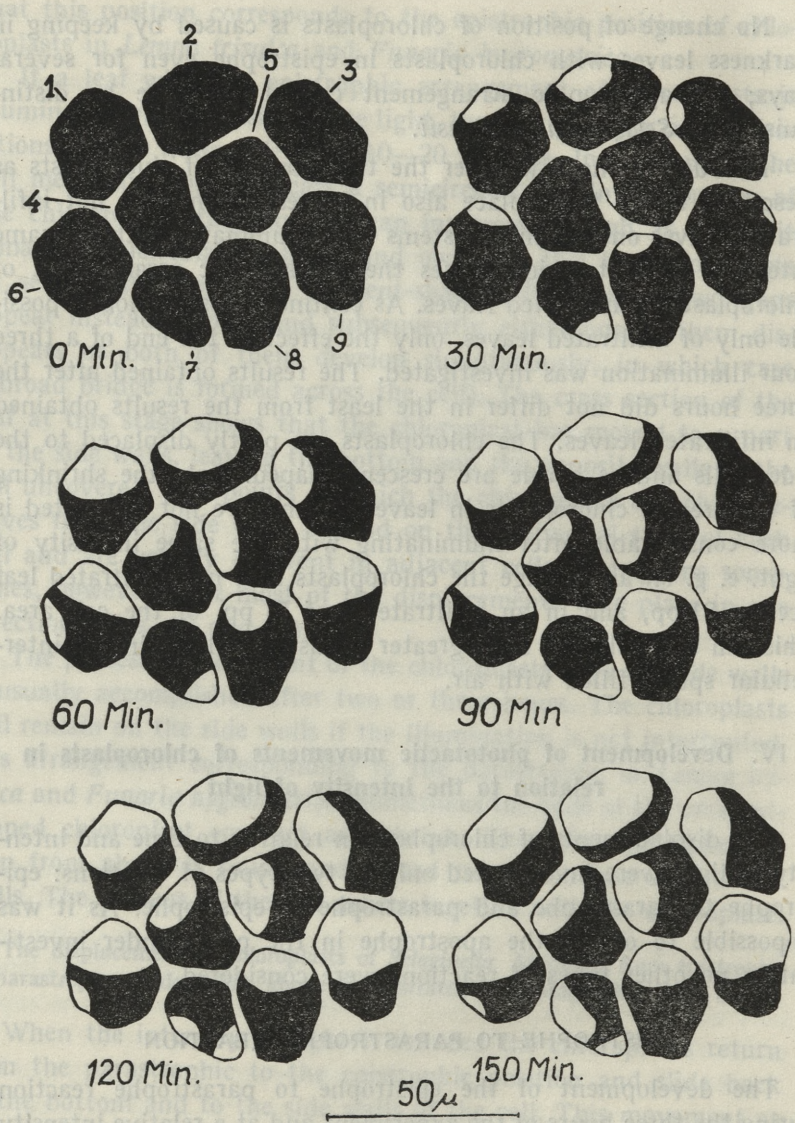

Fig. 2. Change of chloroplast arrangement in 9 cells during the epistrophe to parastrophe reaction, drawn at 30 minute intervals.

of the area was noted in the first 30 minutes after which time the reaction developed rapidly and then stopped gradually (cells 4 and 9). In some instances the area diminished with intervals be-

TABLE I

Current number of cell	0 Min.		30 Min.		60 Min.		90 Min.		120 Min.		150 Min.		180 Min.		210 Min.	
	μ^2	%	μ^2	%	μ^2	%	μ^2	%	μ^2	%	μ^2	%	μ^2	%	μ^2	%
1	532	100·0	450	84·6	348	65·4	248	46 8	246	46 6	254	47·7	249	46·9	252	47·3
2	548	100·0	426	78·6	283	52·2	236	43·5	194	35·8	185	34·2	185	34·1	190	35·0
3	552	100·0	514	93·1	424	76·9	350	63·4	308	55.8	319	57 8	326	59·0	312	56·5
4	461	100·0	461	100 0	354	76·8	352	76·4	349	75·7	314	68 2	309	67·0	316	68·5
5	474	100·0	455	96·0	368	77·6	349	73·6	290	61·2	283	59·7	308	64·9	301	63·5
6	560	100 0	425	75·9	319	57·0	291	52·0	252	45·1	223	39 8	217	38·7	220	39·3
7	339	100·0	288	85·0	290	85·5	282	83·2	216	63·7	189	55·8	181	53·4	173	51·0
8	545	100·0	493	90·5	435	87·8	398	80·3	383	70·3	378	69·4	375	68·8	379	69 6
9	585	100·0	564	96·4	300	51·3	242	41·7	174	29·7	198	33 8	206	35·2	195	33·3
Total	4590	100·0	4076	88·8	3121	68·0	2748	59·8	2412	52·5	2343	51·0	2356	51·3	2338	50·8

tween sudden shrinking (cell 7). Also the percentage of epistrophe at the end of the reaction is different in each cell and varies for the different cells from 30—70%.

With the use of the statistical method of calculating the average percentages of the ractions a very regular curve is obtained, the shape of which resembles the shape of the curve plotted for the

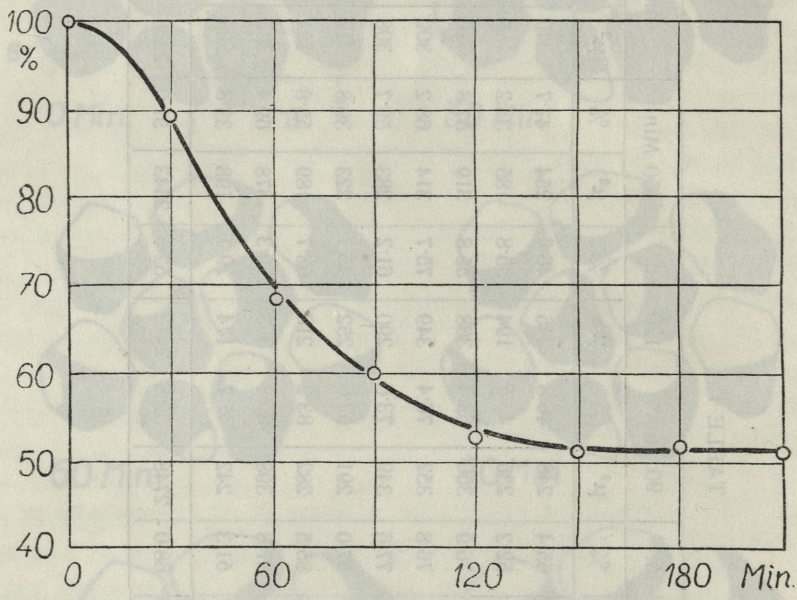

Fig. 3. Graph illustrating the epistrophe — parastrophe reaction according to Fig. 2. Abscissa-time in minutes, ordinates — average area of cell, occupied by chloroplasts expressed as % of the total cell area.

movements of chloroplasts in the epistrophe to parastrophe reaction in *Lemna trisulca* (Zurzycka and Zurzycki 1950). In this paper only the average values calculated in approximately 10 cells will be given (Fig. 3).

With the use of the statistical method the epistrophe to parastrophe reaction was investigated at the different intensities of light quoted below. The intensities are given in relative units (to estimate the intensities the law of squares was applied):

I — 100	IV — 5·5
II — 67	V — 0·71
III — 25	VI — 0·07

TABLE II

Intensity of light	Number of cells	Total cells area μ^2	0 Min.	30 Min.	60 Min.	90 Min.	120 Min.	150 Min.	180 Min.
I	9	4590	100·0	88·8	68·0	59·8	52·5	51·0	51·3
I	8	3255	100·0	82·2	72·3	52·4	52·0	—	52·7
II	10	4962	100·0	89·1	79·7	74·2	70·4	—	63·6
II	10	4183	100·0	87·4	76·5	71·3	68·1	—	66·7
III	8	3438	100·0	94·4	88·8	86·0	78·3	—	75·2
III	14	5335	100·0	93·6	87·7	84·0	73·7	—	73·6
IV	10	4048	100·0	—	93·8	89·5	88·3	—	89·1
IV	9	4395	100 0	97·6	95·2	91·4	89·7	—	90·4
V	10	3862	100·0	97·3	95·8	94·7	95·7	—	96·2

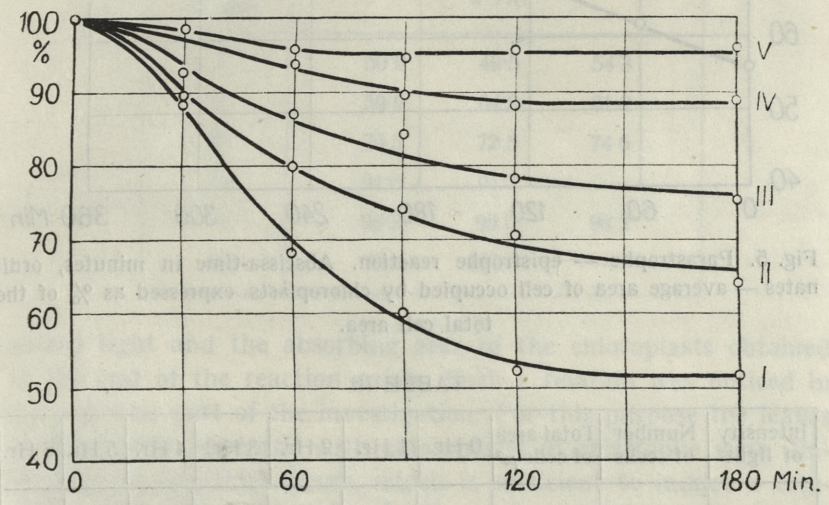

Fig. 4. Epistrophe — parastrophe reaction at the different intensities of light (I—V). Abscissa time in minutes, ordinates — average percentage of area occupied by the chloroplast.

The results are given in Table II and Fig. 4. When the different curves obtained are compared it appears that the rapidity of the reaction increases together with the increase in intensity of light. On the graphs it is expressed by the more rapid dropping of the

curve. Moreover it appears that after 2—3 hours the decrease of the area of the chloroplasts ceases and that a different percentage of the reaction corresponds to each intensity of light.

PARASTROPHE TO EPISTROPHE REACTION

In this reaction the starting position was the parastrophe obtained by illuminating chloroplasts during two hours with light of

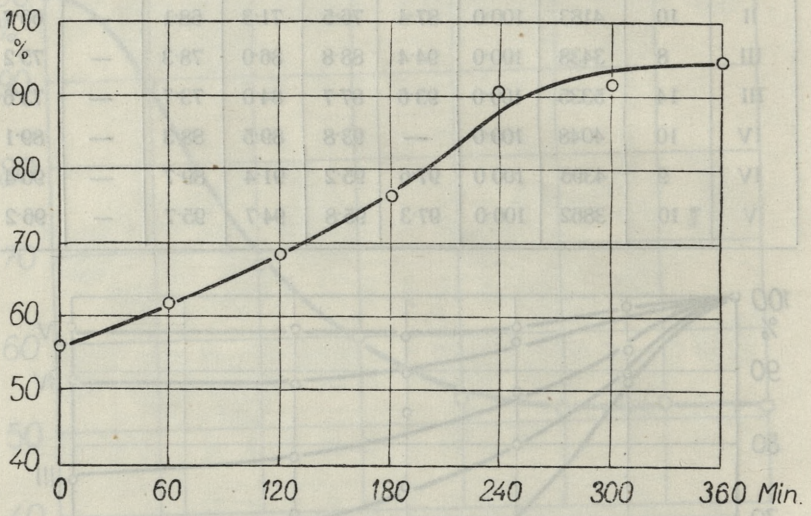

Fig. 5. Parastrophe — epistrophe reaction. Abscissa-time in minutes, ordinates — average area of cell occupied by chloroplasts expressed as % of the total cell area.

TABLE III

Intensity of light	Number of cells	Total area of cells μ^2	0 Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6 Hr.
I → V	10	5790	55·5	61·9	68·3	77·1	90·9	92·3	94·5
I → V	10	4782	62·0	67·4	77·2	85·8	—	95·0	96·4

a relative intensity — 100. After two hours the intensity was reduced to 0·71 (in relative units) and the return of the chloroplasts to the epistrophe was observed. This reaction, as appeared from the first experiments, lasts much longer then the opposite one, hence

the observations and drawings were made at hourly not half hourly intervals. As was mentioned previously when the movements of the chloroplasts were described and as appears distinctly from Fig. 5, this process lasts approximately 5 hours, and even after this time it happens that not the whole chloroplast returns to its starting position. The numerical data for the curve are given in Table III.

V. Relation between the absorbing area of chloroplasts and the intensity of light

The experiments in this part of the investigation were carried out to determine whether a relation between the intensity of absorbed light and the absorbing area of the chloroplasts obtained at the end of the reaction exists. Such a relation was noticed in the previous part of the investigation. For this purpose the leaves of *Selaginella Martensii* with chloroplasts in epistrophe were illuminated during three hours, which is sufficient to induce a complete parastrophe, with light of different intensities. The percentage of the reaction was obtained by calculating the ratio of the area of a chloroplast at the beginning and at the end of the experiment. The experiment was repeated three times in each light intensity. The results obtained are given in Table IV and Fig. 6. The average percentage of reaction obtained in each light intensity differed only slightly one from another, which shows explicitly that the degree in which the chloroplasts react to a stimulus of light that

TABLE IV

Intensity of light	3 Hr.		
I	50·9	49·5	54·3
II	59 9	64·9	61·2
III	76 5	72·5	74·6
IV	91·8	91·7	—
V	98·2	96 0	98·3
VI	99·4	100 0	—

is the decrease of the area of absorption of this stimulus depends completely on the intensity of the stimulating light.

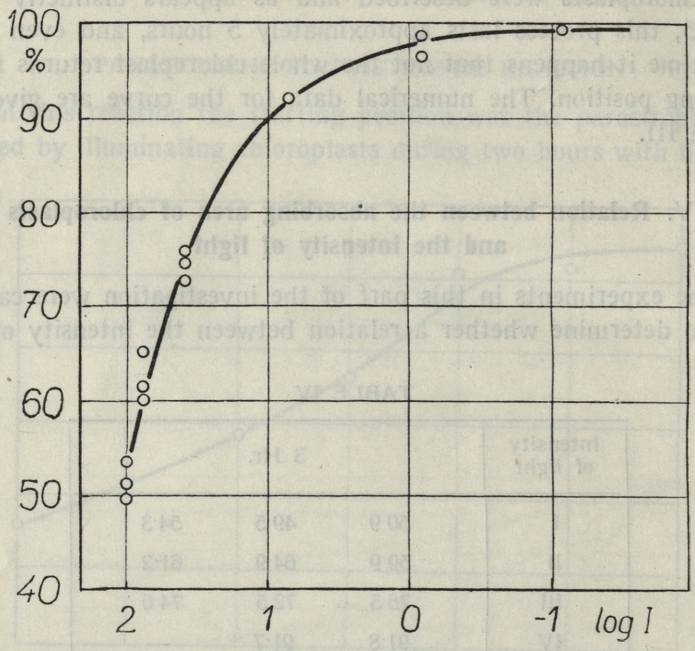

Fig. 6. Average percentage of area occupied by the chloroplast plotted against the intensity of light.

VI. The phototactic movements of chloroplasts induced by red and blue light

The phototactic reactions were investigated only in blue and red light. The reason for experimenting in light of these two colours was that it had been found in the course of previous investigations (Senn 1908, Voerkel 1934, Zurzycka 1951) that only within these ranges of the spectrum may important differences in the course of the reaction be induced. Glass filters were used and their ranges of transparency were

in the blue filter — 425—510 mμ
in the red filter — 592 mμ to the limit of red light.

In experiments with coloured light an Osram 110 V 250 W lamp was the source of light. The intensity of light, which was measured

proximately with a Lange's photocell, was found to be equal to the greatest intensity of white light used in previous experiments.

If the chloroplasts in epistrophe are illuminated with blue or red light then in both cases they will move from the epistrophe to the parastrophe and simultanously their absorbing area will decrease. The shape of the contracting chloroplasts is usually different in light of each of the two ranges of wave lengths (Fig. 7). In blue light the shrinking of the area always begins with the formation of a distinctive inward gap so that the expanse at which the chloroplast adheres to the side walls of the cells is considerable. The chloroplast retains its crescent shape throughout the reaction (in the same way as in intensive white light). It is not till after 3—5 hours that the crescent ends of the chloroplast can in some cases contract together and so cause the chloroplast to assume a circular shape.

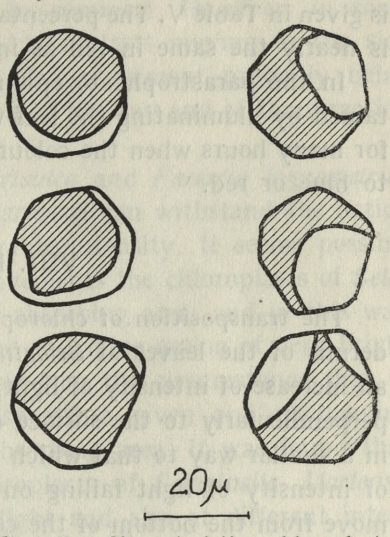

Fig. 7. Characteristic chloroplast shapes in parastrophe. Left in red light, right in blue light.

In red light similar shapes can often be noticed, though the inward gap is never so marked as in blue light. On the whole, however, the transposition of the chloroplasts in red light has a quite different character. The detaching of the chloroplast takes place at first on a short segment of the cell walls and so deep inward

TABLE V

Colour of light	Number of cells	Total cells area μ^2	1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.
Blue	11	5220	100·0	85·6	76·0	70·0	67·6
Blue	10	4682	100·0	87·5	73·4	71·2	69·4
Red.	11	4075	100·0	86·4	75·8	69·8	69·6
Red.	10	4593	100·0	86·5	79 6	75·0	72·5

gap is formed. However, after some time the chloroplast removes itself from the greater part of the side walls. As a result it assumes a more circular shape. In a few rare instances it was noticed that the edge of the chloroplast moved completely away from the walls of the cell. The development of the reaction in blue and red light is given in Table V. The percentage of the reaction when it is finished is nearly the same in red as in blue light.

In the parastrophe to epistrophe reaction the parastrophe obtained by illuminating the leaf with white light remains unchanged for many hours when the colour of the light is changed from white to blue or red.

VIII. Discussion

The transposition of chloroplasts in cells of the upper-face epidermis of the leaves of *Selaginella Martensii* Spring., caused by an increase of intensity of light, if the stimulus of light is directed perpendicularly to the surface of the leaf, develops on the whole in a similar way to that which Senn (1908) described. An increase of intensity of light falling on the cell causes the chloroplast to move from the bottom of the cell — i. e. from epistrophe — to the side walls. The arrangement of chloroplasts on the side walls was defined, similarly as was done in the case of *Lemna trisulca* and *Funaria hygrometrica*, as the parastrophe.

The influence of the direction of light rays on the chloroplast movements were not investigated. Haberlandt's experiments (1905) showed that the principle of the phototactic reactions of chloroplasts is the same independently from the direction of the rays of light.

In 1923 Suessenguth described the changes in the colour of the leaves of *Selaginella serpens* at the different times of the day. This species is grown in hothouses just as often as *Selaginella Martensii*. Suessenguth found out that the changes in the colour of leaves at different times of the day are caused by definite changes in the arrangement and shape of chloroplasts. In the morning the chloroplasts are cup-shaped and placed at the bottom of the epidermis cells causing the leaves to have a lush green colour. In the course of the afternoon or early evening the chloroplasts become round and place themselves at the upper wall of the epidermis

cells, causing the leaves to loose their lush green and changing it to a bluish green. All attempts at finding a similar daily cycle in the changes of shape and arrangement of chloroplasts in hot-house grown plants of *Selaginella Martensii* were unsuccessful which was possibly due to the early season of the year. (Suessenguth's investigations were carried out in summer). However, in some leaves at between 4 and 6 pm. chloroplasts moving to the side and upper walls were observed, this movement probably being caused by the change in the course of the day and of the direction of rays of light.

Like chloroplasts of *Lemna trisulca* and *Funaria hygrometrica* the chloroplasts of *Selaginella Martensii* can withstand the action of light of even a very considerable intensity. It seems possible that owing to the phototactic movements the chloroplasts of *Selaginella* are able to regulate their absorbing area, and in this way they can eliminate or diminish the damaging action of very bright light. This assumption can be based on the circumstance that to a given intensity of light corresponds a given and unchanging percentage of shrinking of the absorbing area. It was found that this phenomenon occurs in chloroplasts of *Selaginella Martensii* at different intensities of white light and also at different intensities of coloured light in *Funaria hygrometrica* (Voerkel 1934) and *Lemna trisulca* (Zurzycka 1951).

Both Voerkel's (1934) and Zurzycka's (1951) investigations show that in coloured light the chloroplasts of *Funaria hygrometrica* and *Lemna trisulca* react differently to coloured light when their arrangement is in profile and differently when it is flat. This can probably be explained by the differences in absorbtion of light caused by the regular arrangement of chlorophyll and carotene molecules in the grana. In the flat slatelike chloroplasts of *Lemna* and *Funaria* nearly all grana are arranged in parallel planes and the reactions in these plants are highly selective in regard to the wave lengths of light. In *Selaginella Martensii* the chloroplasts in epistrophe are cup-shaped and so some of the grana are illuminated in the flat position and others in the profile position. It can be presumed in advance that the differences of the reactions to the colours of light under investigation will not be so marked. The lack of the apostrophe arrangement makes it impossible to demonstrate the difference, if it exists, in a manner similar to that which

was done in investigations on *Lemna trisulca.* However, the pronounced difference in the shape of chloroplasts which was noted in the course of the epistrophe to parastrophe reaction in the experiments with coloured light may possibly be explained by the differences of reaction to light of the middle and the sides of the chloroplasts. It should be noted that the results obtained in the course of experiments with coloured light are only preliminary and should be studied in fuller detail.

IX. Summary

1. A quantitative method of investigating the phototactic reaction of chloroplasts in infiltrated leaves of *Selaginella Martensii* Spring. was described. Measurements were based on the area of the chloroplasts as seen from above, and expressed as the percentage of the area of the cell also as seen from above.

2. Using this method the epistrophe to parastrophe and parastrophe to epistrophe reactions were investigated and it was estabilished that a definite relation exists between the intensity of light and the area of absorption of an illuminated chloroplast.

3. It was demonstrated that phototactic reactions of chloroplasts of *Selaginella Martensii* are caused both by blue and red light. The outlines of the contracting chloroplast differ usually under the influence of the two colours of light. It is possible that this is caused by different action of light on the middle and the edges of the chloroplasts.

The authors wish to express their deep gratitude to Professor F. Górski for his valuable guidance in the course of this work.

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Laboratory Director: Professor F. Górski.

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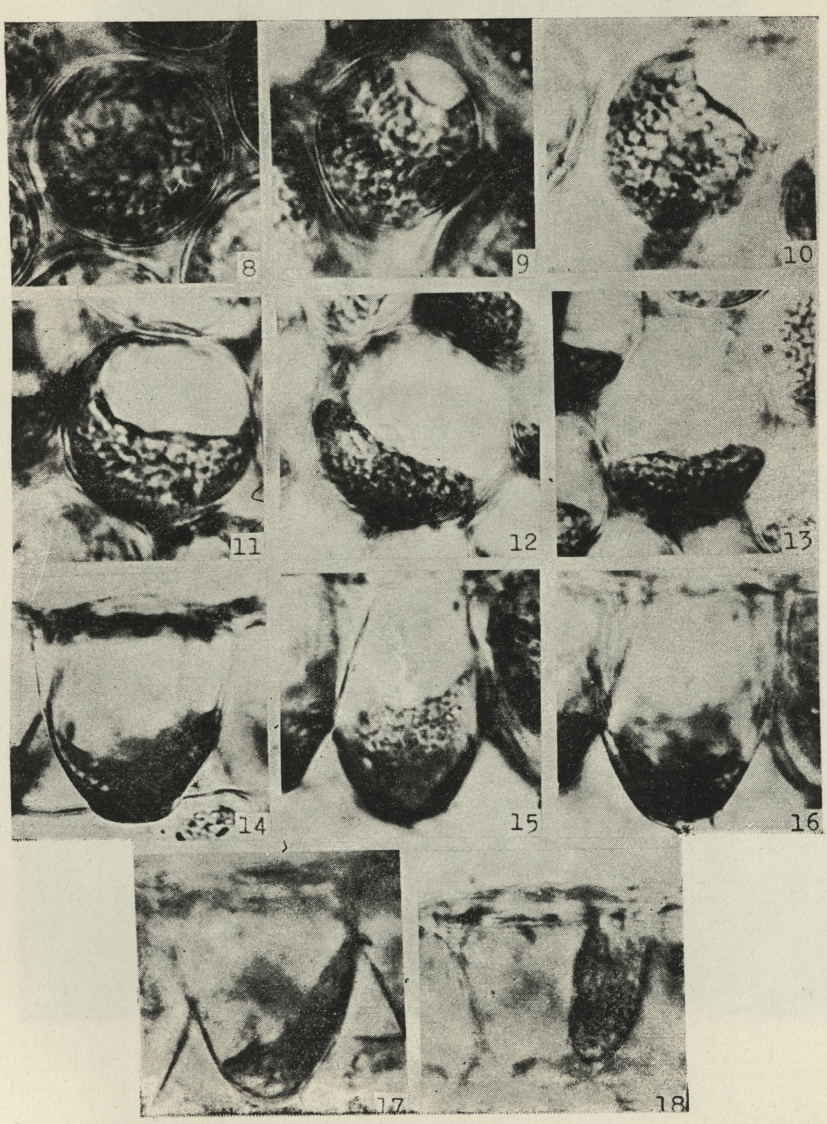

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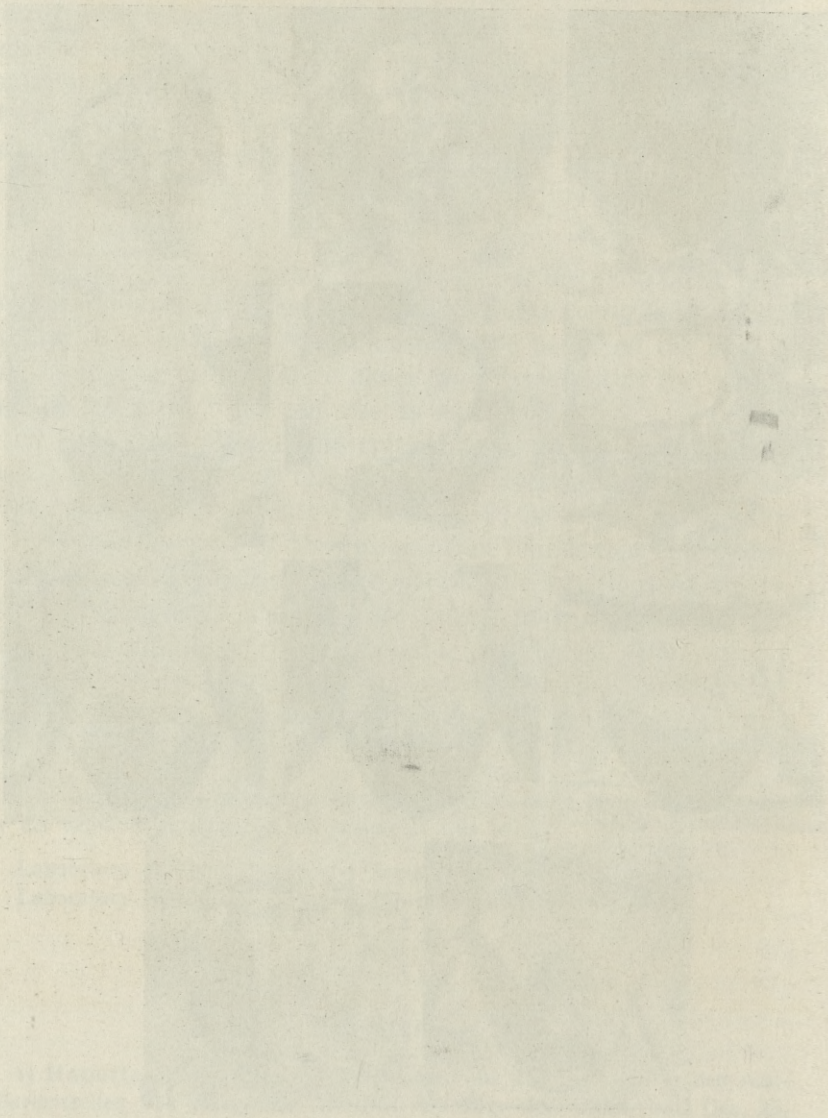

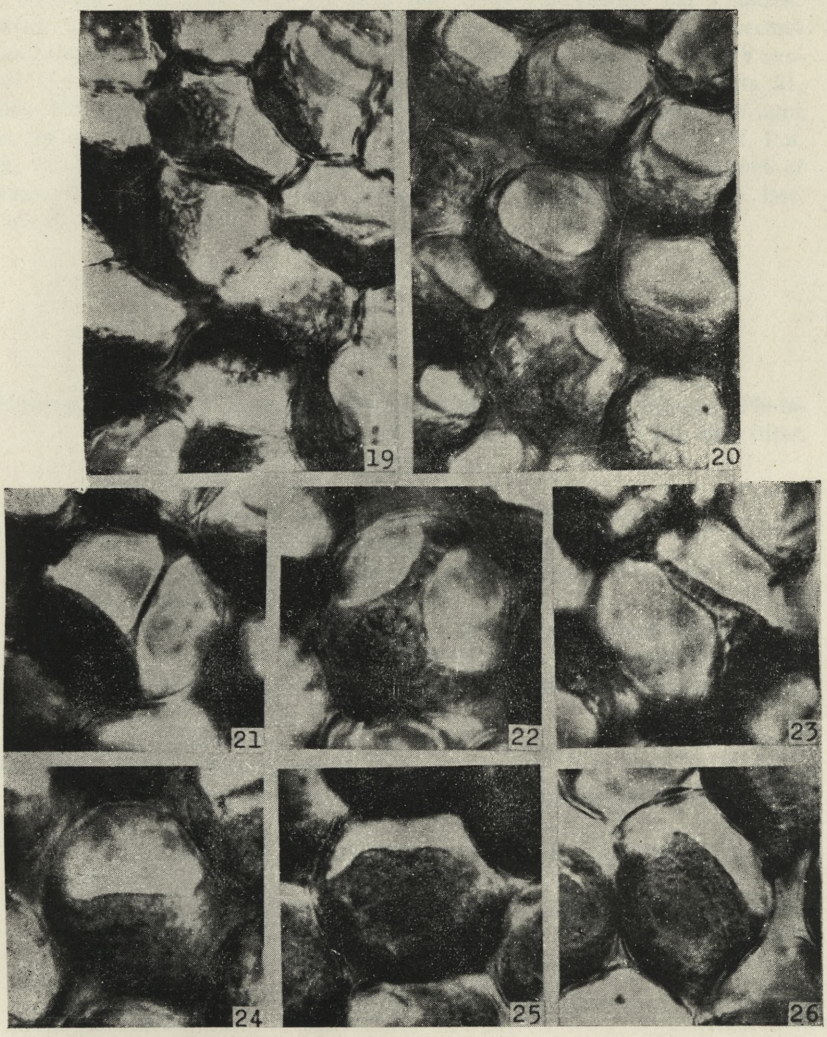

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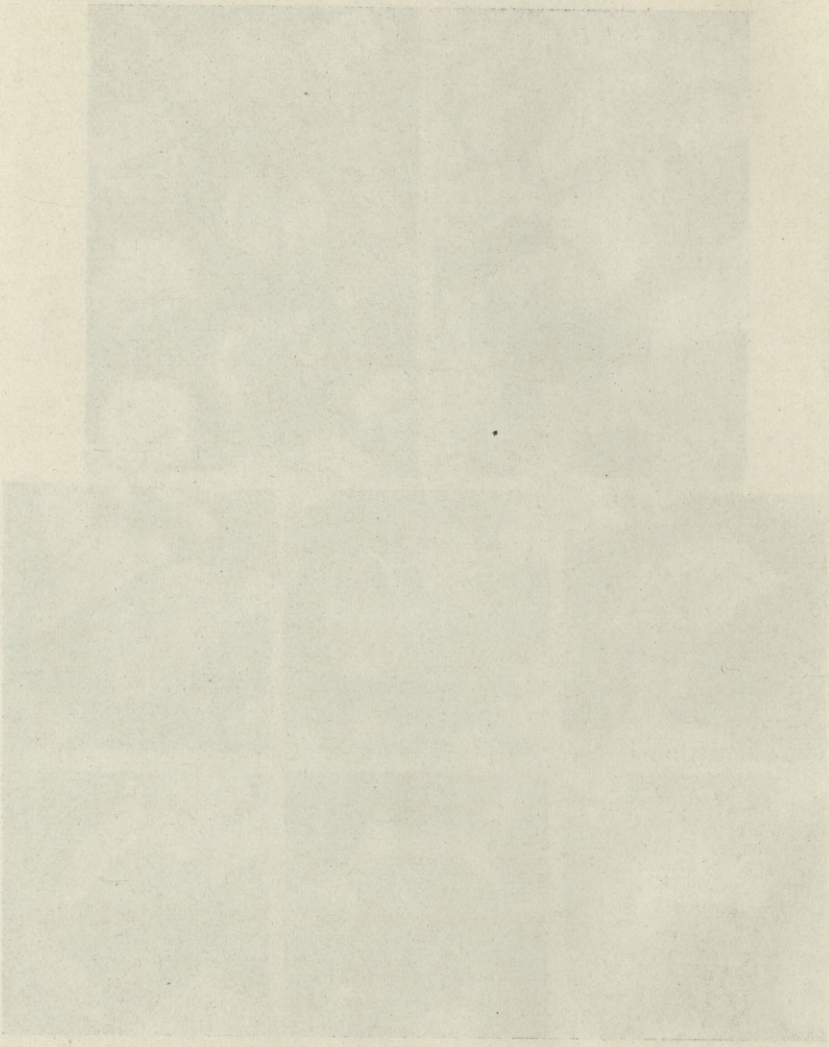

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Explanation of plates

Plate 13. Chloroplasts in living cells of *Selaginella Martensii* Spring. Photographs done with obj. imm. 100×, n. A. 1·25, eyepiece 5×, blue filter.
8—13 — view of cells from above.
8 — complete epistrophe.
9—12 — different stages of transposition of chloroplasts.
13 — rounded outline of chloroplasts in parastrophe.
14—18 — side view of cells in a cross section of the leaf.
14 — epistrophe.
15, 16 — epistrophe — photograph at different levels of the same cell.
15 — the edge of the chloroplast is visible, 16 — optical section.
17 — transposition of chloroplasts in the course of epistrophe — parastrophe reaction.
18 — parastrophe.

Plate 14. 19—25 — view of cells from above.
19 — chloroplasts detaching themselves in different directions in every cell.
20 — chloroplasts detaching themselves in the same direction in all cells.
21—23 — characteristic «bridges» formed by chloroplasts in epistrophe-parastrophe reaction.
24 — shape of chloroplasts in blue light.
25, 26 — shape of chloroplasts in red light.

Badania cyto-ekologiczne nad Poa alpina L. var. vivipara L. — Cyto-ecological studies in Poa alpina L. var. vivipara L.

Mémoire

de M^me^ *M. SKALIŃSKA* m. c.

présenté le 1 Juin 1951, par Mme M. Skalińska m. c. et M. B. Pawłowski m. c.

(Plate 15)

Contents:

Introduction

The purpose of the present work, begun in 1949, was to give a contribution to the knowledge of the cytological differentiation of *Poa alpina* L., a species which manifests a high degree of diversity. Morphological differences among the various biotypes within this species concern general habit, vigour, height of the flowering stem, size and shape of the panicles, etc. The species shows also a complex cytological differentiation: biotypes from various geographic regions, investigated by Müntzing (1932, 1940, 1949), Flovik (1938), Böcher (1938), Böcher and Larsen (1950), Nygren (1950), represent a series of chromosomic types chiefly with aneuploid numbers ranging from 14 to 54 (—57). Further

differences concern the mode of reproduction: the various strains may be sometimes normal sexuals; apomictic or viviparous biotypes however seem to prevail.

Details concerning the chromosome numbers and the mode of reproduction of the various strains of *P. alpina* from Scandinavia, Spitzbergen, Faeroes Islands, Greenland, have been published by the above authors as well as by Håkansson (1943, 1944). In addition, a limited number of Swiss biotypes and a sexual strain probably from South Russia have been studied by Müntzing. On the basis of the results obtained this author expressed the opinion that *P. alpina* shows a geographic differentiation: each geographic region has its special chromosome number (Müntzing, 1949, p. 407).

The present study has been undertaken as an attempt to give a cytological analysis of *P. alpina* from a geographic region which is entirely isolated and remote from the centres explored in the course of the previous research work. Such an isolated centre is found in Poland in the Tatra Mts and the adjacent range of Pieniny. *P. alpina* is represented there by two forms: v. *seminifera* and v. *vivipara* L. Of the two forms, the former is notably rarer in the Tatra Mts than the latter which is extremely common there in a variety of habitats and at different altitudes. The results obtained are based on the study of 111 viviparous biotypes and two strains of v. *seminifera*; they have revealed a high degree of cytological differentiation, the material investigated comprising 7 chromosomic types (Table 1). The range of numbers found in the viviparous biotypes supplements substantially the series known for the northern countries and for Switzerland. It is interesting to note that the numbers found in v. *vivipara* from the Tatra and Pieniny Mts are lower than those reported from the north and arctic.

Material and methods

The main area explored (roughly 20 km in length and 8 to 12 km across) involves the Polish (northern) part of the Tatra Mts: the Western Tatra, chiefly calcareous, and the High Tatra in the south-east, the granitic part. In addition some strains from the foothills of the main range as well as from the range of Pieniny, situated more north-eastward, have been studied. The present study

deals chiefly with v. *vivipara*. It gives data concerning 111 biotypes collected in a variety of natural habitats at different altitudes ranging from 900 to 2300 m o. s. l. in the Tatra Mts and from 550 to 930 m o. s. l. in the range of Pieniny where *P. alpina* v. *vivipara* is of notably rarer occurrence than in the Tatra Mts. In addition, chromosome numbers of two strains of v. *seminifera* from the Tatra Mts have been determined. (For details concerning the habitats see Table II).

The determination of the numbers of chromosomes was based exclusively on root tip mitoses. The material was fixed in the diluted fixative of Navashin. The sections 10 μ thick were stained with Newton's gentian violet. Usually at least three root tips of each plant were studied and in each case for the determination of the chromosome numbers 6 to 10 metaphase plates were analysed. Sometimes however it was necessary to increase the number of the metaphase plates investigated in view of the frequently occuring variation of the chromosome numbers within the meristems of a single root tip. In such cases each germ layer of the growing point was studied separately.

Chromosome numbers

Owing to the high degree of morphological diversity within the viviparous biotypes of *P. alpina* the existence of at least several chromosomic types in the area explored could be anticipated. The cytological study of 107 viviparous clones from the Tatra Mts and their foothills, and of four clones from Pieniny revealed in fact a complex differentiation, since on the relatively small territory the occurrence of 7 different chromosomic types (2n=14, 22, 26, 28, 33, 34, 35) could be observed (Figs. 1, 2, Table I). In addition, the numbers 22 and 33 have been found in two non viviparous strains (Figs. 2 E, F, 6).

It is evident from Table I that among the various types three, viz., those with 22, 26 and 33 chromosomes are particularly common; the types with 28 chromosomes are less frequent, nevertheless this number has been found in 16 clones. The number 14, although exceedingly rare in the ample material studied, deserves special mention since it represents the primary diploid number of the genus *Poa*. On the other hand, the two remaining types 34 and 35,

have been found only sporadically; they represent slight deviations from the type 33 and might have been secondarily differentiated from the respective type.

TABLE I.

Frequency of different chromosomic types of *Poa alpina*.

Number of clones	Chromosome numbers (2n)							Total
	14	22	26	28	33	34	35	
var. vivipara: in the Tatra Mts	1	33	30	16	24	5	2	107
in Pieniny Mts	—	2	2	—	—	—	—	4
var. seminifera: in the Tatra Mts	—	1	—	—	1	—	—	2

The basic chromosome number of the genus *Poa* is 7. Multiples of this number occur in all its species with euploid numbers, e. g. *P. trivialis* 2n=14 (Avdulov, 1931); *P. Chaixii* Vill. 2n=14 (Avdulov, 1931, Nannfeldt, 1937); *P. annua*, subsp. *typica* 2n=28 (de Litardière, 1939); *P. laxa* 2n=28 (Skalińska unpubl.); *P. laxa*, var. *flexuosa* 2n=42 (Nygren, 1950). By contrast, the species *P. alpina* with its viviparous form contains a wide range of chromosomic types. In this series, the extremely rare viviparous strain with the strictly diploid number (Figs 1 A, 4) represents presumably an ancient type. It should be added that this lowest number has been found in a non-viviparous strain by Sokolovskaya and Strelkova (1940); it has been also reported recently by Müntzing (1949) for a peculiar sexual strain with 2 to 8 accessory chromosomes in the germ-tract. The occurrence of the diploid viviparous biotype in the Tatra Mts where strains with 28 chromosomes (Figs 1 D, 8) have been also found, is theoretically important: the higher number could be attained in the way of somatic chromosome doubling; accordingly, the types with 28 chromosomes ought to be considered as primary tetraploids. The results of previous investigations have shown that this strictly tetraploid number is very rare in other centres of distribution of *P. alpina*. A non-viviparous strain with 28 chromosomes native in West-Greenland has been recorded by Böcher and Larsen (1950).

Viviparous strains with this number however have not been found at all in the course of previous investigations.

Another type which is very rare in Switzerland and does not occur at all in the northern countries is represented by strains with 22 chromosomes (Figs 1 B, 5). This number, very common among the viviparous strains of the Tatra Mts, was found by Müntzing (1940) only in a strictly sexual strain from Switzerland. The possible origin of the aneuploid number 22 has been discussed by Müntzing (p. 182) in connexion with the regular chromosome pairing observed by him in this strain at meiosis. According to Müntzing, this number resulted from the change of the basic number 7 into a new (secondary) basic number 11. Thus, strains with the somatic number 22 represent secondary diploids. In the same way biotypes with 33 and 44 chromosomes should be regarded as secondary triploids and tetraploids.

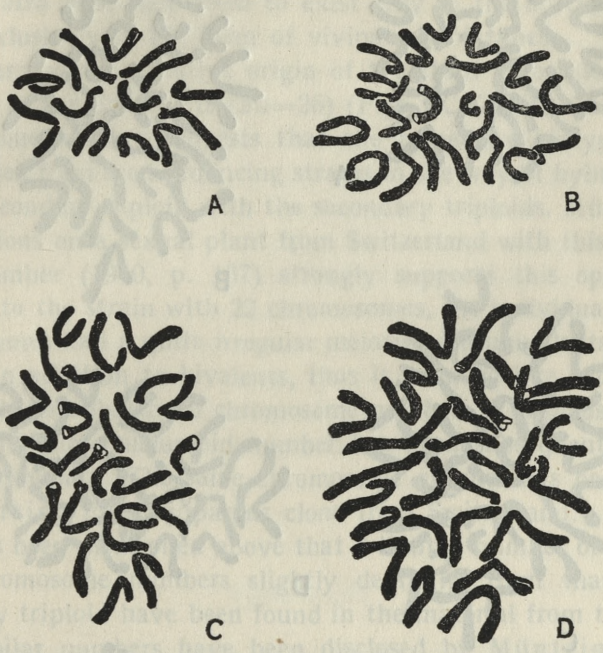

Fig. 1. Somatic metaphase plates of *Poa alpina*, var. *vivipara*. A — 14 chromosomes (clone 176a); B — 22 chromosomes (clone 191); C — 26 chromosomes (clone 131); D — 28 chromosomes (clone 166). (×3000).

The secondary triploid number 2n=33 (Figs 2 A, B, 9) represents one of the three main types of viviparous clones in the Tatra Mts and has been found there also in one of the seed-producing

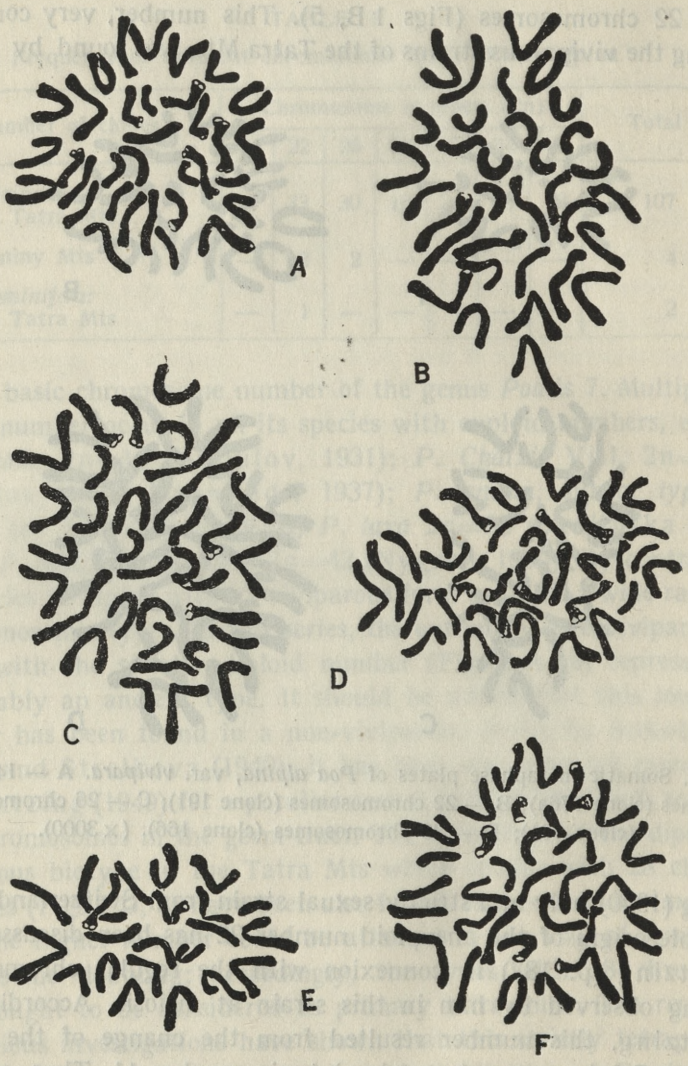

Fig. 2. Somatic metaphase plates of *Poa alpina*, var. *vivipara* (A—D) and var. *seminifera* (E, F): A — 33 chromosomes (clone 51); B — 33 chromosomes (clone 111); C — 34 chromosomes (clone 130); D — 35 chromosomes (clone 32); E — 22 chromosomes (strain 182); F — 33 chromosomes (strain 184). (×3000).

strains (Fig. 2 F). Concerning its general distribution, it is noteworthy that in other regions this chromosomic type is of relatively frequent occurrence: it is represented in the Alps and in the northern countries by partially sexual, apomictic and viviparous strains. The progenies of the former are cytologically heterogenous. On the other hand the secondary tetraploids (2n=44) have been not found in the Tatra Mts; they seem to exist only in the north occurring there exclusively in the form of viviparous biotypes.

Concerning the putative origin of the third chromosomic type common in the Tatra Mts (2n=26) (Figs 1 C, 7), its intermediate chromosome number suggests that the respective biotypes could have arisen from seed-producing strains in the way of hybridization of the secondary diploid with the secondary triploids. Müntzing's observations on a sexual plant from Switzerland with this chromosome number (1940, p. 137) strongly supports this opinion: in contrast to the strain with 22 chromosomes, the individual with 26 chromosomes had a quite irregular meiosis with univalents and trivalents in addition to bivalents, thus it behaved like a cross-product with an unbalanced chromosome number. It is plausible that this intermediate aneuploid number has been subsequently stabilised by vivipary. The same chromosome number has been found by Müntzing in a viviparous clone from Switzerland.

It has been mentioned above that a limited number of biotypes with chromosome numbers slightly deviating from that of the secondary triploid have been found in the material from the Tatra Mts. Similar numbers have been disclosed by Müntzing (1932, 1940) in the heterogenous progenies of sexual biotypes from Switzerland as well as in a viviparous plant. It is evident that in progenies of seed-producing plants such types could have arisen in consequence of meiotic disturbances. On the other hand, the possible origin of such deviating numbers in viviparous strains is rather difficult to explain. Two types from the Tatra Mts, with 34 and 35 chromosomes, belong here (Figs 2 C, D, 10). In the material investigated these two types are very rare, being represented only by 7 viviparous clones. The problem of their putative origin will be discussed farther (p. 276).

It should be added that in some instances the correct determination of the chromosome numbers presented difficulties. This was due to the frequently occurring variation of the chromosome num-

ber within the meristems of a single root tip in some plants. Slight differences usually not exceeding 1—2 chromosomes could be observed in the analysed metaphase plates. In some plants with 33 chromosomes occasionally plates with 34 chromosomes could be found; likewise, plants with 34 chromosomes had a few deviating plates with 35 chromosomes. A deviation from 26 to 25 (thus, a probable elimination of a single chromosome) has been observed with certainty only once, in a periblem cell in a root tip of the strain 161. In plants with 22 chromosomes one extra-chromosome and in rare instances two could be found also chiefly in the periblem. One particular plant (N. 57) with 22 chromosomes manifested a remarkable variation of chromosome numbers in the meristem of one root tip, ranging from 22 to 25, while two other root tips had a stable number. In the first root tip in three successive microtome sections the following numbers have been found in the three germ layers in 11 metaphase plates: dermatogen — 22 (1 plate); periblem, external layers — 22 (2 plates), 24 (1 plate), 25 (1 plate); deeper layers — 22 (1 plate), 24 (2 plates), 25 (1 plate); plerom — 23 (1 plate), 25 (1 plate). The above mentioned deviations represent in general only rare exceptions in the material investigated, occasionally also abnormal anaphases, possibly leading to non-disjunction, could be observed.

Morphology

The various viviparous biotypes of *P. alpina* native in the Tatra Mts manifested a striking morphological diversity; the four strains growing in the range of Pieniny are not identical, either morphologically or ecologically with the strains from the Tatra Mts.

Observations concerning the morphological features of the various biotypes were performed on plants in their natural habitats as well as in approximately uniform external conditions in the experimental field. The results of these studies, combined with the cytological investigations of the respective biotypes, have shown clearly that morphological criteria are inadequate for distinguishing the various chromosomic types not only in nature but also after the elimination of the influence of various external conditions existing in natural habitats.

The morphological diversity concerns chiefly the size and shape of the panicles, the height of the flowering plants as well as their general habit.

Size and shape of the panicles: the length of the panicles ranges in natural habitats from 1 cm to 9 cm. The extremes are rare while the intergrading values (3 to 6 cm) are frequent in all chromosomic types. The shape of the panicles manifests a great diversity, but no correlation with the chromosomic type could be observed: the panicles are loose (with long internodes and long lateral branches) or intermediate, or dense (with very short internodes and short lateral branches); in all main chromosomic types both the extremes and a series of intergrading forms were observed. Likewise, the relatively rare type with a unilateral ramification of the dense panicles could be found in the material studied irrespective of the chromosomic type. In culture the comparison of the panicles of various biotypes has revealed some size differences between the higher (33—35) and the lower (22—28) chromosomic types. (It should be added that the single plant with 14 chromosomes could not be observed in conditions of culture since is hat been collected only in 1951). On the whole, plants with higher chromosome numbers have somewhat larger panicles (4·8 to 8·5 cm long), usually with coarse bulbils, whereas plants with 22, 26 and 28 chromosomes have smaller panicles (3 to 5 cm long) with more gracile bulbils. No sharp line of demarcation however can be drawn between the two groups.

The length of the stem, measured from the bottom to the basis of the developed panicle, is a character strongly influenced by the habitat. In the Tatra Mts, at various altitudes, it varies from 5·3 cm to 28 cm, while in one strain in Pieniny it attained the length of 32 cm. In specimens from culture some differences could be established between the higher (33—35) and the lower (22, 26, 28) chromosomic types: the former are on the whole somewhat shorter, their haulms are only 7 to 20 cm long, rather stout and stiff; the latter are more slender and a little taller, their haulms are 12 to 26 cm long, thinner and more flexible. In general it may be said that the lower chromosomic types have somewhat longer and thinner haulms bearing smaller panicles, whereas in the higher types shorter and stouter haulms are combined with larger panicles. In

view of the strong overlapping of the values, a precise delimitation of the two groups is hardly possible.

Distribution in the Tatra and Pieniny Mts and ecology

A glance at Tables II, III and the map, Fig. 3 shows that the four main chromosomic types differ in their distribution although their areas overlap partially. The differences concern chiefly the altitudinal distribution and the edaphic conditions.

The altitudinal distribution of the biotypes originating from the Tatra Mts has been grouped in four layers, according to Szafer's division into vertical areas of the mountain flora (Szafer, 1929): (1) the higher mountain layer (roughly 900—1200 m o. s. l.); (2) the layer of subalpine forests (1200—1550 m o. s. l.); (3) the layer of *Pinus montana* (1550—1900 m o. s. l.); (4) the alpine layer (from 1900 m o. s. l. upwards). The notably lower range of Pieniny belongs to the lower mountain layer (600—900 m o. s. l.).

As far as the edaphic conditions are concerned, the following details should be given: the High Tatra, representing the south-eastern part of the Tatra Mts, is granitic with very steep cliffs and uncovered rocks. A number of specimens, chiefly those belonging to the alpine layer were found there growing in exposed habitats in cracks of solid granitic rocks. On the other hand, the Western Tatra has chiefly calcareous soils since on its slopes limestones are deposited on the surface of the granitic rocks. Some higher peaks of the main range however have caps of granit. Contrasting with the High Tatra, the Western Tatra has gentler slopes which bear deeper soil layers and have a more abundant vegetation. The range of Pieniny is chiefly built of limestones. The foothills in the north are built of geologically young marls, slates and sandstones with a relatively deep layer of soil on their surface; they are largely covered with woods.

Besides soil and elevation other factors acting in the various habitats (light, moisture of the ground, plant community, etc.) are also important. They influence however rather the local choice of habitat in accordance with the particular ecological requirements of the viviparous clones. All clones, irrespective of their chromosomic type, show a preference for partial shade and at least a moderately moist soil, thus, they occur abundantly on northern slopes and, by

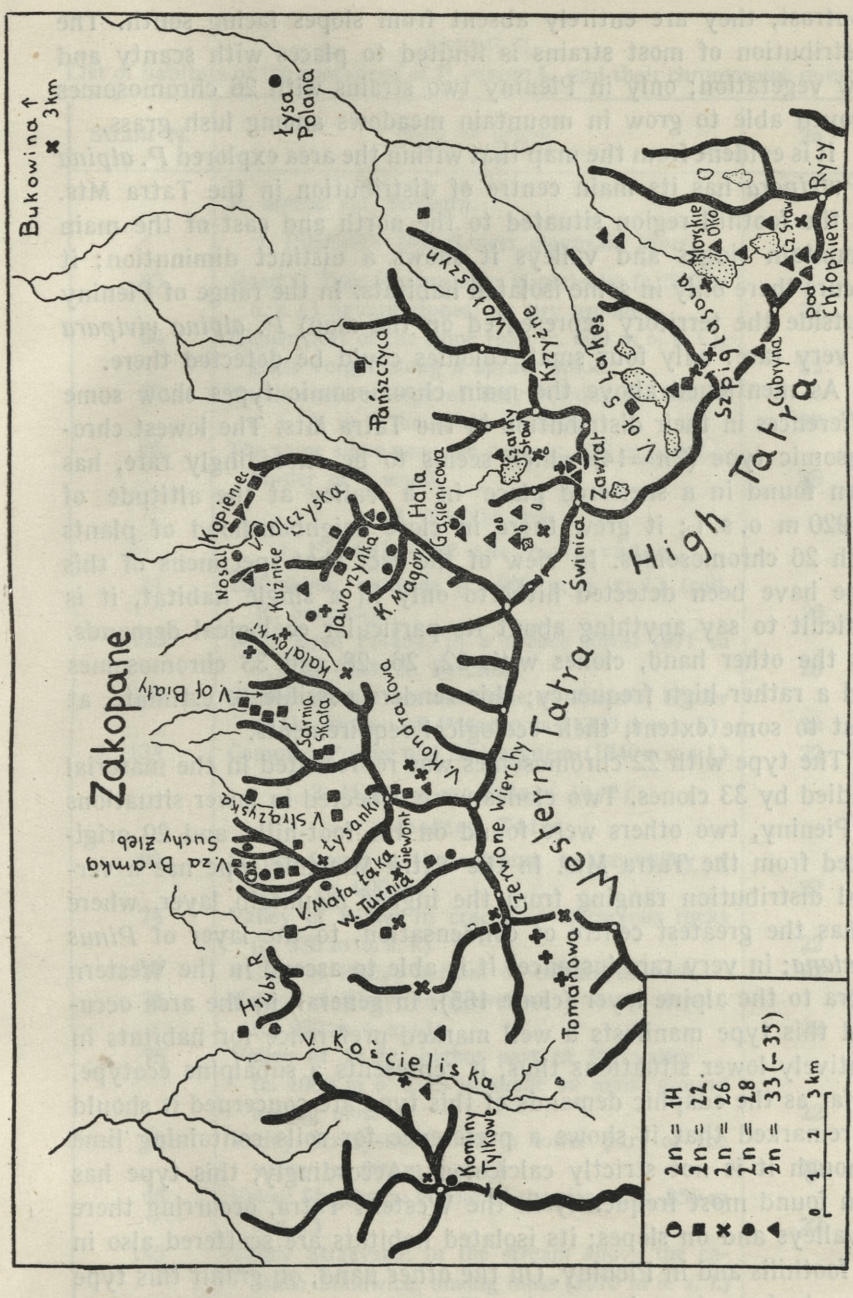

Fig. 3. Map showing the distribution in the Tatra Mts and the foothills of various chromosomic types of *Poa alpina* var. *vivipara*. Solid black lines represent the main mountain ridges.

contrast, they are entirely absent from slopes facing south. The distribution of most strains is limited to places with scanty and low vegetation; only in Pieniny two strains with 26 chromosomes proved able to grow in mountain meadows among lush grass.

It is evident from the map that within the area explored *P. alpina* v. *vivipara* has its main centre of distribution in the Tatra Mts. In the foothill region situated to the north and east of the main mountain ridges and valleys it shows a distinct diminution; it occurs there only in some isolated habitats. In the range of Pieniny (outside the territory represented on the map) *P. alpina vivipara* is very rare: only four small colonies could be detected there.

As mentioned above the main chromosomic types show some differences in their distribution in the Tatra Mts. The lowest chromosomic type (2n=14) which seems to be exceedingly rare, has been found in a sheltered place in a valley at the altitude of c. 920 m o. s. l.; it grew there in close neighbourhood of plants with 26 chromosomes. In view of the fact that specimens of this type have been detected hitherto only in a single habitat, it is difficult to say anything about its particular ecological demands. On the other hand, clones with 22, 26, 28 and 33 chromosomes had a rather high frequency; this renders possible to estimate, at least to some extent, their ecological requirements.

The type with 22 chromosomes was represented in the material studied by 33 clones. Two clones were collected in lower situations in Pieniny, two others were found on the foot-hills, and 29 originated from the Tatra Mts. In the Tatra Mts this type has a vertical distribution ranging from the higher mountain layer, where it has the greatest centre of condensation, to the layer of *Pinus montana*; in very rare instances it is able to ascend in the Western Tatra to the alpine layer (clone 185). In general, in the area occupied this type manifests a well marked preference for habitats in relatively lower situations thus, it represents a subalpine ecotype. As far as the edaphic demands of this type are concerned it should be remarked that it shows a preference for soils containing lime although it is not strictly calcicolous. Accordingly, this type has been found most frequently in the Western Tatra, occurring there in valleys and on slopes; its isolated habitats are scattered also in the foothills and in Pieniny. On the other hand, on granit this type seems to be extremely rare (see Table II, clones 89, 90). Within

TABLE II

List of habitats of the specimens of *P. alpina* L. and their chromosome numbers

Strain N.	Place of origin	2n
	P. alpina var. *vivipara*:	
	I. PIENINY Mts (Lower mountain layer)	
63	Road to Trzy Korony over Krościenko (c. 550 m o. s. l.) among stones, on dry ground.	22
64	Higher part of the same road (c. 600 m o. s. l.); grass verges along a spruce wood.	22
65	North-western slope of Trzy Korony (c. 880 m o. s. l.), in a mountain meadow.	26
66	The same slope near the peak (c. 930 m o. s. l.) border of a wood.	26
	II. TATRA Mts	
	1. Foothills of the main range	
41	Bukowina; roadside (c. 960 m o. s. l.) (coll. K. Satczek).	26
125	Near Łysa Polana, in a spruce forest (971 m o. s. l.) (coll. K. Satczek).	28
133	Bottom of the western slope of Kosista; border of the stream of Pańszczyca (1300 m o. s. l.)	22
134	Common «Polana pod Wołoszynem» (1240 m o. s. l.)	22
	2. Higher mountain layer	
	Western Tatra	
95	Spruce forest at the entrance to the valley of Biały (c. 900 m)	22
74	Valley of Biały; in cracks of calcareous rocks (c. 930 m o. s. l.)	22
75	Valley of Biały; bottom of calcareous rock	22
76	Valley of Biały; in gravel on a steep slope (c. 970 m o. s. l.)	22
78	Valley of Biały; higher part of the valley (c. 1000 m o. s. l.) in shade on moist ground among moss	22
45	Valley Strążyska: wood in lower part of the valley (c. 900 m o. s. l.)	22
46	Valley Strążyska: border of the stream (c. 950 m o. s. l.)	22
145	Valley Strążyska: in the stream above the cascade Siklawica, among moss (1010 m o. s. l.) in rapidly running water	22

Strain N.	Place of origin	2n
148	Valley Strążyska; in cracks of calcareous rocks near Siklawica	22
175	Suchy Żleb: border of the stream in a narrow gorge with slopes covered by a beech wood (c. 930 m o. s. l.); very damp habitat	28
191	Entrance to the valley Za Bramką; bottom of calcareous rock in sheltered habitat (c. 920 m o. s. l.)	22
176 a, b	Valley Za Bramką; in cracks of a limestone rock and on its bottom (c. 920 m o. s. l.); (2 clones)	14, 26
137	Valley Za Bramką, among rock debris near the stream	28
179	Valley Za Bramką, higher part of the valley, near the stream (c. 960 m)	22
138	Slope above the valley Za Bramką, in a mixed wood on moist ground (c. 1100 m)	22
54	Valley of Mała Łąka, in a mixed wood (c. 1000 m o. s. l.)	22
80	Valley Kościeliska near Hala Pisana, in moss by a permanent spring; swampy ground (1015 m o. s. l.)	34
122	Valley Kościeliska near Hala Pisana, dry ground near the stream (coll. K. Satczek and H. Wcisło)	26
121	Near the entrance to Valley Kościeliska, at the high-road (coll. K. Satczek a. H. Wcisło)	22
155	Hruby Regiel, lower part (coll. E. Banach and H. Wcisło)	28
96	Way from V. Kościeliska to Hala Tomanowa; border of stream (c. 1100 m)	26
144	At the bottom of Krokiew, in the dry bed of a torrent (c. 950 m)	26
40, 157, 158	Valley Jaworzynka, near the road (1000—1095 m) (3 clones)	33, 26, 28
163	South-western slope over Jaworzynka, in a wood	22
166, 124	Valley Olczyska, border of the stream (2 clones)	28, 28
61	Over Kuźnice, way to Kalatówki among stones (c. 1050 m)	26
	3. Layer of subalpine forests	
	a) Western Tatra	
52, 114	Slope above the valley Strążyska; border of a beech wood (c. 1200 m) and of a spruce forest (c. 1300 m) (2 clones)	22, 22

Strain N.	Place of origin	2n
180, 181	Peak of Sarnia Skała (1377 m) and slope c. 50 m below (2 clones)	22, 22
139, 116	Peak of Łysanki (1440 m) and c. 40 m below (2 clones)	28, 26
96	Way to Hala Tomanowa from Valley Kościeliska: on a stony slope near the stream (c. 1250)	26
99	Hala Tomanowa — grazing ground (1360 m)	26
100	Czerwony Żleb over Hala Tomanowa; among loose blocks of limestone (c. 1450 m)	26
156	Gładkie Upłaziańskie (c. 1500 m) (coll. E. Banach a. H. Wcisło)	26
187	Upłaz — on a grassy slope (c. 1300 m) (coll. Z. Kotońska)	33
5, 55	Kobylarz on the way to Czerwone Wierchy; grassy slope facing west (1400 m) (2 clones)	22, 28
162	Slope of Skupniów Upłaz over Valley Jaworzynka; open space with low vegetation (c. 1300 m)	22
168	Slope of Kopa Magóry, facing north-east; spruce wood, moist ground among moss and stones (c. 1350 m)	28
169	The same slope, higher (c. 1400 m), open space, drier ground	28
51	Woody slope of Boczań (c. 1200 m)	33
161	Kalatówki, border of a spruce wood (c. 1200 m)	26
60	Valley Kondratowa, among stones (1330 m)	26
104	Way to the pass Kondracka, border of the path (c. 1400 m)	26
110,111,112	Peak of Nosal (1206 m) and somewhat below (3 clones) border of a spruce wood (2 habitats); steep cliff (1 hab.)	33, 33, 34
143	Peak of Kopieniec Wielki (1328 m), among low vegetation	22
123	Kopieniec Mały (western slope) (coll. K. Satczek)	34
	b) High Tatra	
119	High-road near the valley of the lake Morskie Oko; border of a spruce wood, on dry ground (c. 1400 m)	33
120	The same road; border of a stream (c. 1400 m	33
93	Valley of Morskie Oko; eastern border of the lake (c. 1400 m)	33

Strain N.	Place of origin	2n
149	Valley of Morskie Oko; western border of the lake (c. 1400 m)	26
	4. Layer of *Pinus montana*	
	a) Western Tatra	
3, 26	Peak of Kominy Tylkowe (1820) (coll. A. Kłaput) (2 clones)	26, 28
57	Eastern slope of Wielka Turnia, covered with limestone scree and grass (c. 1800 m)	22
59	Eastern slope of Małołączniak (group of Czerwone Wierchy), c. 100 m below the peak in low grass (c. 1900 m)	28
172	At the bottom of the north-western cliff of Wielka Turnia, near the pass Siwarowa (c. 1500 m)	26
101	Higher part of Czerwony Żleb (c. 1550), among stones	26
159	Pass Tomanowa (1686 m) (coll. H. Wcisło and E. Banach)	26
106	In a glacial cirque «Piekło» on the south-eastern slope of Giewont; in humus-filled cracks of rocks (c. 1600)	33
107, 108	Slopes of Giewont above the pass Kondracka, among rocks (c. 1750 m) and on stony soil with scanty vegetation (c. 1800 m) (2 clones).	22, 22
42	Skupniów Upłaz, slope above the road, in cracks of limestone rocks (c. 1500 m)	22
43 a, b	Pass «Między Kopami», on a slope above the road (1501) (2 clones).	28, 35
44	The common Karczmisko, along the road (1560)	28
	b) High Tatra	
47, 48, 49	Hala Gąsienicowa, among stones near the road (1520 m) (3 clones)	28, 33, 33
50	Border of the lake Czarny Staw, in moist soil among stones (1620 m)	33
10	Valley of Stawy Gąsienicowe: border of lake Litworowy, among moss and grass, swampy ground (c. 1600 m)	33
86	Western higher part of the same valley, border of a stream, moist soil (c. 1700 m)	33
85	Eastern part of the same valley, near path to Kasprowy; among stones in a stream (c. 1700)	33

Strain N.	Place of origin	2n
67	Path to pass Świnicka from the same valley; on a slope facing north (c. 1650 m)	26
70, 129	Path below the lake Zmarzły Staw, among granitic blocks (c. 1700 and 1760 m) (2 clones)	26, 26
89	Valley of Five Polish Lakes, border of the lake Wielki Staw (1665 m) (coll. E. Banach and H. Wcisło)	22
90	North-eastern slope of Miedziane, over the Valley of Five Polish Lakes (c. 1870 m) (coll. E. Banach a. H. Wcisło)	22
91	Way to Pass Szpiglasowa from the Valley of Five Polish Lakes (c. 1750 m) (coll. H. Wcisło a. E. Banach)	33
29	Path from the lake Morskie Oko to Czarny Staw (c. 1550 m)	33
30	Slope above the eastern border of the lake Czarny Staw over Morskie Oko (c. 1590 m)	33
153	Path from Czarny Staw to Rysy, near a patch of permanent snow (c. 1630)	26
	5. Alpine layer	
	a) Western Tatra	
185	Below the peak of Ciemniak in the group of Czerwone Wierchy; western slope, Triassic limestone (c. 2030 m) (coll. Z. Kotońska)	22
186	Below the peak of Ciemniak; northern slope (c. 2050 m) (coll. Z. Kotońska)	26
	b) High Tatra	
79	Pass Krzyżne (2113 m) (coll. J. Winnicka)	33
128, 132	Pass Szpiglasowa (2114 m) (2 clones)	26, 26
14	Path Orla Perć near pass Zawrat; northern slope; in cracks of granitic rocks (c. 2180 m)	33
32	Northern slope of Mały Kozi Wierch below pass Zawrat among granitic rocks (c. 2150 m)	35
15, 130, 131	Pass Zawrat (2159 m) (3 clones)	34, 34, 26
62	Kazalnica, below the pass «Pod Chłopkiem» (2150 m) (coll. A. Bajer)	33
27	Higher part of the path to the pass «Pod Chłopkiem» (c. 2200 m) (coll. A. Bajer)	33
28	Pass «Pod Chłopkiem» (2300 m) (coll. A. Bajer)	33

Strain N.	Place of origin	2n
136	Pass between Cubryna and Zadni Mnich (2180 m) (coll. Z. Paryska)	33
	P. alpina var. *seminifera*:	
182	Western Tatra: Kominy Tylkowe (1820 m) (coll. Z. Paryska)	22
184	High Tatra: near the lake Zmarzły Staw (1785 m) (coll. Z. Kotońska)	33

their region of distribution the biotypes with 22 chromosomes seem to possess a high degree of adaptability to various external conditions: in the calcareous part of the Tatra Mts this type grows most abundantly in valleys in a variety of habitats: both on open and shady places, on dry and moist soils, in cliffs of rocks, in wood clearings, along roads and banks of streams; in the different habitats the vigour and habit of the plants manifest a high degree of variability; particularly vigorous plants have been found in an extreme habitat: on the top of a cascade in rapidly running water (clone 145).

The type with 26 chromosomes is represented in the material investigated by 30 strains, two originating from Pieniny and one from the foothill range, while the remaining 27 strains were native in the Tatra Mts. Ecologically, this chromosomic type seems to represent a non-uniform, differentiated group, since the altitudinal distribution of its strains is different in the granitic and in the calcareous parts of the Tatra Mts. In the Western Tatra it is represented by subalpine clones which have their greatest centre of condensation in the layer of subalpine forests; in the High Tatra the type with 26 chromosomes is almost entirely restricted to the two upper layers; in the alpine layer it ascends to the altitude of 2159 m o. s. l. and occurs there together with the alpine type with 33 chromosomes; this suggests the existence of alpine biotypes also within this chromosomic type. In their natural habitats in the Tatra Mts the plants are of moderate performance. They occupy rather dry habitats, frequently on slopes with stony ground, on roadsides; in general they are found in places with low and scanty vegetation. Two subalpine strains of this type in Pieniny (clones 65 and 66) represent an entirely different ecological type: the plants grow there in mountain meadows among lush grass, thus in a plant

community in which *P. alpina* is never found in the Tatra Mts. The above details permit to assume the existence of a differentiation into alpine and various subalpine biotypes within this group.

The type with 28 chromosomes which presumably represents the tetraploid form of the extremely rare type with 14 chromosomes, is the least frequent among the four main types. In the material studied this type has been found 16 times: one strain was native in the foothills (clone 125), 14 biotypes have been found in the Western Tatra on limestone and only one strain originated from the granitic part of the Tatra Mts. According to the results obtained, the vertical distribution of this type ranges from the higher mountain layer to the layer of *Pinus montana*, where it replaces partially the subalpine biotypes with 26 chromosomes. It should be added that this type seems to be almost entirely limited to the limestone area, approaching in this respect the type with 22 chromosomes. Likewise, the primary diploid (2n=14) has been found on limestone (clone 176a). The single plant with 28 chromosomes collected in the granitic part, had its habitat close to the limit of the limestone area where from the bulbils could be easily spread. Some clones of this type were found in habitats with a notably higher degree of moisture, both in the soil and in the air, than the habitats of type 26.

The strains with chromosome numbers ranging from 33 to 35 seem to represent a single ecological group in spite of slight karyological differences. 31 biotypes of this group native in the Tatra Mts have been found in the material studied. Their vertical distribution extends from the higher mountain layer where however representatives of this group seem to appear only exceptionally, to the alpine layer where they grow abundantly. Most of the clones are distinctly alpine ecotypes. Concerning the edaphic demands, this type shows a well marked preference for acid soils; accordingly, in contrast with all other types, this group is rather rare in the calcareous part of the Tatra and represents the most frequent type in the High Tatra on granit. In the granitic part it occurs chiefly in the two highest layers, constituting a common component of their flora. In the alpine layer at elevations exceeding 2000 m o.s.l. these plants are found on cliffs, among scree, in cracks of rocks; in these extreme high-mountain habitats they are able to form large tufts and well developed panicles. In the layers of *Pinus*

montana and of subalpine forests they are found frequently in moist habitats (in swampy ground at the borders of lakes), on banks of streams, as well as in water of mountain streams among stones. As mentioned above, in the limestone part of the Tatra Mts this type is rather rare, scattered there only in form of isolated spots. In relatively dry limestone habitats plants of this group show a rather poor development and are notably exceeded in vigour by plants with 28 chromosomes growing in the same habitats (e. g. clones 43 a, b). In some particular moister habitats on limestone however these plants seem to be well established and show there a quite normal development; a clone with 34 chromosomes (N. 80) grows abundantly in the Valley Kościeliska in swampy ground among mosses by a permanent spring; another clone with 33 chromosomes (N. 106) was found in a glacial cirque on the slope of Giewont in humus-filled cavities of rocks. In consequence of these particular local conditions the surface layers of the soil may become faintly acid.

Observations concerning the edaphic requirements of non-viviparous strains were limited to two biotypes only: the first (182) with a low chromosome number (2n=22), originated from the limestone in the Western Tatra; the second (184), with a high number (2n=33) has been found on granitic rocks in the High Tatra. Although the study of only two strains does not give any adequate basis for general conclusions concerning the ecological demands of the seed-producing strains, the results obtained suggest that a differentiation of the chromosomic types is parallel to that observed in the viviparous clones.

Differences in the composition of populations of *P. alpina* v. *vivipara* in the subsequent vertical layers are represented on Table III. In the lower mountain layer and on the foothills only biotypes with 22, 26 and 28 chromosomes have been found. In the higher mountain layer almost all chromosomic types are present, but the three types mentioned are distinctly dominant. In the next layer — that of subalpine forests, these types are still abundant; the higher chromosomic type however (2n=33) found in the higher mountain layer only in form of a few isolated spots, becomes gradually more frequent and reaches a well marked prevalence in the layer of *Pinus montana*. In the alpine layer it is this type which becomes distinctly dominant — together with the alpine ecotypes of the type with

26 chromosomes, while the strains with 22 and 28 chromosomes show a rather abrupt diminution.

TABLE III.

Altitudinal distribution of chromosomic types of *P. alpina* L. var. *vivipara* L.

Vertical areas:	Chromosomic types (2n):							Total numbers of biotypes:
	14	22	26	28	33	34	35	
Lower mountain layer	—	2	2	—	—	—	—	4
Foothills	—	2	1	1	—	—	—	4
Higher mountain layer	1	15	6	6	1	1	—	30
Layer of subalpine forests	—	7	9	4	7	2	—	29
Layer of Pinus montana	—	6	8	5	10	—	1	30
Alpine layer	—	1	4	—	6	2	1	14
Total	1	33	30	16	24	5	2	111

The following general remarks concerning the interrelations of the main chromosomic types in their distribution should be added. Some small areas are occupied entirely by only one chromosomic type. Clones with 22 chromosomes are the only representatives of *P. alpina* in some limestone valleys of the Western Tatra. Clones with 26 chromosomes have been found along some mountain roads ascending from the higher mountain layer to the layer of *Pinus montana*. In the granitic part some regions in the layer of *Pinus montana* and in the alpine layer are occupied solely by the type with 33 chromosomes. On the other hand, in some other regions two types were found side by side in the same habitat. The common occurrence of the following main types could be observed: 22 with 26, 22 with 28, 26 with 28, 26 with 33, and 28 with 33. It should be emphasized that the types 22 and 33 have been never found in the same habitat, probably owing to their contrasting edaphic and altitudinal preferences. An interesting case is represented by the striking diversity of the population of *P. alpina* var. *vivipara* in a short and narrow limestone valley «Za bramką»; it is composed of four different types; in sheltered habitats among rocks and along the stream clones with 14, 22, 26 and 28 chromosomes are crowded in this little valley on an area of less than 1 km. The bio-

type with 14 chromosomes was found there in close neighbourhood of type 26. The above details show that the main chromosomic types are not sharply separated; their areas overlap partially.

In the general distribution of the various types in the Tatra Mts, one point still remains obscure: the occasional occurrence of one extreme chromosomic type within the area of another type in the form of isolated spots (clones 80, 106, 187). The well known peculiar mode of propagation of the viviparous clones favours the dispersal of the bulbils rather on short distances; the vertical distribution may be partially extended downwards since the bulbils may be brought from higher situations by mountain streams and in this way the young plants may establish themselves in new habitats at lower altitudes; the bulbils may be also easily displaced and subsequently pressed in into the soil by tourists as well as by sheep flocks going up and down on mountain slopes and along roads and valleys. These mechanisms of dispersal may be responsible for the occasional transport of the bulbils of a few clones with 22 chromosomes into the valley of five Polish Lakes and there from onto some of the surrounding slopes along a road leading there from the foothills (clones 89 and 90). This mode of dispersal however does not afford any explanation for the occurrence of viviparous clones with 33 and 34 chromosomes in isolated remote habitats. It is possible however that these isolated spots of the highest type found in the Western Tatra should be considered as remnants of a regressing population. The secondary triploids (2n=33) could have arisen in several points of the area of the secondary diploids (2n=22) by functioning of unreduced gametes of sexual strains. The increase of the chromosome number followed by the differentiation of alpine types enabled the latter to expand over new territories, extending in this way the altitudinal and edaphic range of the species. At this evolutionary stage, crosses between the two types could lead to the production of intermediate types, the fittest of which (2n=26) stabilized by vivipary could establish themselves at various altitudes both in the Western Tatra and in the High Tatra; after a partial withdrawal of the secondary triploids from habitats dominated by the intermediate type 26, only isolated spots of the higher type have persisted in the most suitable habitats.

Discussion

In spite of the fact that *Poa alpina* has been already investigated by a number of research workers (Turesson 1927, Müntzing 1932, 1940, 1949, Flovik 1938, Böcher 1938, Böcher and Larsen 1950, Nygren 1950) it still deserves further studies in view of the complexity of problems dealing with its intra-specific differentiation regarding chromosome numbers, geographic and ecological distribution of the various types, morphology and the mode of reproduction. The present work based chiefly on the study of viviparous clones from the Tatra Mts and the range of Pieniny, has added further evidence to the knowledge of the remarkable diversity within this species.

The chromosome numbers found in the material investigated in the course of the present study represent a distinctly discontinuous range. Its various numbers may be classified as follows:

The types with 14 and 28 chromosomes and those with 22 and 33 chromosomes represent two different series: the first consists of diploids and tetraploids with the basic number 7, while the second is composed of diploids and triploids with the secondary basic number 11. The former series has evidently a more limited general distribution than the latter. In the Tatra Mts the primary diploid (2n=14) represents undoubtedly the most ancient and rarest type among the viviparous clones. In other geographic regions viviparous biotypes with this number are unknown hitherto; seed-producing strains with 14 chromosomes have been reported from the Caucasus by Sokolovskaya and Strelkova (1940) and from mountains of South Russia by Müntzing (1949). The primary tetraploid (2n=28) in the Tatra Mts is not as frequent as the other main types; nevertheless, it has been found there in 16 habitats. In other geographic regions it is extremely rare: a non-viviparous strain has been found by Böcher and Larsen (1950) in West-Greenland. The second series including types with the secondary basic number (x=11) has a notably wider distribution in the Tatra Mts: it is represented there by secondary diploids (2n=22) and secondary triploids (2n=33); viviparous biotypes with these numbers are common; in addition also non-viviparous strains, very rare in the Tatra Mts, belong to the same chromosomic types. The secondary diploids represent subalpine ecotypes which occur also in lower

situations in the range of Pieniny while the secondary triploids represent alpine ecotypes. In other geographic regions the secondary diploids are rare: they are represented only by sexual strains in Switzerland (Müntzing 1940). On the other hand, strains with the exactly triploid secondary number have a very wide general distribution; they occur in the form of viviparous or seed-producing biotypes in Switzerland, Scandinavia and Greenland.

The clones with 26 chromosomes have presumably a hybrid origin. Ecologically, they represent in some respects intermediate forms between their putative ancestors: the secondary diploids and the secondary triploids. The ecological requirements of this non-uniform group are in good accordance with its presumable hybrid origin. This type which is frequent in the Tatra Mts and occurs also in Pieniny, is rather rare in other geographic regions: till now it has been recorded only from Switzerland (Müntzing 1940) where both sexual and viviparous strains were found.

The relatively rare clones with 34 and 35 chromosomes presumably represent occasional numerical deviations from the type 33; these aneuploids may have arisen in the Tatra Mts by a secondary differentiation of that type. It is however difficult to explain the possible origin of such deviating numbers in viviparous strains. It is assumed in general that plants representing the vegetative progenies of these strains have exactly the same chromosome numbers as their mother plants; this assumption however has not been verified hitherto on a large scale by studies of the offsprings raised from bulbils. Therefore, the possibility of an occasional occurrence of slightly deviating numbers in the vegetative progenies should not be overlooked. It is well known that meiotic and mitotic irregularities may be induced by external factors; in natural exposed habitats on higher elevations the frequently occurring sudden changes of temperature may cause disturbances leading to somatic non-disjunction in early stages of the development of the bulbils; these processes may be responsible for a subsequent intra-clonal cytological differentiation. The occurrence in the Tatra Mts of plants with deviating chromosome numbers in the same habitats as the type with 33 chromosomes is in favour of the above assumption. In the material from Switzerland, likewise, the slightly deviating number c. 34 has been found by Müntzing (1940, p. 144) among plants with 33 chromosomes of a viviparous clone from Arosa.

The frequently occurring variation of the chromosome numbers within the meristem of a single root tip observed in some plants (see p. 260) affords another kind of evidence of the correctness of the above considerations.

It should be remarked that the present observations were limited to roots of adult plants transplanted from their natural mountain habitats. Further studies aiming at the elucidation of the cytological mechanism of the production of deviating chromosome numbers should deal with panicles and bulbils exposed to the influence of local external conditions in the natural habitats. An experimental attack of this problem is also possible: laboratory experiments on the effect of sudden temperature changes in early stages of the development of the bulbils might also throw light upon the origin of plants with deviating chromosome numbers.

The mode of reproduction of *P. alpina* occurring in the Tatra and Pieniny Mts is particularly interesting. According to Gustafsson (1947) in numerous species sexual forms may possess a lower chromosome numbers than those found in apomicts. It should be emphasized that in *P. alpina* from the Tatra not only the higher chromosomic types but also biotypes with the lowest numbers (14 and 22) have acquired the viviparous mode of reproduction. In other centres of distribution of *P. alpina* these types have been found only in the form of sexual strains (Müntzing 1940, 1949, Sokolovskaya and Strelkova 1940). The seed-producing forms of *P. alpina* are very rare in the Tatra Mts; their mode of reproduction (sexual or apomictic) has not been investigated hitherto.

The general geographic distribution of the viviparous types of *P. alpina* shows a well marked differentiation; this is evident from the comparison of the chromosome numbers known from the Alps and the Tatra Mts with those from the areas of distribution of this species in the north. According to Müntzing (1940) in Switzerland the viviparous strains have 26, 33 and 34 chromosomes; the results of the present study have shown in viviparous strains from the Tatra Mts the occurrence of seven chromosomic types: 14, 22, 26, 28, 33, 34, 35. On the other hand, in the northern centre of distribution of *P. alpina* the chromosome numbers of the viviparous biotypes are higher: according to Flovik (1938), a viviparous clone from arctic Norway has 33 chromosomes while one from

Spitzbergen has 44 (or 42+2ff); thus, the former representing a secondary triploid and the latter a secondary tetraploid. In the Faeroes Islands Böcher (1938) found a viviparous clone with a still higher number: 2n=48. Nygren (1950) found that in Sweden and Norway the chromosome numbers of viviparous strains range from 36 to 54(—57). The range of chromosome numbers of non-viviparous strains investigated hitherto is more limited since it does not exceed 38. The lowest number, 2n=14 has been found in strains from the Caucasus by Sokolovskaya and Strelkova (1940). In Central-European mountains non-viviparous strains with the numbers 22, 26, 33, 37 have been found by Müntzing in Switzerland; two of the above numbers occur also in strains from the Tatra Mts. In Sweden apomictic strains have 33, 35 and 38 chromosomes (Müntzing 1940), while those in Greenland have 28 and 33 chromosomes (Böcher 1938, Böcher and Larsen 1950). The strains with lower chromosome numbers (14 to 26) seem to have a more limited geographic distribution since they are not found in higher latitudes. Thus, the results of the investigations in *P. alpina* give further support to the opinions of Hagerup (1931), Tischler (1935), Flovik (1940), Rozanova (1947) as well as Löve and Löve (1949) that, in spite of some exceptions, in general in the majority of plant genera the proportion of types with higher chromosome numbers increases in higher latitudes.

According to Szafer (1949, p. 187), *P. alpina* is a representative of the arctic-alpine Eurasiatic element in the Polish flora. Its general area of distribution is discontinuous in Europe and Asia, with one centre in the north and other centres in high mountains of Europe and Asia. It represents a species of presumably mountain origin which migrated northwards in the postglacial time and proved able to colonize new territories in Scandinavia and in the Arctic. This species shows a complex differentiation concerning the morphology, chromosome numbers, the mode of reproduction as well as edaphic and altitudinal preferences; it represents a mixture of various ecotypes. According to Turesson (1927) rather early Scandinavian immigrants of this species are represented by the hardiest alpine ecotypes, whereas other ecotypes (e. g. the lowland ecotype) probably migrated northwards in a much later and warmer period. It should be added that this opinion of Turesson was expressed prior to any cytological studies in *P. alpina*. Müntzing

(1940) whose investigations have thrown light upon the complex cytological differentiation existing in this species, assumes that different chromosomic types could have migrated in various climatic periods. It is interesting to note in connexion with the above opinions that only selected chromosomic types among those occurring in the mountains of Central Europe proved able of an expansion to the northern countries, namely types with higher numbers. In the Tatra Mts viviparous biotypes with 33 chromosomes represent chiefly alpine ecotypes; judging from the choice of their habitats they seem to be hardier than the types with lower chromosome numbers. On the other hand, the latter types seem to have been secluded from the process of migration. It is probable that the northern expansion of the fittest ecotypes could have taken place before the vivipary had developed in the course of evolution, since vivipary does not favour the migration over vast territories.

In view of the differentiation of the apomictic strains with regard to their chromosome numbers, Müntzing (1949) expressed the opinion that each geographic region has its special number (p. 407). The results of the present study however show that in some geographic regions considered as the putative centres of origin of the species, not a single selected type but on the contrary a series of types occur together. A region of this kind is represented by the Tatra Mts, where at least 7 different viviparous types are found in the relatively small area. In addition to a cytological differentiation, a differentiation into ecotypes with different edaphic and altitudinal preferences can be observed within the multiform population. In this centre various successful types with a high degree of adaptability had evolved; the presumably oldest type with 14 chromosomes, on the contrary, seems to represent an almost extinct form without any possibility of a further expansion; its occurrence is limited to very sheltered places. It is possible that the study of a larger number of biotypes from the Alps would disclose also there a higher degree of differentiation than that known hitherto. On the other hand, in the areas of immigration the opinion expressed by Müntzing seems to be perfectly correct: in these regions the biotypes have been subjected to a strong selection which has played an important part in the limiting of the existing diversity.

The results of the present study have added further evidence concerning the high intraspecific differentiation of *P. alpina*; they have shown also that cytological investigations combined with studies in the geographic distribution of various chromosomic types may throw light upon the past history of migration of this species.

Summary

The present study gives a cyto-ecological analysis of *Poa alpina* L. from the Tatra and Pieniny Mts, a geographic region which is entirely isolated and remote from centres investigated previously. *P. alpina* is represented there by two forms: var. *vivipara* L. which is extremely common in a variety of habitats and at different altitudes, as well as the notably rarer var. *seminifera* L. The results obtained are based on the study of 111 viviparous biotypes and in addition of two strains of var. *seminifera*.

In the area explored *P. alpina* var. *vivipara* shows a high degree of cytological differentiation. It is represented in the range of Pieniny by two types, with 22 and 26 somatic chromosomes; in the Tatra Mts seven different types were found: with 14, 22, 26, 28, 33, 34 and 35 chromosomes. Among these types those with 22, 26 and 33 chromosomes are particularly common; the type with 28 chromosomes is also relatively frequent, while the three remaining types are rare. The two seed-producing strains have the numbers 22 and 33.

The above chromosome numbers may be classified as follows: the types with 14 and 28 chromosomes and those with 22 and 33 chromosomes represent two different series: the first consists of diploids and tetraploids with the basic number 7, while the second is composed of diploids and triploids with the secondary number 11. The clones with 26 chromosomes could have arisen from seed-producing strains in the way of hybridization of the secondary diploids (with 22 chromosomes) with the secondary triploids (with 33 chromosomes), the intermediate aneuploid number being subsequently stabilized by vivipary. A limited number of biotypes with numbers slightly deviating from that of the secondary triploids (with 34 and 35 chromosomes) have possibly arisen by a secondary intraclonal differentiation of that type.

The various viviparous types show differences in their edaphic and altitudinal preferences although their areas overlap partially.

The lowest chromosomic type, evidently representing the most ancient form was found in a limestone valley in a very sheltered habitat at a relatively low altitude. The strains with 22 and 28 chromosomes represent subalpine ecotypes with a distinct preference for soils containing lime. The strains with 26 chromosomes represent a mixture of subalpine and alpine ecotypes which occur both on limestone and on granit. The hardiest alpine ecotypes growing on granit in exposed habitats at high elevations are represented by the strains with 33—35 chromosomes.

In the Tatra Mts, the viviparous mode of reproduction is not limited to strains with the higher chromosome numbers; even the lowest chromosomic types (14 and 22) are viviparous. On the other hand, in other centres of distribution of *P. alpina*, viviparous strains with the above chromosome numbers are unknown; the respective chromosomic types being represented only by sexual strains.

The general geographic distribution of the viviparous types of *P. alpina* shows a well marked differentiation. In Central Europe (the Alps and the Tatra Mts) it is represented by types with lower chromosome numbers (14 to 35) while in its northern centre of distribution (Scandinavia, Spitzbergen, Faeroes Islands) the chromosome numbers of the viviparous biotypes are higher, ranging from 33 to 54(—57).

P. alpina is regarded as a species of mountain origin which migrated northwards in the postglacial time. The total absence of lower types from the north suggests that only selected chromosomic types, namely those with higher numbers, proved able of an expansion to the northern countries, while the lower types seem to have been secluded from the process of migration.

Contrary to the areas of immigration in which each geographic region may possess its special chromosome number (Müntzing 1949, p. 487), in the Tatra Mts, a geographic region considered as one of the putative centres of origin of this species, not a single selected type but a series of chromosomic types is represented in the mixed population.

The author's thanks are due to all persons who helped in collecting the plant specimens for the present study. The research grants of the Polish Academy of Sciences in 1949, 1950 and 1951 are gratefully acknowledged.

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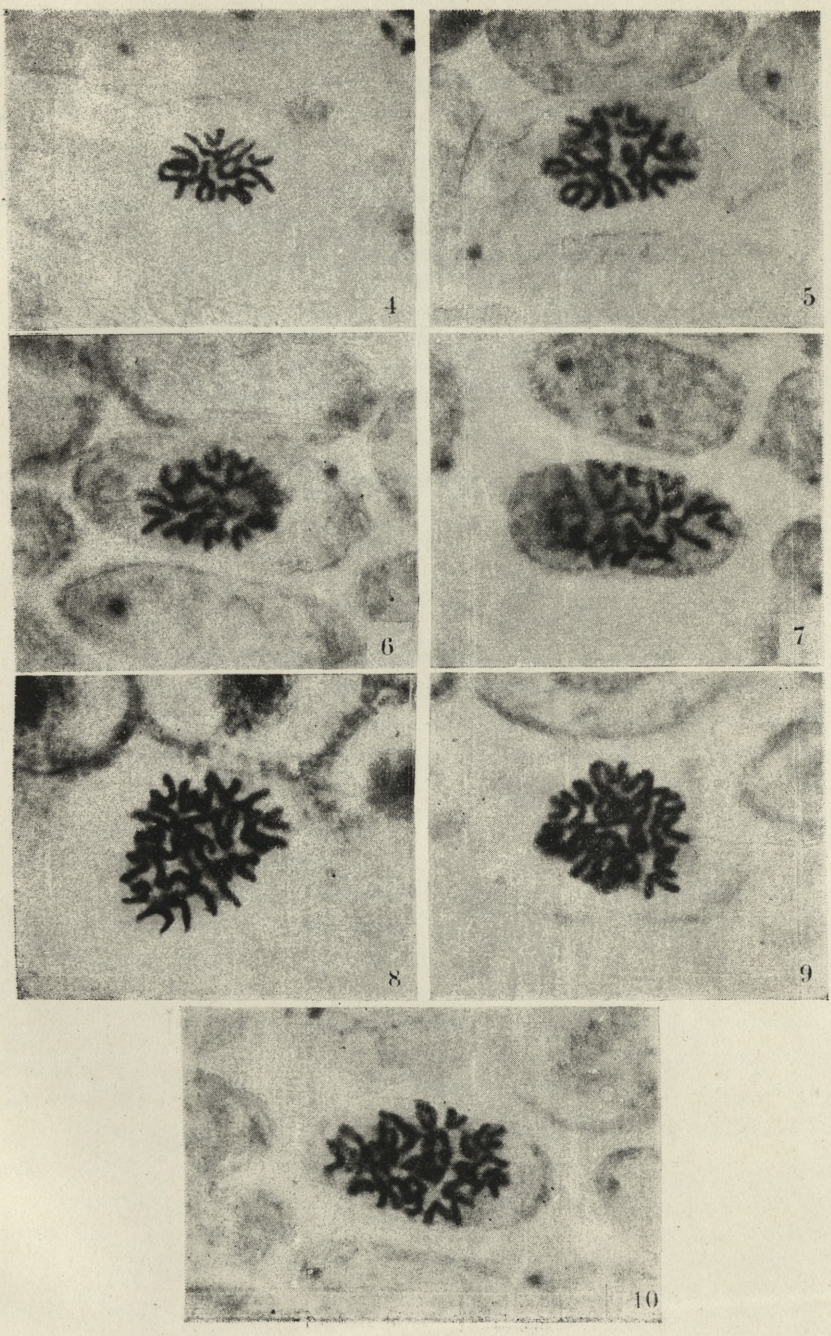

M. Skalińska

Explanation of the plate

All figures except Fig. 6 represent microphotos of root tip metaphases of *Poa alpina* var. *vivipara*; Fig. 6 represents a somatic metaphase of var. *semini-fera*. The microphotos have been taken with Leitz Makam combined with the camera Practiflex with the use of a Leitz oil immersion lens ×100 and a Leitz periplan eyepiece ×10. Their magnification is c. 1700×.

Fig. 4 — 2n=14 (clone 176a); the same plate as Fig. 1 A.
Fig. 5 — 2n=22 (clone 191); the same plate as Fig. 1 B.
Fig. 6 — 2n=22 (strain 182); the same plate as Fig. 2 E.
Fig. 7 — 2n=26 (clone 131); the same plate as Fig. 1 C.
Fig. 8 — 2n=28 (clone 166); the same plate as Fig. 1 D.
Fig. 9 — 2n=33 (clone 51); the same plate as Fig. 2 A.
Fig. 10 — 2n=35 (clone 32); the same plate as Fig. 2 D.

Badania cytologiczne nad występującymi w polskich Karpatach gatunkami rodzaju Soldanella L. — Cytological studies in species of the genus Soldanella L. from the Polish Carpathians.

Mémoire

de M[lle] *K. SATCZEK*

présenté le 1 Juin 1951, par Mme M. Skalińska m. c. et M. W. Szafer m. t.

(Plate 16)

Introduction

Studies in the genus *Soldanella* L. done hitherto concerned chiefly systematic-geographical and anatomical problems (Kamieński 1876, Engler and Prantl 1897, Vierhapper 1904, 1926, Pax 1905, Wettstein 1933, Pawłowski 1929, 1930, Hegi 1927), whereas the cytology of this genus was not investigated. This study gives chromosome numbers of species of *Soldanella* occurring in the Polish Carpathians.

The genus *Soldanella* is regarded as a genus of tertiary (arctic-tertiary, Hegi 1927) origin. It occurs exclusively in the mountain regions of Central and Southern Europe, extending from the Pyrenees in the west to the Eastern Carpathians and the Balkan Mountains in the east and from Babia Góra in the north to the mountains of Calabria and Pindus in the south. The opinion of Kusnezow (1902 quoted after Vierhapper) that this genus has its representatives also in Asia, namely in Armenia, is queried by Vierhapper (1926) and needs to be verified.

Vierhapper considers the species of *Soldanella* as mountain endemics of Central and Southern Europe.

The vertical distribution of the genus extends from the lower mountain layer up to the alpine layer; the greatest variety of forms

occurs in the alpine layer where they show the highest degree of condensation.

The areas of the various species and forms may overlap or even totally cover one another giving possibilities of intercrossing (Vierhapper 1904, Pawłowski 1930, Hegi 1927). On the other hand some species give examples of vicarismus: *S. carpatica*, occurring in the western Carpathians is replaced in the central Alps by *S. pusilla* (Braun-Blanquet 1930); the alpine species *S. alpina* is replaced in lower mountain situations in the Alps by *S. montana* (Wettstein 1933).

The species of the genus *Soldanella* occur both on granitic and calcareous ground, in more or less moist habitats, on soils rich in humus; they are frequent in mixed fir and spruce forests with an abundance of mosses; they may be also found on meadows and on peaty grassland in the lower mountain layer as well as on mountain pastures; they use also to grow in moist mountain clefts where snow remains for a long time in the spring.

There is a disagreement of opinions concerning the taxonomy of *Soldanella*. Some authors distinguish within the genus only 4 species (Engler and Prantl 1897) others not less than 10 species (Vierhapper 1926). They agree however in subdividing the genus (according to Borbás 1901) into two sections *Tubiflores* and *Crateriflores* on the basis of the flower morphology.

The present study deals with the cytology of the following species belonging to the section *Crateriflores*: *S. carpatica* Vierh., *S. montana* Willd., *ssp. eumontana* Lüdi, *S. montana* W. *ssp. hungarica* (Simk.) Lüdi *var. major* (Neilr.) Vierh. According to Pawłowski the distinction between these three forms is based on some morphological and anatomical characters (Table I), mainly on the size, number and durability of glands occurring on the petioles, shoots and flower stipulae, as well as on the shape of the teeth of the capsules (Szafer and Pawłowski 1930).

Material and Methods

The material for this study originated exclusively from natural habitats in the Tatra Mts, both in their calcareous and granitic parts, and from the western Carpathians (Babia Góra). Living plants were collected in a variety of habitats with different edaphic

TABLE I.

Character differences between the three forms investigated.

Characters	*S. carpatica*	*S. montana ssp. eumontana*	*S. montana ssp. hungarica var. major*
The leaf:			
Width of the blade	7—47 mm	15—45 mm.	21—57 mm
	Blade dark green, usually thick and leathery; dried leaves concentrically wrinkled. Margins usually almost entire, sometimes slightly crenate	Blade lighter, thinner; dried leaves with smooth surface. Margins usually distinctly crenate.	Blade light green, thin; dried leaves with smooth surface. Margins usually distinctly crenate.
venation:	indistinct	distinct	distinct
Glands:			
on petioles	sessile, in young developing leaves sparse, and of short duration; in wholly developed leaves petioles nearly glabrous.	on stalks, rather slender (c. 0·5 mm long). The stalk notably longer than the head, built of 3—4 distinctly elongated cells. Distribution very dense, usually partially maintained on the petioles of older leaves.	on stalks, short (not exceeding 0·2 mm) 1·5—5 (6) times longer than the head, built of 3—4 short or only slightly elongated cells. Glands usually vanishing in later stages.
on stipulae	on short stalks, the length of the stalk equal to the size of the head or 2 (—3) times longer.	on stalks, (2—) 3 times or more longer than the head.	length of the stalks equal to the size of the head or 2(—3) times longer.
Diameter of the pollen grains	12—24 μ (Mo = 21 μ)	12—21 μ (Mo = 18 μ)	12—21 μ (Mo = 18 μ)
Capsules: shape of the teeth	rounded	truncate	truncate
General geographic distribution	endemic species of the western Carpathians: Tatra Mts, Chocz, Fatra, Lower Tatra, (Pawłowski 1929), Babia Góra (Pawłowski 1929, Walas 1933).	north-eastern Alps and their foreland to the Danube, Böhmerwald, Southbohemian-Moravian Mts; very rare in the Transsilvanian Alps and Stara Planina (Hegi 1927), Carpathians (Szafer and Pawł. 1930)	Eastern part of the northern calcareous Alps, Carpathians, eastern Balcan Mts (Vierhapper 1904, 1926, and Hegi 1927).

Characters	*S. carpatica*	*S. montana ssp. eumontana*	*S. montana ssp. hungarica var. major*
Vertical distribution in the Polish Tatra	from the higher mountain layer to the alpine layer (up to 2470 m o. s. l.) (Pawłowski 1929, Pawłowski, Sokołowski, Wallisch 1928).	isolated habitats chiefly in the higher mountain layer.	almost exclusively in the higher mountain layer and the layer of subalpine forests; very rarely ascending to the layer of *Pinus montana* (up to 1650 m o. s. l.) (Pawłowski 1928, 1929, 1930).
In the Tatra Mts the areas of the three forms overlap partially (Tables II—IV and Fig. 3).			

conditions, situation and altitude. The habitats are situated at various altitudes ranging from 800—2159 m over sea level.

The somatic chromosome numbers were studied on root-tips; these investigations were sometimes supplemented by studies of mitoses found in the leaf buds which proved to be an unexpectedly good material for cytological studies.

For the fixing of the root-tips and the leaf-buds the Navashin's fixative (modified according to Delaunay) and sometimes the Lewitsky's fixative (5:5) were used. Especially well spaced metaphase plates were obtained after cooling on ice blocks the objects plunged already in the fixative. During the excursions the Navashin's fixative diluted with distilled water in the proportion of 1:1 was used (Manton 1942). The flower-buds were for the most part fixed in nature in the diluted Navashin's fixative with a pre-treatment with acetic alcohol. The most favourable time to fixing was between 11·00 and 12·30 o'clock.

Microtome sections, 8—10 μ thick, were stained with Newton's gentian violet and differentiated in clove oil or in the solution of phenol in xylene in the proportion of 1:3.

The drawings have been done with the Reichert oil immersion lens 100×, N. A. 1·30 in combination with Zeiss compensating eyepiece 20× and the Zeiss-Abbè camera lucida. The magnification of the drawings is c. 2700×. The microphotographs have been done with the Zeiss apochromatic oil immersion lens 90×, N. A. 1·30,

Zeiss compensating eyepiece 15×, with the help of Leitz Makam combined with the Practiflex photographic camera. Their magnification is c. 2600×.

Results

a) SOMATIC CHROMOSOME NUMBERS

As stated already in a preliminary report (Satczek, in Skalińska 1950) all the three forms investigated have the same chro-

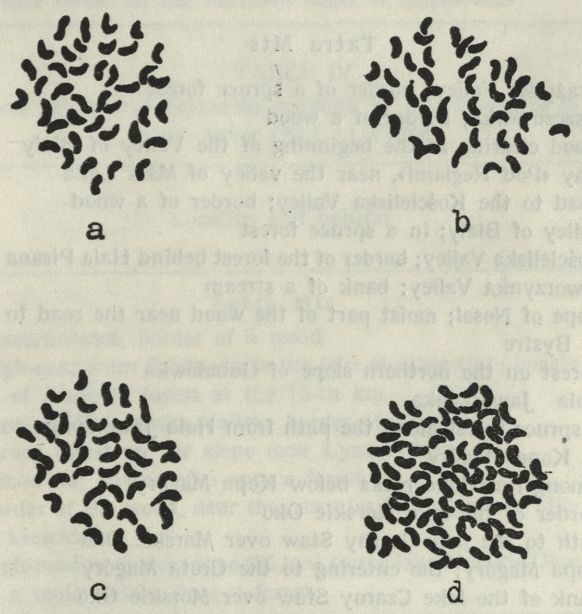

Fig. 1. Somatic plates in root-tips. a) *Soldanella carpatica* (2n=40 ; b) *Soldanella montana* ssp. *eumontana* (2n=40); c) *Soldanella montana* ssp. *hungarica* var. *major* (2n=40); d) *Soldanella carpatica*, a cell with doubled chromosome number (2n=ca 80).

mosome number: 2n=40 (Figs 1 a—c, 4—6). For *S. carpatica* the above number (Figs 1 a, 4) was established on specimens originating from 30 habitats (Table II) on altitudes ranging from 800—2159 m o. s. l. *S. montana ssp. eumontana* (Figs 1 b, 5) has been studied on plants from 4 habitats (Table III) while *ssp. hungarica var. major* (Figs 1 c, 6) from 7 habitats (Table IV) which exceed

TABLE II

Localities and habitats of *Soldanella carpatica* Vierh.

No of habitat	Locality and habitat	Altitude over sea level
	Western Carpathians	
43	Road from Zawoja to Babia Góra, border of a forest	c. 800
42	Babia Góra, below the peak of Diablak, among rock debris	c. 1580
	Tatra Mts	
1	Strążyska Valley; border of a spruce forest	c. 900
12a	Jaszczurówka; border of a wood	c. 908
5	Wood clearing at the beginning of the Valley of Biały	c. 920
19	Way «Pod Reglami», near the valley of Mała Łąka	c. 930
55	Road to the Kościeliska Valley; border of a wood	c. 930
18	Valley of Biały; in a spruce forest	c. 960
16	Kościeliska Valley; border of the forest behind Hala Pisana	c. 1015
33	Jaworzynka Valley; bank of a stream	c. 1090
50	Slope of Nosal; moist part of the wood near the road to Bystre	c. 1100
52	Forest on the northern slope of Gubałówka	c. 1100
32	Hala Jaworzynka	c. 1150
31	A spruce forest along the path from Hala Jaworzynka to Kopa Magóry	c. 1200
3	Among limestone rocks below Kopa Magóry	c. 1300
27	Border of the lake Morskie Oko	1395
28	Path to the lake Czarny Staw over Morskie Oko	c. 1450
30[1]	Kopa Magóry; the entering to the Grota Magóry	1490
48	Bank of the lake Czarny Staw over Morskie Oko	1582
30	Northern slope of Kopa Magóry	c. 1600
46	Peatty border of the lake Litworowy Stawek	1618
13	Slope of Miedziane; stony dry bed of a stream	c. 1800
14	Way to the peak of Mnich; stony dry bed of a stream	c. 1800
39	Path near Wielka Turnia; among stones	c. 1850
47	Pass Karb; in grassland among stones	1852
44	Way to the pass Świnicka	c. 1900
49	Slope of Kasprowy; near the way among rock debris	c. 1900
45	Path from the Szpiglasowa pass to the Valley of Five Polish Lakes	c. 2000
38	Peak of Małołączniak, in grass	2095
41	Kazalnica below the pass Pod Chłopkiem	2159

TABLE III

Localities and habitats of *Soldanella montana* Willd. *ssp. eumontana* Lüdi

No of habitat	Locality and habitat	Altitude over sea level
	Tatra Mts	
6	Jaszczurówka; border of a wood	c. 908
17	Jaszczurówka; border of a moist forest	c. 910
54	Road to the Kościeliska Valley; border of a wood	c. 930
7	Spruce forest on the northern slope of Gubałówka	c. 1100

TABLE IV

Localities and habitats of *Soldanella montana* W. ssp. *hungarica* (Simk.) Lüdi var. *major* (Neilr.) Vierh.

No of habitat	Locality and habitat	Altitude over sea level
	Tatra Mts	
56	Jaszczurówka; border of a wood	c. 908
8	High-road from Zakopane to the lake Morskie Oko; border of a spruce forest at the 13-th km	c. 930
51	Road to Kościeliska Valley; border of a wood	c. 930
9	Spruce forest on the slope over Łysa Polana	c. 970
35	Bukowina; border of a spruce forest	c. 970
25	Border of the wood, near the cascades Wodogrzmoty Mickiewicza	c. 1100
10	High-road from Zakopane (25 km) to the lake Morskie Oko; a moist ditch along a forest	c. 1106

only slightly the lower limit of the higher mountain layer (908—1106 m o. s. l.). The established chromosome number of *S. carpatica* was checked on meioses in the embryosac mother cells and in pollen mother cells.

The somatic chromosomes of all the forms studied are relatively short and thick. Their length ranges from 0·9 to 1·3 μ. The occurrence of chromosomes with trabants was stated but their exact number could not be established; probably in the chromosome set there are 2 pairs of chromosomes with trabants; they are somewhat longer than the remaining ones: their length attains about 1·7 μ.

The studied species of the genus *Soldanella* proved to be karyologically uniform in spite of the notable morphological diversity within each form and of various ecological conditions of the specimens studied. Only occasionally one could find in root tip meristems of *S. carpatica* some groups of polyploid cells with doubled chromosome numbers. The more exact analysis of such metaphase plates allowed to establish the chromosome number 2n=c. 80 (Figs 1 d, 7). This phenomenon of a somatic polyploid mutation has been observed in two plants originating from the Tatra Mts: 1. from the border of a spruce forest on a slope above Hala Jaworzynka (Table II, habitat 31), and 2. from patches of grass among rocks near Wielka Turnia (Table II, habitat 39). It should be noted that these plants were collected in the summer season of 1949 shortly after a snow fall.

b) MEIOSIS

Studies of meiosis were done both on pollen mother cells and on embryosac mother cells of *S. carpatica*. This species proved to be especially suitable for the study of meioses in view of its pretty long time of flowering resulting from its considerable vertical range. On the other hand, the flowering time of *S. montana* (*ssp. eumontana* and *ssp. hungarica var. major*) is relatively short; this creates difficulties in providing the material of young flower buds from natural habitats early in the spring.

The course of meiosis both in the pollen mother cells and in the embryosac mother cells is regular in general. In diakinesis 20 bivalents are observable, giving evidence of a complete chromosome pairing. In metaphase I the pairs of chromosomes are grouped in the equatorial plate of the spindle (Figs 2 a, b, 8—10). Like in diakinesis, bivalents usually with one terminal chia ma, represent also in this stage the prevailing chromosome configuration. Polyvalents have not been observed. The I anaphase, the I telophase (Fig. 2 c) and the homeotypic division have a normal course. Only exceptionally some disturbances in meiosis could be observed; viz.: the elimination of single chromosomes or groups of them during the I ana-telophase. As a result of these phenomena polyads containing sometimes even 10 nuclei of various sizes

could be observed occasionally instead of normal tetrads. Their diameter ranged from that of the nuclei of normal primary pollen grains to that of microcytes (Figs 2 e, 2 f).

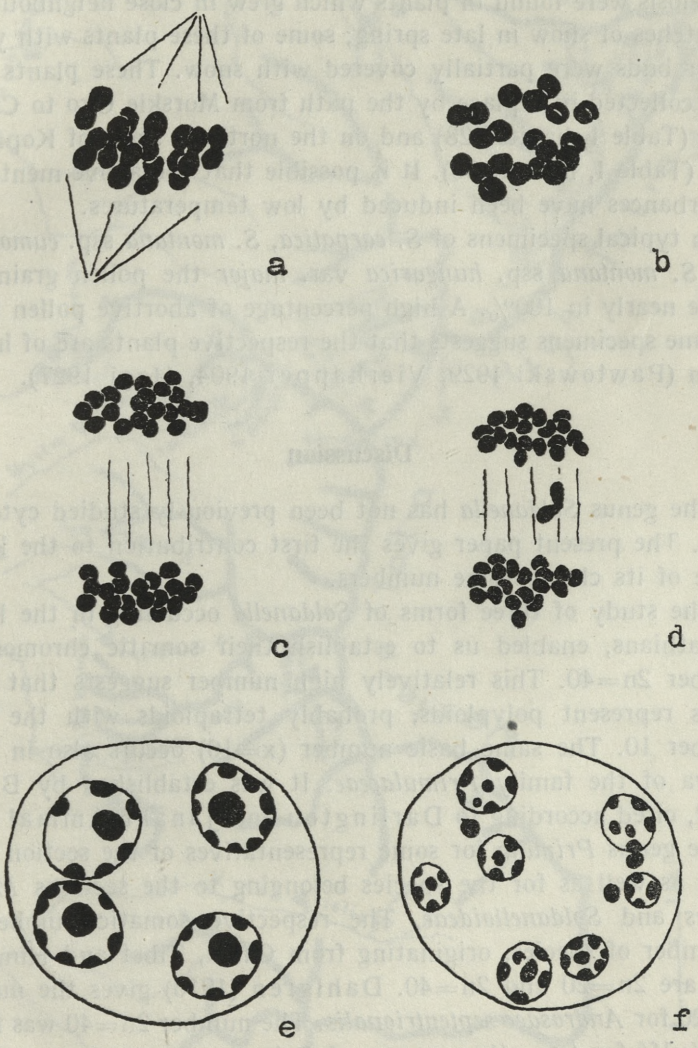

Fig. 2. *Soldanella carpatica.* a) I Metaphase in P. M. C. in side view; b) I Metaphase in P. M. C. in polar view; c) I Telophase in P. M. C. in side view; d) Abnormal I ana-telophase in E. M. C. in side view; e) II Normal P. M. C.; (4 nuclei); f) Abnormal P. M. C. with extra nuclei.

Similar disturbances consisting in the elimination of some chromosomes were also observed in the I ana-telophase of the embryosac mother cells (Figs 2 d, 11).

It should be noted that the irregularities observed in the course of meiosis were found in plants which grew in close neighbourhood of patches of snow in late spring; some of these plants with young flower buds were partially covered with snow. These plants have been collected in a place by the path from Morskie Oko to Czarny Staw (Table I, habitat 28) and on the northern slope of Kopa Magóry (Table I, habitat 30). It is possible that the above mentioned disturbances have been induced by low temperatures.

In typical specimens of *S. carpatica*, *S. montana* ssp. *eumontana* and *S. montana* ssp. *hungarica* var. *major* the pollen grains are fertile nearly in 100%. A high percentage of abortive pollen found in some specimens suggests that the respective plants are of hybrid origin (Pawłowski 1929, Vierhapper 1904, Hegi 1927).

Discussion

The genus *Soldanella* has not been previously studied cytologically. The present paper gives the first contribution to the knowledge of its chromosome numbers.

The study of three forms of *Soldanella* occurring in the Polish Carpathians, enabled us to establish their somatic chromosomes number 2n=40. This relatively high number suggests that these forms represent polyploids, probably tetraploids with the basic number 10. The same basic number (x=10) occurs also in other genera of the family *Primulaceae*. It was established by Bruun (1932, cited according to Darlington and Janaki Ammal 1945) in the genus *Primula* for some representatives of the section *Farinosae* as well as for the species belonging to the sections *Muscarioides* and *Soldanelloideae*. The respective somatic numbers of a number of species, originating from China, Tibet and Himalaya Mts, are 2n=20 and 2n=40. Dahlgren (1916) gives the number 2n=20 for *Androsace septentrionalis*. The number 2n=40 was found by Wulff for two other genera of *Primulaceae*: *Hottonia palustris* (1938) and *Anagallis arvensis* (1937). These results give further support to the assumption that the three forms of *Soldanella* investigated in the course of the present work represent tetraploids.

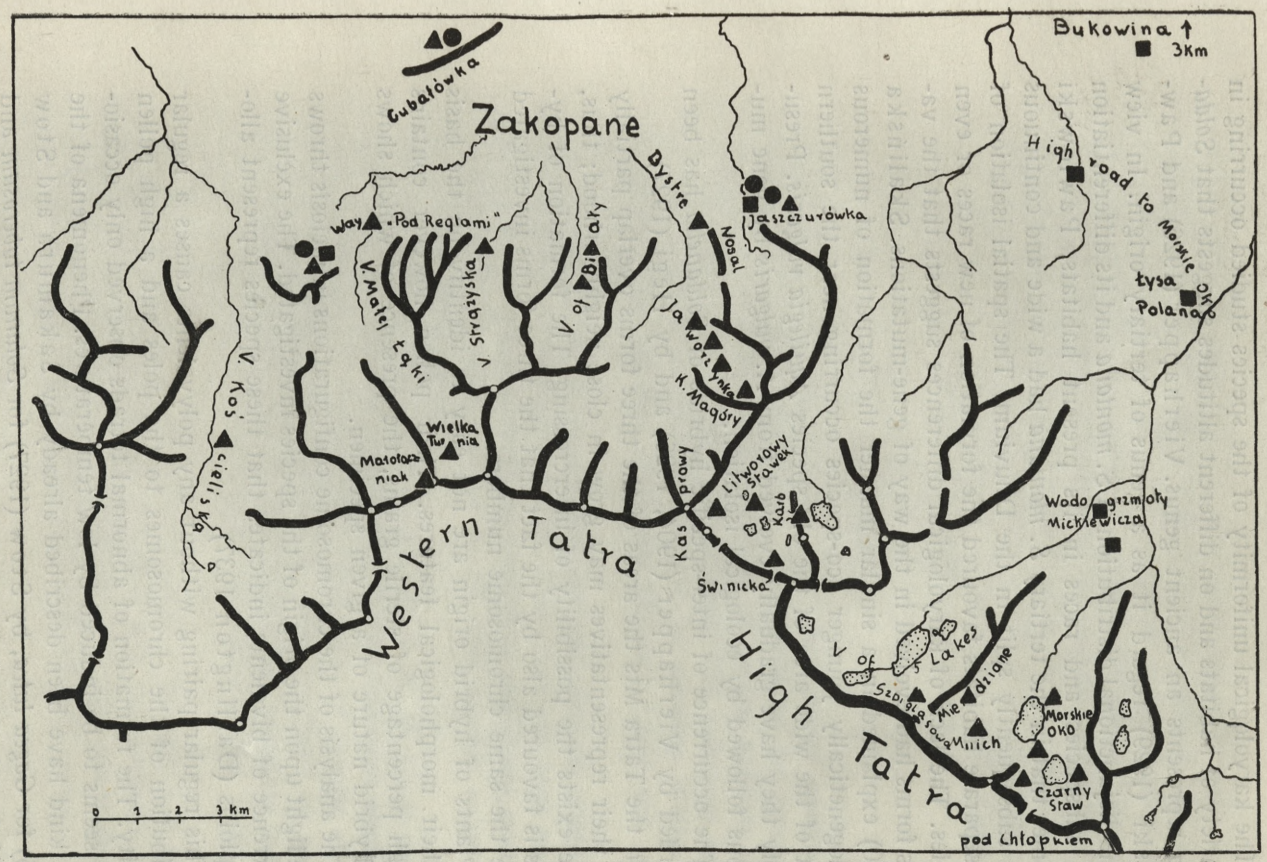

Fig. 3. Map of habitats of the studied specimens (Tatra Mts). Two habitats from Babia Góra are outside the territory represented in the map. Triangles — *Soldanella carpatica* Vierh.; circles — *Soldanella montana* W. ssp. *eumontana* Lüdi; squares — *Soldanella montana* W. ssp. *hungarica* (Simk.) Lüdi var. *major* (Neilr.) Vierh. Solid lines represent mountain ridges.

The karyological uniformity of the species studied occurring in a variety of habitats and on different altitudes suggests that *Soldanella* represents an ancient genus. Vierhapper (1926) and Pawłowski (1929) regard it as a genus of tertiary origin. In view of the disjunctional distribution of *S. montana* and its differentiation into subspecies and races in its present habitats, Pawłowski assumes that in the tertiary *S. montana* had a wide and continuous area subsequently split in the Diluvium. The spatial isolation of the separate habitats favoured the formation of new races or even species. The lack of karyological differences suggests that the various forms had evolved in the way of gene-mutations. Skalińska (1940) explained in a similar manner the formation of numerous phylogenetically younger eco-species occurring near the southern limit of the wide area of the old species *Aquilegia vulgaris*. Presumably they have gradually evolved from *A. vulgaris* by gene mutations followed by ecological isolation.

The occurrence of inter-specific hybrids of *Soldanella* has been recorded by Vierhapper (1904, 1926) and by Hegi (1927).

In the Tatra Mts the areas of the three forms overlap partially and their representatives may grow in close neighbourhood; thus, there exists the possibility of intercrossing. The formation of hybrids is favoured also by the fact that the three forms investigated have the same chromosome numbers.

Plants of hybrid origin are not easy to identify on the basis of their morphological features. Their pollen however contains a high percentage of sterile grains, the presence of which shows the hybrid nature of a given specimen.

The analysis of the chromosome configurations in meiosis throws some light upon the origin of the species investigated. The exclusive occurrence of bivalents indicates that these species represent allopolyploids (Darlington 1937).

This regular pairing without any polyvalents causes a regular distribution of the chromosomes to the poles and a high pollen fertility. The formation of abnormal tetrads observed only occasionally seems to be induced by low temperatures. Phenomena of the same kind have been described already by Sakamura and Stow (1926) for *Gagea lutea*, by Stow (1927) for *Solanum tuberosum* and by Michaelis (1926) for *Epilobium hirsutum*.

The occasional occurrence of groups of polyploid cells in root-tip meristems of plants fixed soon after snow-falls in the summer season 1949 belongs to the same category of phenomena bound with thermal shocks. It should be added that similar phenomena of spontaneous chromosome doubling in somatic tissues have been also observed in some other plants fixed in the same period in the Tatra Mts by other workers of the Institute of Plant Anatomy and Cytology (*Gentiana Clusii* — Skalińska 1951, *Cardamine Opizii* — Banach, *Ranunculus glacialis* — Bauer).

Summary

This study gives for the first time chromosome numbers of species of the genus *Soldanella* L. The specimens for cytological investigations originated from a variety of natural habitats in the Tatra Mts and the Western Carpathians. The following species have been studied: *S. carpatica* Vierh., *S. montana* Willd. ssp. *eumontana* Lüdi and ssp. *hungarica* (Simk.) Lüdi var. *major* (Neilr.) Vierh.

In all these forms the somatic chromosome numbers established on root-tip mitoses proved to be 40. This relatively high number suggests that the species investigated represent polyploids, probably tetraploids with the basic number x=10. This number occurs also in some other *Primulaceae*.

The course of meioses in P. M. C's and in E. M. C's is normal in general. Only bivalents are formed. In rare instances studies of root-tip mitoses have revealed the occurrence of groups of cells with doubled chromosome numbers (c. 80), scattered in normal tissues. These polyploid somatic mutations were probably induced by low temperatures after a sudden snow-fall in the Tatra Mts in summer 1949.

The occasional disturbances in the course of meiosis in P. M. C's were also due probably to the action of low temperatures, since they could be observed only in flower-buds of two plants growing in close neighbourhood of areas still covered with snow in late spring.

The present studies were carried out during the years 1949—1951 in the Institute of Plant Anatomy and Cytology of the Jagiellonian University, Kraków. I desire to express my deep gratitude to Professor M. Skalińska, Head

of the Institute for her friendly interest in my investigations and encouragement as well as for valuable advice and suggestions during the course of my work. My thanks are also due to all persons who helped me in collecting the plant specimens for investigations and to Dr A. Bajer for the microphotos.

I am indebted to the Polish Academy of Sciences for the research grant which has greatly facilitated the field work.

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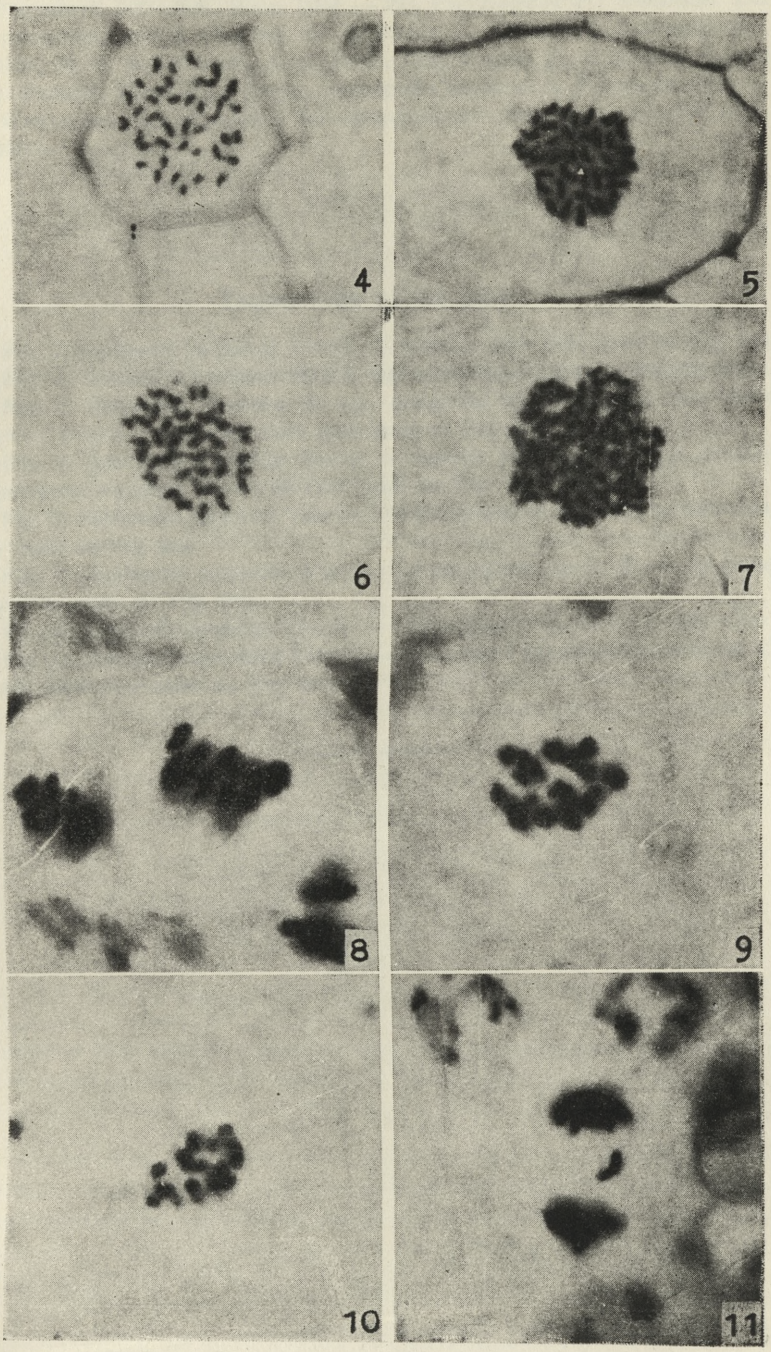

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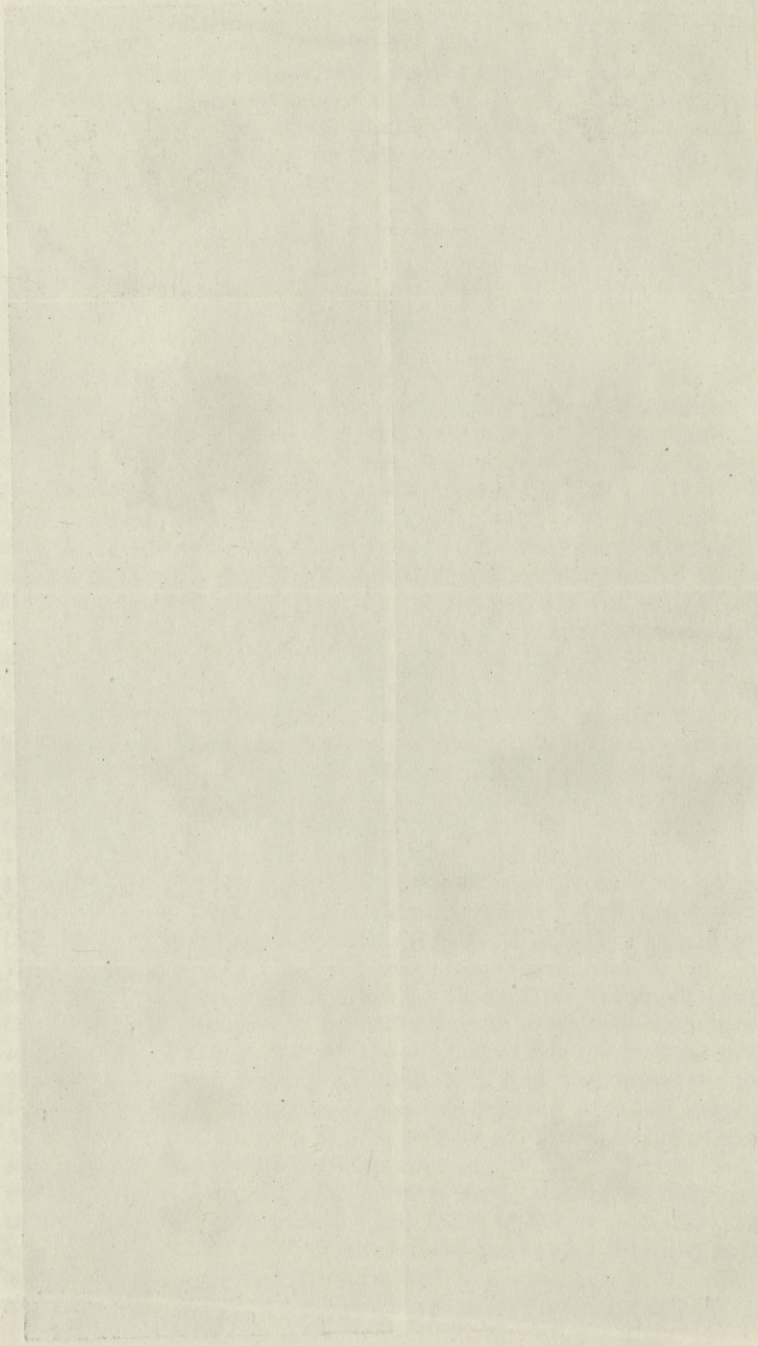

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Explanation of the plate

Fig. 4. *Soldanella carpatica* Vierh. — somatic plate (the same as Fig. 1a).

Fig. 5. *Soldanella montana* Willd. ssp. *eumontana* Lüdi — somatic plate.

Fig. 6. *Soldanella montana* W. ssp. *hungarica* (Simk.) Lüdi var. *major* (Neilr.) Vierh. — somatic plate (the same as Fig. 1c).

Fig. 7. *Soldanella carpatica* Vierh. — somatic plate, a cell with doubled chromosome number, 2n=ca 80 (the same as Fig. 1d).

Fig. 8. *Soldanella carpatica* Vierh. — I Metaphase in P. M. C. in side view (the same as Fig. 2a).

Fig. 9. *Soldanella carpatica* Vierh. — I Metaphase in P. M. C. in polar view (the same as Fig. 2b).

Fg. 10. *Soldanella carpatica* Vierh. — I Metaphase in E. M. C. in polar view.

Fig. 11. *Soldanella carpatica* Vierh. — Abnormal I ana-telophase in E. M. C. in side view (the same as Fig. 2d).

Nowe przywrotniki karpackie i bałkańskie. — Alchemillae carpaticae et balcanicae novae.

Mémoire

de M. ***B. PAWŁOWSKI***

présenté le 1 Juin 1951, par M. B. Pawłowski m. c. et M. W. Szafer m. t.

In itineribus meis carpaticis nec non balcanicis Alchemillas plures collegi, quae cum nulla specie adhuc descripta arcte congruunt et ideo hoc loco ut species novae describuntur. Quarum nonnullas e locis pluribus et in speciminum copia satis magna collectas habeo. Aliae, quarum specimina non nisi pauca legi, differentias tam graves ostendunt, ut haud dubitem, quin eae sint species novae. Praeter alias notas etiam florum figuram accuratissime perscrutatus sum. Quae florum figura quamquam saepe differentias maioris momenti aeque inter species singulas ac inter series et subsectiones praebet, tamen adhuc in descriptionibus plerumque non sufficienter indicatur. Pars observationum mearum, quae mensuris et numeris exprimi possunt, in tabulis dissertationi insertis exponitur.

Sectio BREVICAULON Rothm.
Subsectio CALYCANTHUM Rothm.
Series ELATAE Rothm.

1. **Alchemilla Achtarowii Pawł. n. sp.**

Planta perennis, sat magna, dilute subcoerulescenti-viridis; coloratio aestivalis obsoleta, dilute violescenti-brunnea. Caules complures erecti vel ima basi leviter arcuati 35—45 cm longi validi in internodiis 4—6 i. e. usque ad $^1/_2$—$^3/_4$ altitudinis — inferne densius, superne sparsius — patenter breviter hirsuto-pilosi, superne glabri. Laminae foliorum radicalium magnae, orbiculari-reniformes vel suborbiculares, ad 10 cm longae, ad 12 cm latae, sinu basali aperto, 9- vel incoplete 11- lobae, manifeste plicatae, subtus pallidiores,

nervis 1. et 2. ordinis in utraque pagina (in sicco) albidis valde conspicuis, subtus prominulis; pagina superior in vicinitate sinus basalis et ad nervos, saepe etiam in plicis, sparse vel sparsissime adpresso pilosa, ceterum glabra, rarissime in foliis nonnullis exterioribus tota sparse pilosa, pagina inferior tota dense patenter pilosa; lobi in foliis exterioribus ad $^1/_7$—$^1/_6$, in interioribus (maioribus) ad $^1/_5$—$^2/_7$ radii longit. incisi, arcuati vel semiorbiculares usque late semielliptici, marginales a sese distantes, omnes circumcirca dentati; dentes in utroque latere — in lobis tribus mediis[1] — (7) 8—10 (12) oblique semiovato-triangulares acutiusculi saepe latiusculi, saepe dorso convexi, apice breviter penicillati; apicalis paulo minor; petioli longissimi, (10) 15—30 cm longi, firmi, dense patenter pilosi. Folia caulina numerosa et pro portione magna quamquam rosularibus multo minora, inferiora late subreniformia sinu basali valde improfundo latissimo vel fere semiorbicularia basi ± truncata, 5- vel incomplete 7- loba, lobis vix ad $^1/_8$—$^1/_5$ laminae incisis, arcum humilem formantibus, in utroque latere dentibus 5—7 acutis vel obtusiusculis praeditis; folia caulina superiora basi late cuneata vel truncata, ad $^1/_6$—$^1/_4$ radii incisa, omnia eodem modo ac folia rosularia pilosa vel — praecipue superiora — in pagina superiore etiam basi glabra; stipulae latissimae, inferiores improfunde inaequaliter dentatae, superiores profundius sed vix plus quam ad $^1/_4$ in lacinulas numerosas acutas sat regulariter partitae. Inflorescentia sat magna multiflora, saepe iam in caulis dimidio incipiens, sat densa quamquam cymis singulis sat laxis; rami erecti sub angulo acutissimo abeuntes, 1 vel 2 (rarius 3) infimi versus basin ± patenter pilosi, ceterum aeque ac rami superiores glabri; ramuli divaricati, sparse longe patenter pilosi; pedicelli glabri vel nonnulli sparsissime pilosi, urceolis 1·5—4× longiores vel (supremi) eis aequilongi. Flores virescenti-flavi speciosi, (4) 4·5—6·5 (usque 7·2) mm lati; urceoli basi sparse pilosi vel glabri, fructiferi nonnisi ca. 1—1·5 mm longi; sepala glaberrima ovato-oblonga acuta; episepala eis plerumque multo longiora, ovato oblonga vel late oblonga acuta, in floribus omnibus (100% florum examinatorum) omnia (id est 4), raro uno excepto (i. e. 3 in uno flore), rarissime duobus exceptis (i. e. 2 in uno flore) denticulis 1—4 (5) i. e. in uno vel in utroque latere 1—2 (3)

[1] In omnibus descriptionibus numerus dentium solummodo in lobis tribus medianis indicatur.

parvis, sed bene conspicuis ornata; episepala margine integra nonnisi 8% exhibent. — Nux urceolo manifeste excedens (Fig. 1).

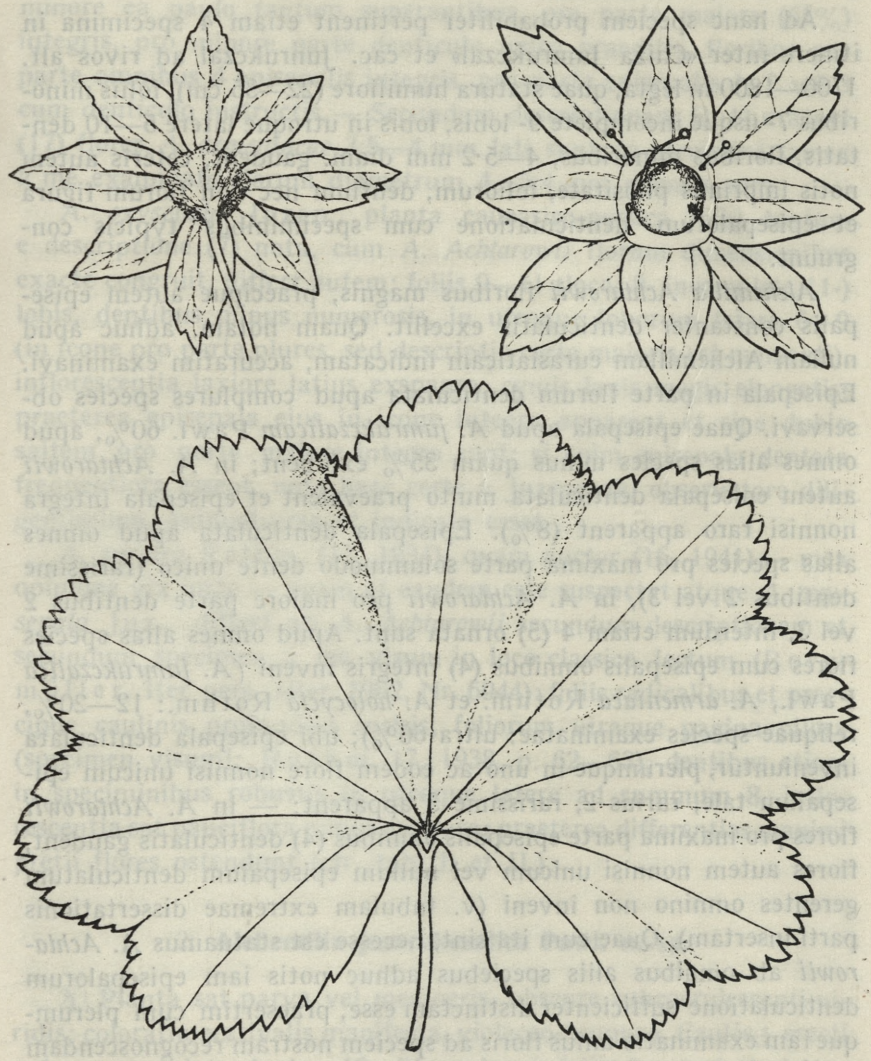

Fig. 1. *Alchemilla Achtarowii* Pawł. Flores: $^{9}/_{1}$; folium: $^{1}/_{1}$. — Pili in folio omissi. — Figuras omnes (1—13) ad naturam delnneavit T. Tacik.

Bulgaria, montes «Centralna Stara Płanina»: in valleculo infra cac. Jumrukczal, ad rivum, solo cristallinico, alt. ca. 1830 m s. m.,

30. 8. 1948, legi ipse. — Typus Cracoviae in Herb. Ac. Soc. Polon.

Planta in honorem Domini Borisii Achtarow, botanici bulgarici illustrissimi, denominata.

Ad hanc speciem probabiliter pertinent etiam 4 specimina in itinere inter «Chiża Jumrukczal» et cac. Junrukczal ad rivos alt. 1700—1800 m legta, quae statura humiliore (22—35 cm), foliis minoribus 7- usque incomplete 9- lobis, lobis in utroque latere 5—10 dentatis, floribus minoribus, 4—5·2 mm diam. gaudent, ceteris autem notis imprimis pilositate, foliorum, dentium nec non florum figura et episepalorum denticulatione cum speciminibus typicis congruunt.

Alchemilla Achtarowii floribus magnis, praecipue autem episepalis constanter denticulatis excellit. Quam notam, adhuc apud nullam Alchemillam eurasiaticam indicatam, accuratim examinavi. Episepala in parte florum denticulata apud complures species observavi. Quae episepala apud *A. jumrukczalicam* Pawł. 60%, apud omnes alias species minus quam 35% exhibent; in *A. Achtarowii* autem episepala denticulata multo praevalent et episepala integra nonnisi raro apparent (8%). Episepala denticulata apud omnes alias species pro maxima parte solummodo dente unico (rarissime dentibus 2 vel 3), in *A. Achtarowii* pro maiore parte dentibus 2 vel 3, interdum etiam 4 (5) ornata sunt. Apud omnes alias species flores cum episepalis omnibus (4) integris inveni (*A. jumrukczalica* Pawł., *A. armeniaca* Rothm. et *A. holocycla* Rothm.: 12—20%, reliquae species examinatae: ultra 60%); ubi episepala denticulata inveniuntur, plerumque in uno ac eodem flore nonnisi unicum episepalum tale, rarius 2, rarissime 3 apparent; — in *A. Achtarowii* flores pro maxima parte episepalis omnibus (4) denticulatis gaudent, flores autem nonnisi unicum vel nullum episepalum denticulatum gerentes omnino non inveni (v. tabulam extremae dissertationis parti insertam). Quae cum ita sint, necesse est statuamus *A. Achtarowii* ab omnibus aliis speciebus adhuc notis iam episepalorum denticulatione sufficienter distinctam esse, praesertim cum plerumque iam examinatio unius floris ad speciem nostram recognoscendam sufficiat.

Inter species seriei *Elatae* duae plantae — *A. armeniaca* Rothm. et *A. oxysepala* Juzep. — *A. Achtarowii* proximae esse videntur. *A. armeniaca* Rothm., Anatoliae (et Caucasi?) incola, differt tamen ab *A. Achtarowii*: statura permagna, foliis incomplete 11- ad 13-

lobis, dentibus utrinque tantum 7—9, inflorescentia laxiore, urceolis densiuscule pilosis, sepalis et episepalis angustioribus (lanceolatis), episepalis sepala pro multo maiore parte subaequantibus, pro parte minore ea paulo tantum superantibus, pro parte maiore (65%) integris, pro minore parte denticulo unico praeditis, floribus pro parte omnibus 4 episepalis integris, pro parte episepalis 1, 2 vel 3 cum denticulo instructis. — Secundum descriptionem Rothmaleri (17) flores *A. armeniacae* 3·5—4 mm lati sunt, in specimine tamen a me examinato florum diametrum 4—5·4 mm mensus sum.

A. oxysepala Juzep., planta caucasico-persica mihi tantum e descriptione (7) nota, cum *A. Achtarowii* florum dimensionibus exacte congruit, differt autem: foliis 9—11 (loco: 9- incomplete 11-) lobis, dentibus minus numerosis, in utroque loborum latere 6—9 (in icone pro parte plures, sed descriptio certe maioris est momenti), inflorescentia laxiore latius exspansa, cymis laxis magis elongatis; praeterea episepala eius in icone integra apparent et sine dubio saltem pro parte maiore integra sunt; si enim episepala dentata frequentiora essent, nota haec certe a Juzepczuk, observatore diligentissimo, animadversa et indicata esset.

A. persica Rothm. (11, 1934), quam auctor (16, 1941) — mea opinione vix iuste — unam et eandem esse suspectat atque *A. oxysepala* Juz., differt ab *A. Achtarowii* secundum descriptionem et secundum specimen a me visum in loco classico lectum (Bornmüller, Iter pers. alter. 1902, Nr. 6944): foliis radicalibus et praecipue caulinis profundius incisis, foliorum utraque pagina pilosa (specimen visum!; Rothm. 17, 1938, p. 62—63), dentibus etiam in speciminibus robustis in utroque latere ad summum 8, inflorescentia sat pauciflora minus expansa; praeterea differentias maximi pretii flores ostendunt (cfr. tab. I. et II.).

2. **Alchemilla jumrukczalica Pawł. n. sp.**

4. Planta sat parva vel mediocris, obscure subcoerulescenti-viridis; coloratio aestivalis manifesta, violaceo-brunnea. Caules ± erecti vel arcuato-adscendentes, 10—21 cm longi, foliis 2× (vel plus) longiores, in internodiis 2—4 inferioribus i. e. ad $^1/_3$—$^2/_3$ altitudinis — inferne dense, supra sparsius — breviter hirsuto pilosi, superne glabri. Laminae foliorum radicalium reniformes vel (exteriores) orbiculari-reniformes, sinu basali aperto (in foliis infimis angusto

vel fere clauso), ad 4·9 cm longae, ad 5·9 cm latae, subtus vix paululum pallidiores, siccae ± plicatae, 7- vel incomplete 9- lobae; pagina superior in foliis infimis tota sat dense pilosa, in reliquis in parte basali, saepe etiam ad nervos vel etiam ad plicas sparse pilosa, ceterum glabra vel sparsissime pilosa; pagina inferior tota dense patenter pilosa; lobi in foliis omnibus ad $^2/_9$—$^1/_3$ radii longitud. incisi, semiorbiculares vel late semielliptici, circumcirca dentati; dentes in utroque latere (5) 6—7 (8) subaequales, mediocres, obtusiusculi vel obtusi, subconniventes; petioli ad 8 cm longi, dense patenter pilosi. Folia caulina haud numerosa et haud magna, ad $^1/_5$—$^1/_3$ incisa, eodem modo ac folia radicalia pilosa vel suprema in pagina superiore glabra; stipulae ad $^1/_3$ in lacinias acutas partitae. Inflorescentia sat parva et haud multiflora, ramis sub angulo acuto abeuntibus; ramuli congesti, sparse longe patenter pilosi; florum glomeruli sat densi; pedicelli glabri vel nonnulli sparsissime pilosi, inferiores urceolis usque ter longiores, superiores eis breviores. Flores virescenti-flavi, mediocres, plerumque 3·5—4·5 mm diam.; urceoli brevissime campanulati, glabri vel saepius parce pilosi; sepala late oblongo-triangularia acuta, episepala eis subaequilonga vel paulo longiora, ovato-oblonga, acuta; in floribus fere omnibus (88% flor. examinatorum) (1) 2 vel 3 (rarius omnia i. e. 4) episepala denticulis 1—2 (in uno latere 0—2) minutis praedita. Nux urceolo manifeste excedens.

Bulgaria, montes «Centralna Stara Płanina»: in itinere inter «Chiża Jumrukczal» et cac. Jumrukczal, ad rivum, alt. 1700—1800 m, 30. 7. 1948, legi ipse. Typus Cracoviae in Herb. Acad. Sc. Polon.

Differt ab *A. Achtarowii* Pawł. et *A. armeniaca* Rothm. statura parvula, foliis profundius lobatis, exterioribus in pagina quoque superiore densiuscule pilosis, lobis et dentibus minus numerosis, dentibus obtusis subconniventibus, foliis caulinis minoribus et minus numerosis, floribus densiuscule glomerulatis, inflorescentia pauciflora. Ab *A. Achtarowii* differt praeterea floribus minoribus, episepalis sepala subaequantibus vel paulo (nunquam multo) longioribus, sat multis (40%) integris, pro parte autem (40%) 1-, rarius (20%) 2- dentatis. — Ab *A. armeniaca* differt praeterea urceolis glabris vel parce pilosis et episepalorum denticulatorum numero maiore.

3. **Alchemilla peristerica Pawł. sp. n.**

4. Planta sat magna, subflavido-griseoviridis; coloratio aestivalis ut videtur nulla. Caules complures, erecti, superne saepe paululum flexuosi 30—40 cm alti, firmi sed sat graciles, in internodiis 4—6 inferioribus i. e. ad $^2/_5$—$^2/_3$ longitudinis — infra dense, supra sparsius vel sparsissime — patenter pilosi, magis superne glabri. Foliorum radicalium laminae sat magnae ± orbiculares, 5—8×6—9 cm, sinu basali plerumque angustissimo vel clauso et tunc lobis basalibus haud raro sese obtengentibus, 9—10- vel incomplete 11- lobae, ± plicatae, subtus pallidiores, nervis 1. et 2. ordinis in utraque pagina (in sicco) bene conspicuis albidis, subtus prominulis; pagina superior glaberrima vel versus basim et versus marginem loborum basalium sparse pilosa, pagina inferior tota dense molliter et in nervis patenter pilosa; lobi ad $^1/_2$—$^2/_5$ radii longitudinis incisi, semielliptici, semiovati, vel e basi subrectangulari usque subtrapezoidali rotundati usque obtuse triangulares, ima basi spatio 2—5 mm integri, supra dentati; dentes in utroque latere (8) 9 (11), in foliis exterioribus graciles acuti, in interioribus grossiores latiores obtusiusculi, omnes apice penicillati, a basi versus apicem loborum regulariter accrescentes, terminalis vicinis multo minor; petioli ad 17 cm longi, dense patenter pilosi. Folia caulina multo minora, sparse pilosa, subtus molliter pilosa, reniformia sinu basali late aperto vel basi ± truncata, inferiora et media 7- loba, lobis ad $^1/_3$—$^1/_2$ radii incisis, lateribus basi 2·5—5 mm longa integerrimis, superne rotundatis et graciliter dentatis, dentibus acutis, in utroque latere (in foliis inferioribus) 5—7; stipulae iam mediae sat profunde acute dentatae, superiores ad vel ultra medium in lacinias inaequales acutas partitae. — Inflorescentia haud multiflora, ramis erecto patentibus, 1 vel 2 infimis ima basi ± pilosis, ceterum una cum ramis superioribus glabris; glomeruli densiusculi; pedicelli glaberrimi, inferiores urceolis aequilongi vel ad 3× longiores. Flores luteoli mediocres, 3·5—4·5 mm lati; urceoli basi sparse pilosi vel glabri; sepala triangulari-lanceolata; episepala eis subaequilonga, rarius paulo longiora, urceolis multo longiora, lanceolata acuta, integra, rarius nonnulla denticulo unico minuto praedita. — Fig. 2.

Macedonia occidentalis, montes Perister prope opp. Bitolj, in declivitate septentr. supra pag. Rotino, ad rivum, ca. 1400 m s. m.,

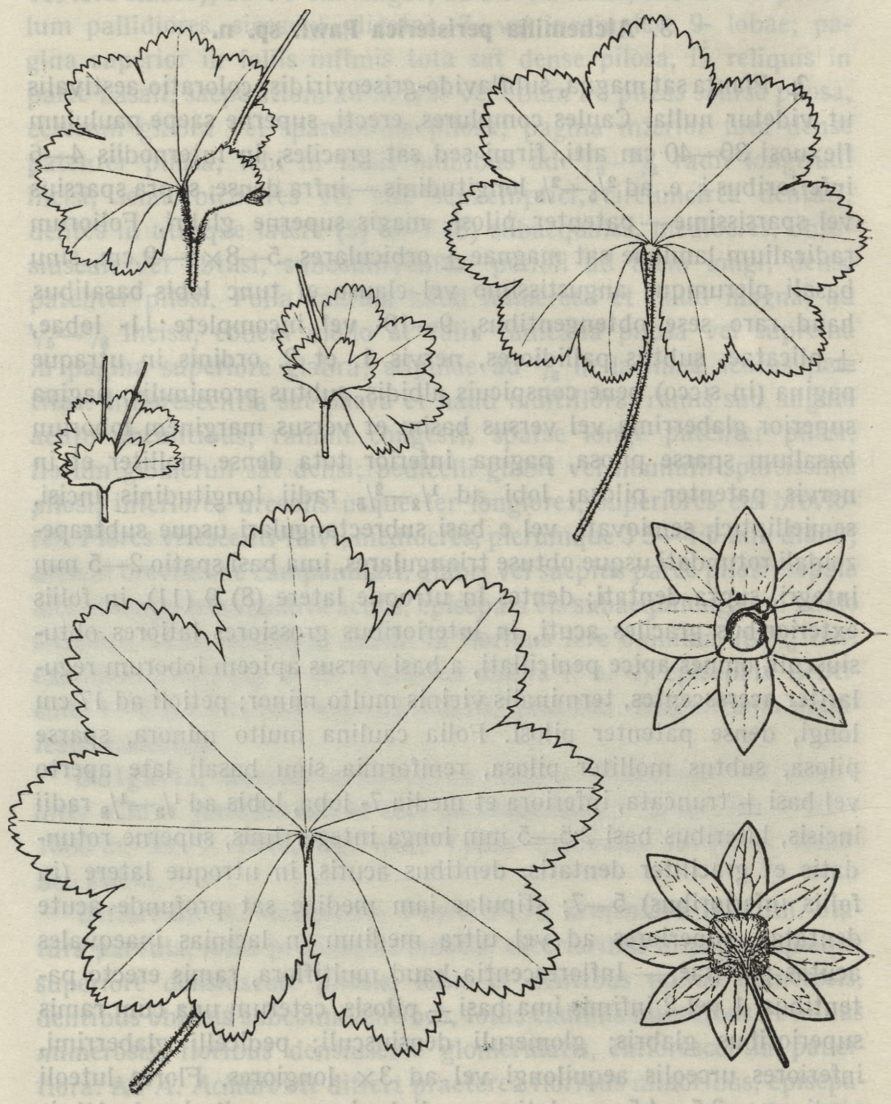

Fig. 2. *Alchemilla peristerica* Pawł. Flores: $^{9}/_{1}$; folia 2 basalia et 3 caulina: $^{1}/_{1}$. Pili in foliis nonnisi in petiolis delineati.

19. 7. 1938, lg. S. P. et B. P.[1]. — Typus in Herb. Universit. Jagell. Cracoviensis.

[1] Collectores: B. P.=B. Pawłowski; S. P.=S. Pawłowska; Kor.=A. et J. Kornaś; J. Kor.=J. Kornaś.

Planta nostra ab omnibus fere *Alchemillis* e subsect. *Calycanthum* ser. *Elatae* quae caulibus et peliolis patenter pilosis gaudent, iam foliis profundius incisis lobisque basi conspicue integris distincta apparet. Ab *A. speciosa* Buser, quae notas ambas indicatas prae se fert, *A. perisierica* differt optime foliis supra glabris vel fere glabris, ad $^1/_3$—$^2/_5$ (nec ad $^2/_5$—$^1/_2$) incisis, pilis in caulibus pedicelisque horizontaliter (nec erecto) patentibus, ramis inflorescentiae pedicellisque glabris aliisque notis. — *A. Bornmülleri* Rothm. quae lobis lateribus integris gaudet, differt a nostra specie statura graciliore, foliis radicalibus nonnisi ad $^1/_6$ incisis, supra dense pilosis, lobis truncatis, dentibus paucis.

4. **Alchemilla Zapałowiczii Pawł. n. sp.**

♃. Planta sat magna, obscure viridis. Caulis 3—4 dm altus, totus — ramis supremis inflorescentiae exceptis — pilis patentibus vel pro parte paulum reflexis obtectus. Foliorum radicalium laminae magnae, rotundato-reniformes, ad 11×12 cm, sinu basali plerumque late aperto, 9- (imperfecte) 11- lobae, saepe maculis pallide rubro-violaceo-brunneis notatae, in utraque pagina dense, praecipue subtus patentim pilosae; lobi ad ($^1/_4$) $^1/_3$—$^2/_5$ laminae incisi, breviter vel oblongo semielliptici vel leviter triangulari-semielliptici, ab ima basi dentati, dentes in utroque latere 7—11 grossi, latiusculi, obtusiusculi, a basi ± ad $^2/_3$ longitud. loborum accrescentes, terminalis minor; petioli ut caulis dense pilosi. Folia caulina sat magna, sat profunde incisa, inferiora longiuscule petiolata. — Inflorescentia angusta; glomeruli florum modice densi; pedicelli omnes glaberrimi, floribus breviores usque aequilongi. Flores pallide luteo-virides, mediocres vel maiusculi, 3·5—5 mm diam., etiam post anthesim ± stellatim expansi; urceoli pro parte glabri, pro parte pilis paucis praediti; sepala pro rata parte magna et manifeste lata, latissime ovata, obtusiuscula vel subacuta; episepala eis pro maiore parte subaequilonga sed evidenter ($1^1/_3$—2×) angustiora, aeque ac sepala urceolis paulo longiora, glabra vel apice pauciciliata, nervis anastomosantibus percursa. Fructus discum manifeste superans. — Fig. 3.

In associationibus ordinis «*Calamagrostidetalia villosae*» in Carpatis boreo-orientalibus: Montes Czywczynenses: Valea Purului, 915 m, *Petasitetum Kablikiani*, 28. 7. 1935, lg. S. P. et B. P. (typus in herb. Ac. Sc. Pol. Cracoviae); Komanowa, 1680 m, *Calamagrosti-*

detum pocuticum, 22. 8. 1934 lg. S. P. et B. P. — Etiam specimen e Montibus Czarnohora: in convalle inter «Kozły Wielkie» et «Szpyci»

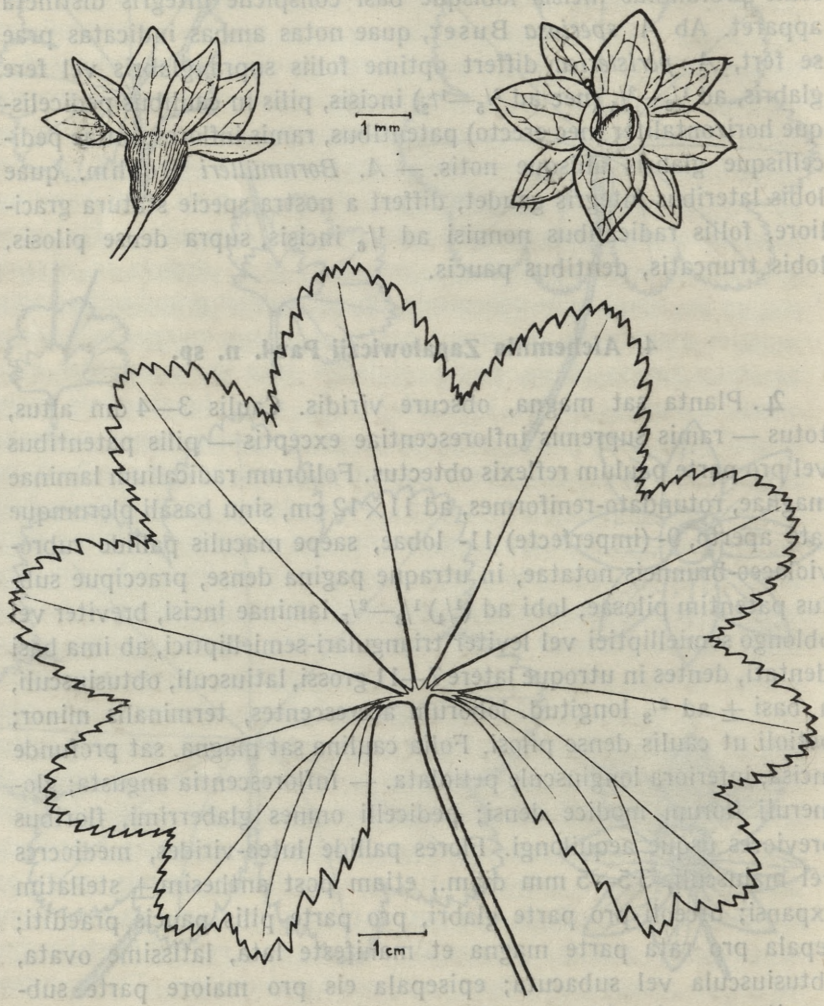

Fig. 3. *Alchemilla Zapałowiczii* Pawł. Pili in folio omissi.

1750 m, *Poëto-Deschampsietum*, 9. 8. 1935, lg. S. P. et B. P., verosimiliter huc pertinet.

Planta insignis, inter subsectionem *Calycanthum* seriem *Elatae* Rothm. et subsect. *Heliodrosium* subser. *Hirsutae* Lindb. fil. quasi

medium tenens. Ab omnibus mihi notis speciebus seriei *Elatae* sepalis et episepalis urceolos vix paulum superantibus sepalisque latis pro portione brevibus abest. A. subser. *Hirsutae* calycis et epicalycis longitudine nec non nuculis discum manifeste excedentibus distincta.

In montibus Pirin in Bulgaria (supra vallem Banderica 2320 m) plantam valde similem legi, sed foliis minus (ad $^1/_4$—$^1/_3$) incisis, in facie superiore pro parte glabris, pro parte solummodo in zona marginali et in plicis pilosis, lobis incisuris integris separatis, glomerulis laxioribus. Quae verisimiliter species est nova, serius describenda.

5. **Alchemilla gorcensis Pawł. n. sp.**

♃. Planta satis magna, saturate vel coerulescenti — in sicco saepe subflavescenti — viridis, plerumque multicaulis. Coloratio aestivalis manifesta, pulchre violascenti-purpurea. Caules 25—60 cm alti, ima basi paululum arcuati, ceterum ± erecti, rigidiusculi, in internodiis 3—6 (rarissime usque ad 8) inferioribus i. e. ± ad $^2/_5$ usque $^3/_4$ (raro solummodo ad $^1/_4$ vel usque ad $^4/_5$) longitudinis laxe vel praecipue inferne densius subadpresso pilosi, superne glabri; pars pilosa inflorescentiam non attingens vel pili saepius usque ad 1., rarius ad 2., rarissime usque ad 4. ramum in caule obvii. Laminae foliorum radicalium in vivo prius modo flabelli plicatae, late infundibuliformes, serius ± planae, reniformes vel late reniformes vel haud multo plus quam semiorbiculares, 2·4—11·3 cm longae, 3·2—14·5 cm latae, supra saturate vel plerumque coerulescenti-virides, subtus pallidiores, nervis 1. et 2. ordinis in sicco subtus valde conspicuis, supra saepius palidis; longitudo: latitudo laminae= (0·65) 0·70—0·81 (0·85); sinus basalis valde improfundus, angulum latissimum, saepius 90—140°, formans — vel sinus profundior, sed tunc quoque late (raro minus late) apertus; nervi subtus toti vel in dimidio superiore adpresso pilosuli, ceterum laminae in utraque pagina glabrae, rarius lobi basilares (=extremi) subtus adpresso pilosuli; laminae 9—11- lobae; lobi vix ad ($^1/_9$) $^1/_8$—$^2/_9$ radii laminae incisi, circumcirca dentati, arcuati vel saepius ± triangulares; dentes in utroque latere 6—9 (10) parvi graciles ± conniventes, vel mediocres latiusculi, acuti vel obtusiusculi, omnes subaequales, apice manifeste penicillati, termi-

nalis lateralibus paulo minor; petioli saepe valde elongati (ad 36 cm), omnes vel infimis exceptis subadpresso pilosi. Folia caulina numerosa, pro portione magna (usque ad 5·5×8 cm), late reniformia vel semiorbicularia, vix ad $^1/_{10}$—$^1/_5$ incisa, 5—7- usque incomplete 9-loba, nervis exceptis subtus glabra; petioli eorum praecipue inferiores saepius sparse subadpresso pilosi; stipulae mediae et superiores amplae, inaequaliter improfunde inciso dentatae. — Inflorescentia magna et multiflora sed pro rata parte angusta, ramis sat brevibus suberectis; glomeruli breviter racemosi, laxiusculi (sed sat conferti) vel densiusculi; pedicelli glaberrimi, floribus plerumque breviores. Flores flavidi, mediocres usque maiusculi, 3·5—5 mm diam.; urceoli glaberrimi, breviter conici, 1—1·5 mm longi; sepala latiuscula ovato- vel late ovato triangularia, acuta vel obtusiuscula, glaberrima, rarius nonnulla apice pilis paucis praedita, urceolis longiora, etiam post anthesin late stellato expansa; episepala eis paulo longiora vel subaequilonga, subaequilata vel paulo tantum (ad summum $1^1/_2$×) angustiora, ovato oblonga, acuta, interdum nonnulla in floribus singulis denticulis 1—2 praedita. Fructus disco manifeste exsertus. (Fig. 4).

Habitat in Carpatorum Occidentalium tractu «Gorce» dicto nec non in Regione Subtatrica et in Tatris in pratis paludosis, ad fontes et rivos, praecipue in assoc. «*Valerianeto — Caricetum flavae*», in regione Fagi, inter 785 et 1040 m s. m.

Montes Gorce: vallis «Spod Ciosków» 930 m, abundanter, 24. 7. 1950, lg. Kor. (typus in Herb. Ac. Sc. Polon. Cracoviae); ibid. 19. 6. 51, lg. J. Kor. et B. P.; — in eadem valle, 785—850 m, 19. 6. 51, lg. (iid.); — vallis rivi Łopuszna 860 et 840 m (iid.); — Furcówka 825 m, 19. 6. 51, lg. Kor.; — vallis rivi «Kluszkowski Potok» (iid.).

Regio Subtatrica: Bukowina, 7. 7. 1938, lg. J. Kor.; — Bukowina «Do Odewsia» infra fontem «Studnia Walasowa» 900 m, 10. 7. 51, lg. S. P. et B. P.; in latere sept.-occ. cacum. Wysoki Wierch supra pag. Bukowina, 940 m, 28. 7. 52 (iid.); — Brzegi, versus vallem rivi «Koziniec» 970—990 m, 20. 7. 51 (iid.); — Cyrhla nad Białką, supra pag. Brzegi 1035 m (iid.); — Polana Poroniec, ad rivum, 1040, 28. 7. 52 (iid.); — Huciska ad Olcza 840 m, 21. 7. 51 (iid.; hoc loco specim. minus typica, caulibus nonnullis vix ad $^1/_5$ pilosis). Tatri Occidentales: vallis «Dolina Strążyska», 930 m, 4. 6. et 7. 52, lg. A. Jasiewicz et B. P.

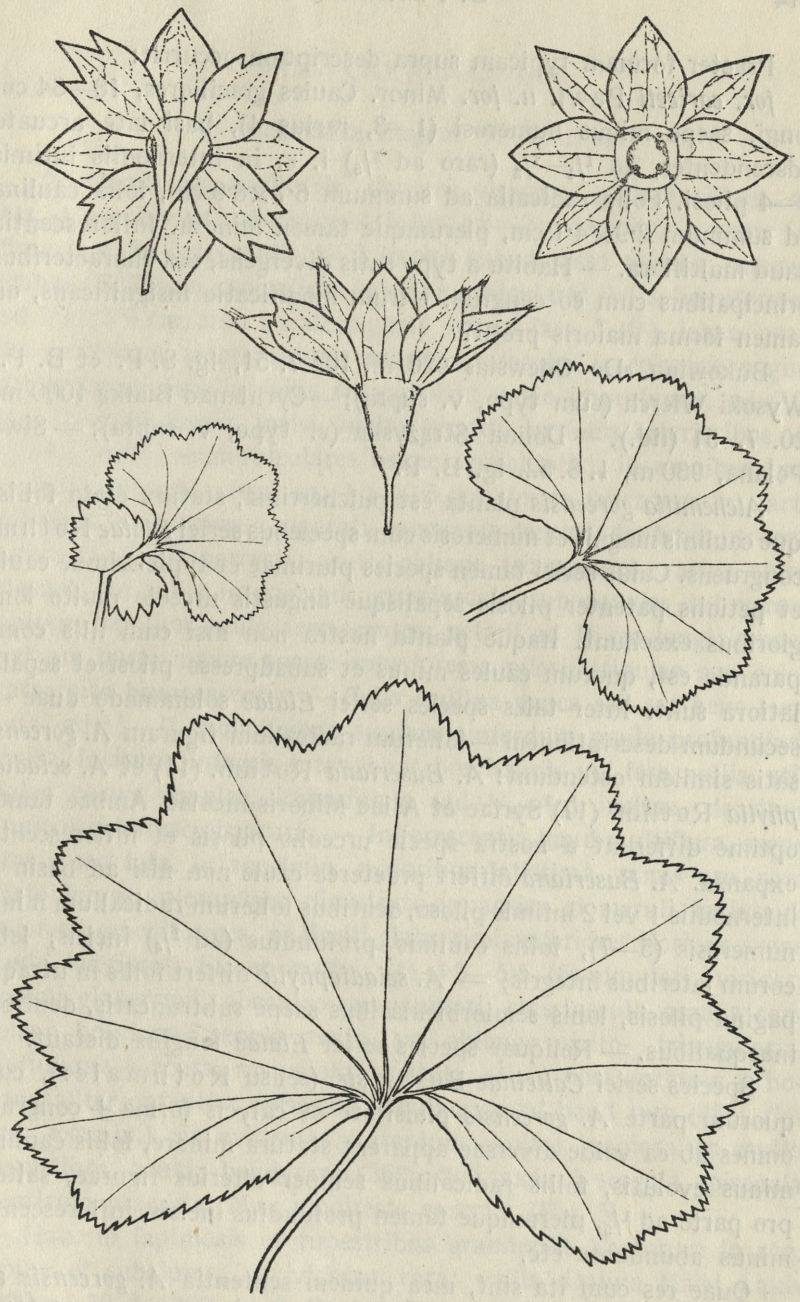

Fig. 4. *Alchemilla gorcensis* Pawł. Flores: $^{8}/_{1}$; folium radicale et 2 caulina: $^{1}/_{1}$. Pili in foliis omissi.

Praeter formam typicam supra descriptam occurrit:

for. cariceti Pawł. *n. for.* Minor. Caules graciliores, 18—34 cm longi, saepe minus numerosi (1—3, rarius 4), basi late arcuato adscendentes, ad $^1/_4$—$^1/_2$ (raro ad $^3/_5$) i. e. in internodiis infimis 2—4 pilosi. Folia radicalia ad summum 6·7×8·9 cm, folia caulina ad summum 2·9×4·1 cm, plerumque tamen minora. Inflorescentia haud multiflora. — Habitu a typo satis divergens, sed characteribus principalibus cum eo congrua. Utrum modificatio insignificans, an tamen forma maioris pretii?

Bukowina «Do Odewsia» 920 m, 10. 7. 51, lg. S. P. et B. P.; Wysoki Wierch (cum typo, v. supra); — Cyrhlanad Białką 1000 m, 20. 7. 51 (iid.), — Dolina Strążyska (c. typo, v. supra); — Siwa Polana, 930 m, 1. 8. 52. lg. B. P.

Alchemilla gorcensis planta est pulcherrima, statura elata foliisque caulinis magnis et numerosis cum speciebus seriei *Elatae* Rothm. congruens. Cuius seriei tamen species plurimae et typicissimae caule et petiolis patenter pilosis sepalisque angustis urceolo multo longioribus excellunt. Itaque planta nostra non nisi cum illis comparanda est, quorum caules minus et subadpresso pilosi et sepala latiora sunt. Inter tales species seriei *Elatae* solummodo duae — secundum descriptionem — foliorum radicalium figuram *A. gorcensi* satis similem ostendunt: *A. Buseriana* Rothm. (11) et *A. sciadiophylla* Rothm. (17) Syriae et Asiae Minoris incolae. Ambae tamen optime differunt a nostra specie urceolis pilosis et inflorescentia expansa. *A. Buseriana* differt praeterea caule non nisi ad basin in internodiis 1 vel 2 infimis piloso, dentibus foliorum radicalium minus numerosis (5—7), foliis caulinis profundius (ad $^1/_4$) incisis, lobis eorum lateribus integris; — *A. sciadiophylla* differt follis in utraque pagina pilosis, lobis semiorbicularibus saepe subtruncatis, dentibus inaequalibus. — Reliquae species seriei *Elatae* longius distant.

Species seriei *Calicinae* Bus. *s. str.* (sensu Rothmaler), cum quorum parte *A. gorcensis* pilositate et calycis forma ± congruit, omnes ab ea valde diversae apparent statura minore, foliis caulinis minus evolutis, foliis radicalibus semper alterius figurae, saltem pro parte ad $^1/_5$, plerumque tamen profundius incisis, inflorescentia minus abundante etc.

Quae res cum ita sint, mea quidem sententia *A. gorcensis* adnumeranda est seriei *Elatae* in qua tamen positionem approximatam seriei *Calicinae* tenet.

Series: CALJCINAE Bus.

6. **Alchemilla Eugenii Pawł. n. sp.**

4. Parva vel mediocris, obscure viridis. Caulis 8—37 cm altus, ± erectus vel adscendens, foliis 1·5—2× longior, in parte infima i. e. in internodiis 1—2 sparse subadpresso pilosus, ceterum glaber. Foliorum radicalium laminae orbiculares vel subreniformes, ad 7·5×8·9 cm, sinu basali angusto vel sat lato, 7-(9-)- lobae, praeter nervorum apices subtus adpresse pilosulos in utraque pagina glaberrimae; lobi in foliis exterioribus valde improfunde, ad ($^1/_8$) $^1/_6$—$^1/_5$ radii longit. incisi, inalte arcuati, in foliis interioribus ad $^1/_4$—$^1/_3$ incisi, semiorbiculares usque semielliptici, in omnibus basi incisura integra parva ca. 1·5—2·5 mm longa interdum in parte foliorum vix conspicua separati, super eam dentati; dentes in utroque latere 5—7 (9) porrecti, acuti, oblique triangulares, rarius obtusiusculi, in foliis interioribus grossiusculi, subaequales vel versus apicem loborum paulo accrescentes; petioli ad 16 cm longi; omnes vel pro parte maiore sparse subadpresso pilosi; stipulae non coloratae cito brunnescentes. — Folia caulina pauca sed sat bene evoluta, ad $^1/_6$—$^1/_3$ (solummodo suprema interdum paulo profundius) incisa, incisuris integris inter lobos brevissimis vel fere nullis; stipulae eorum amplae, improfunde (ad $^1/_7$—$^1/_4$) incisae, dentibus numerosis latiusculis acutis. — Inflorescentia haud multiflora, saepe brevis sed lata, in specimin. maioribus latissima, ramis pro rata parte longis, plerumque divaricato-expansis; glomeruli laxiusculi saepius umbelliformes; pedicelli glaberrimi, inferiores floribus saepe paulo longiores. Flores magni, (4) 4·5—5·5 (6) mm lati, virides; urceoli glaberrimi, obconico-campanulati, crassiusculi, sepalis conspicuo breviores; sepala ovato- vel oblongo ovato- triangularia, plerumque ± acuta, rarius obtusiuscula, etiam post anthesin ± horizontaliter patentia; episepala aequilonga vel haud raro (48% florum examin.) eis evidenter (interdum multo) longiora et multo angustiora, acuta; haud raro (36% flor. exam.) singula episepala denticulo praedita. Nux manifeste exserta. (Fig. 5).

Hab. in lapidiosis et rupestribus graminosis Tatrorum in reg. alpina et subalpina, ut videtur rara: vallis «Dolina Małej Łąki» 1720 m, 20. 7. 1950, lg. S. P. et B. P. (typ. in Herb. Ac. Sc. Pol. Cracoviae); — Krzesanica 2110 m (iid.); in utroque loco solo cal-

careo; — infra «Skośny Wodospad» s. lacum Morskie Oko, ca. 1410 m, 19. 7. 1951, lg. Z. Paryska.

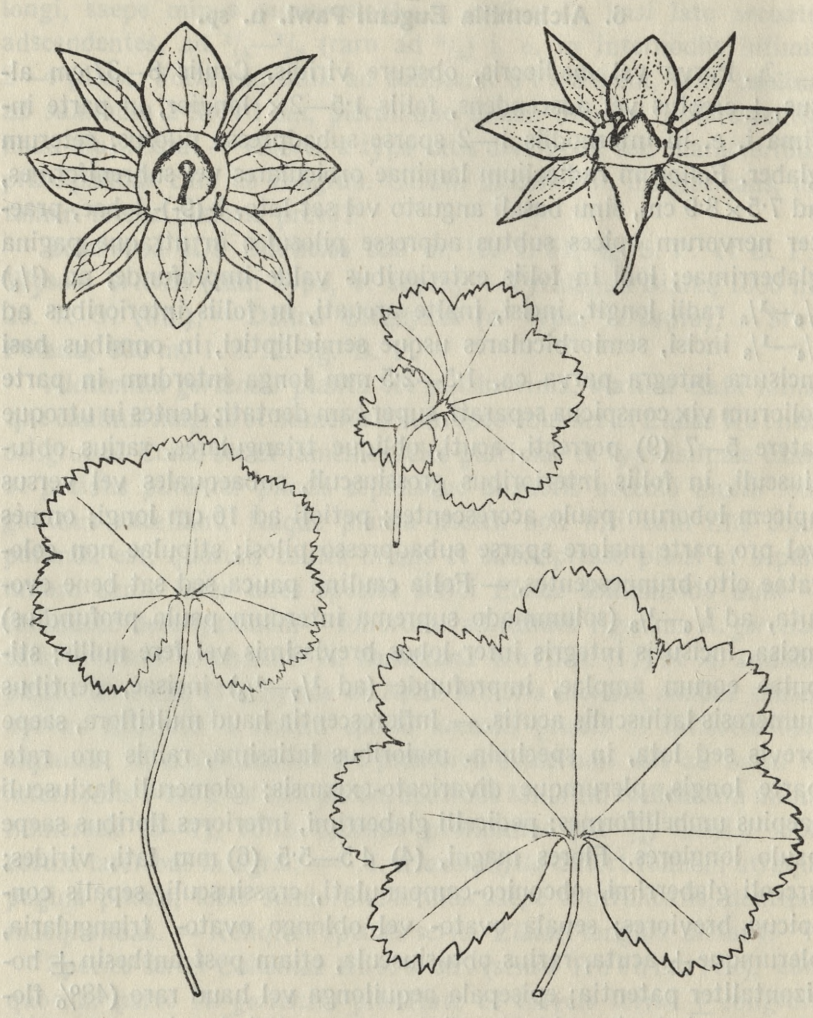

Fig. 5. *Alchemilla Eugenii* Pawł. Flores: $^{8}/_{1}$; folia 2 radicalia et 1 caulinum: $^{1}/_{1}$. Pili in petiolis omissi.

Planta in honorem fratris mei denominata, proxima esse videtur duabus speciebus, quarum una — *A. cuspidens* Bus. — in Alpibus occident. et australibus, altera — *A. dura* Bus. — in Caucaso occurrit. — *A. cuspidens* mihi e descriptione R. Kelleri (ap. Schinz et

Keller 19) et Rothmaleri (17) nota est; praeterea vidi 5 specim. ex Alpibus a Buser ipso determinata. Planta haec differt a nostra foliis profundius incisis (ad $^1/_4$ usque fere ad $^2/_5$), dentibus subconniventibus, incisura integra inter lobos plerumque magis conspicua, inflorescentia minus expansa nec non floribus: urceoli basi magis sunt angustati, sepalis non nisi paululum breviores, episepala sepalis multo rarius (solummodo 20% flor. exam.) longiora plerumque tamen subaequilonga, semper integerrima, una cum sepalis post anthesin erecta; flores sunt paulo minores, (3·5) 4—5 (rarissime ad 5·5) mm lati.

A. dura Bus., mihi tantum e descriptione (Rothmaler 17, Juzepczuk 7) nota, differt a planta nostra statura robustiore, foliis omnibus ad $\pm ^1/_4$ incisis (Rothmaler), foliis caulinis magnis, dentibus magis elongatis, inflorescentia angusta (Juzepczuk 7).

Aliae species affines: *A. debilis* Juzep. (Caucasus, Asia Minor), *A. retinervis* Bus. (patria eadem) et *A. asteroantha* Rothm. (Stara Płanina in Bulgaria) praeter alias notas iam foliis (saltem interioribus) subtus in lobis basalibus nec non in tota longitudine nervorum pilosis sufficienter ab *A. Eugenii* distinguuntur.

Subsectio: HELIODROSIUM Rothm.

Series: GLABRAE Rothm. pro subserie

Inter 6 species novas huc adnumeratas solummodo apud 2 *(A. Żmudae* et *A. Sokołowskii)* caules petiolosque semper glabros inveni. In ceteris speciebus saltem in parte speciminum pili hic illie in caulis parte infima vel in parte petiolorum adsunt. Itaque differentia inter hanc et sequentem seriem non nisi gradualis est: in serie *Glabrae* specimina caulibus et petiolis omnibus glabris aut — in parte specierum — sola occurrunt, aut — in reliquis speciebus — numero praevalent; contra, in subserie *Subglabrae* specimina ista aut omnino desunt, aut multo minus frequenter occurrunt quam specimina caulibus et petiolis saltem pro parte pilosis.

7. **Alchemilla Stanislaae Pawł. n. sp.**

♃. Planta mediocris, sat obscure (in pagina foliorum inferiore paululum coerulescenti-) viridis; coloratio aestivalis haud manifesta, sordide et obscure purpureo-violaceo- brunnea. Caules ± erecti 15—

30 cm alti, glaberrimi. Laminae foliorum rosularium manifeste plicatae, in vivo facile radialiter disrumpentes, 7- 9- lobae; in foliis exterioribus suborbiculares, 1·6—4·6×1·8—5·1 cm, inconspicue — vix ad $^1/_{10}$—$^1/_7$ radii — lobatae, sinu basali angusto vel clauso vel lobis basalibus sese obtegentibus, in foliis interioribus reniformes, 3—6·2×4—8·2 cm, sinu basali latissimo angulum apertum formante, improfunde — ad $^1/_6$—$^1/_5$ ($^1/_4$) — lobatae; lobi omnes late rotundate, circumcirca dentati, dentibus in utroque latere 5—7 (8) sat grossis, ± acutis, apicem versus accrescentibus, terminali ± aequilongo; nervi subtus in parte apicali adpresso pilosuli, ceterum laminae in utraque pagina glabrae; stipulae saepius leviter rubescentes, margine ± ciliolatae, ceterum glabrae; petioli omnes glabri (in uno specimine petiolus unus interior parce pilosus). Folia caulina bene evoluta, inferiora et media multo latiora quam longa, lobis plerumque inconspicuis vel vix conspicuis, etiam superiora lata et vix ad $^1/_5$ vel $^1/_4$ incisa; stipulae eorum latae, inprofunde late dentatae, etiam supremae haud ultra $^1/_5$—$^1/_4$ incisae. — Inflorescentia sat angusta; glomeruli laxiusculi; pedicelli — infimis exceptis — floribus multa breviores, omnes glaberrimi. Flores virescentes, glaberrimi, 3·5—4·7 mm diam.; urceoli elongato turbinato-conici, 1·8—2·7 mm longi, basin versus sensim angustati et in pedicellum inconspicue transeuntes; sepala late cordato-triangularia, acutiuscula vel acuta, urceolis manifeste breviora, post anthesin sat late aperta; episepala lineari oblonga vel oblonga, acuta, sepalis pro parte maiore subaequilonga (sed multo angustiora), pro parte minore paulo breviora; haud raro 1, raro 2 vel 3 episepala in eodem flore denticulo praedita. — Fl. mense VII. (Fig. 6).

Habitat in rupestribus graminosis uberioribus in reg. Mughi et alpina montium Tatrorum solo granitico: Mięguszowiecki Szczyt nad Czarnym Stawem, 2040 m, 27. 7. 1950, lg. B. P. (typus Cracoviae in Herbar. Ac. Sc. Polon.); Mięguszowiecki Szczyt Środkowy, 2140 m, 10. 9. 1926, lg. B. P. (sub «*A. coriacea* var. *straminea*»); supra «Skośny Wodospad» s. lacum Morskie Oko, ca. 1550 m, 29. 7. 51, lg. Z. Paryska.

Planta ab omnibus speciebus seriei *Glabrae* foliis valde improfunde incisis et florum figura valde insigni aberrans. Praeterea differt ab *A. Żmudae* Pawł. foliis interioribus magis reniformibus sinu basali latius aperto, dentibus acutioribus, glomerulis sublaxio-

ribus submaioribus, floribus submaioribus, post anthesin magis apertis, statura maiore, caulibus erectis folia vix superantibus; — ab

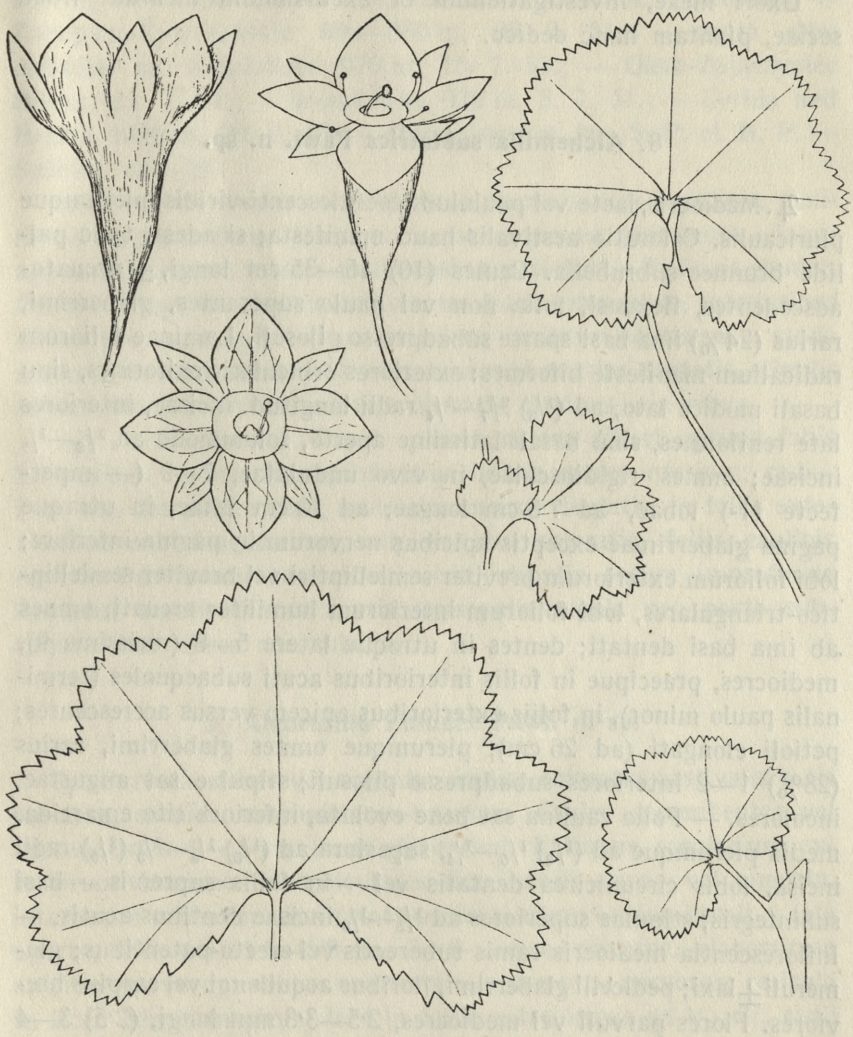

Fig. 6. *Alchemilla Stanislaae* Pawł. Flores: $^{8}/_{1}$; folium radicale exterius (supra ad dextram) et interius (infra ad sinistram) et folia 2 caulina: $^{1}/_{1}$.

A. inconcinna Bus. (1) colore laetius viridi, foliis 7—9- (nec 9- 11-)-lobis, dentibus angustioribus acutioribus non mammiformibus, inflorescentia angustiore (nec effusa), glomerulis minus laxis, urceolis

in pedicellum sensim angustatis, caulibus semper et petiolis fere semper glaberrimis. — Ceterae species magis distant.

Uxori meae, investigationum et excursionum mearum fideli sociae, plantam hanc dedico.

8. **Alchemilla subtatrica Pawł. n. sp.**

4. Mediocris, laete vel paululum coerulescenti-viridis, plerumque pluricaulis. Coloratio aestivalis haud manifesta; si adest, tunc pallide brunneo-subrubella. Caules (10) 15—35 cm longi, ± arcuato-adscedentes, flexuosi, folia non vel paulo superantes, glaberrimi, rarius (24%) ima basi sparse subadpresso pilosuli. Laminae foliorum radicalium manifeste biformes: exteriores orbiculari-reniformes, sinu basali modice lato, ad (1/3) 2/7—1/4 radii longitud. incisae, interiores late reniformes, sinu basali latissime aperto, solummodo ad 1/6—1/5 incisae; omnes rigidiusculae, in vivo undulatae, 7—9 (— imperfecte 11-) lobae, ad 7·5 cm longae, ad 10 cm latae, in utraque pagina glaberrimae exceptis apicibus nervorum in pagina inferiore; lobi foliorum exteriorum breviter semielliptici vel breviter semielliptico-triangulares, lobi foliorum interiorum humiliter arcuati, omnes ab ima basi dentati; dentes in utroque latere 5—8 (rarissime 9), mediocres, praecipue in foliis interioribus acuti subaequales (terminalis paulo minor), in foliis exterioribus apicem versus accrescentes; petioli elongati (ad 26 cm), plerumque omnes glaberrimi, rarius (28%) 1—2 interiores subadpresso pilosuli; stipulae sat angustae, incolores. — Folia caulina sat bene evoluta, inferiora cito emarcida, media plerumque ad (1/6) 1/5—1/4, superiora ad (1/5) 1/4—1/3 (2/5) radii incisa, lobis circumcirca dentatis vel — in foliis supremis — basi subintegris; stipulae superiores ad 1/5—1/3 incisae dentibus acutis. — Inflorescentia mediocris ramis suberectis vel erecto-patentibus; glomeruli ± laxi; pedicelli glaberrimi, floribus aequilongi vel saepius breviores. Flores parvuli vel mediocres, 2·5—3·3 mm longi, (2·5) 3—4 (4·5) mm lati, glaberrimi, viriduli; urceoli parvi, campanulati, basi ± abrupte contracti, sepalis ± aequilongi; sepala late ovato-triangularia, subacuta, post anthesin erecto-patentia, integra, raro (7% sepalorum examinat.) singula vel omnia 4 in eodem flore apice 1—2 dentata; episepala ovato- vel oblongo lanceolata, sepalis breviora vel subaequilonga, acutiuscula, fere semper integra.

Hab. in Regione subtatrica in pratis siccis *(Gladioleto-Agrostidetum)* vel in paludibus silvaticis: Antołówka supra Zakopane, 930 m, 18. 7. 1951 (typus in Herb. Acad. Sc. Polon. Cracoviae); — Zakopane-Żywczańskie 860—880 m, 25. 7. 51.; — supra «Nędzówka» ad Kościelisko 970 m, 15. 7. 51.; — Olsza-Zajączyniec 880 m, 21. 7. 51.; — Weszkówka 930 m, 8. 7. 51.; — Cyrhla nad Białką 1020 m, 20. 7. 51. — Omnia specim. leg. S. P. et B. P. — Specim. ex.: 29.

Inter omnes species seriei *Glabrae* planta nostra praecipue similis est *A. inconcinnae* Bus. et aeque atque ea plerumque glabra apparet, interdum tamen caulis basi et petiolis 1—2 pilosis gaudet. Differt tamen ut videtur sufficienter foliis biformibus, interioribus minus profunde incisis quam exteriora, dentibus acutis nullo modo mammiformibus, foliis caulinis minutius et acutius dentatis, stipulis superioribus minoribus acutius dentatis, floribus minoribus.

Ab *A. Sokołowskii* Pawł. planta nostra differt optime foliis biformibus, exterioribus profundius incisis quam interiora, radialiter non disrumpentibus, lobis circumcirca dentatis, in foliis exterioribus subtriangularibus, stipulis non coloratis, foliis caulinis superioribus minus profunde incisis, stipulis eorum improfunde dentatis, sepalis minus acutis, episepalis sepala pro parte subaequantibus, disco non rubello.

9. **Alchemilla Żmudae Pawł. n. sp.**

♃. Parvula, dilute vel sordide viridis; coloratio aestivalis satis obsoleta, sordide rubroviolaceo-brunnea. Caules decumbentes vel adscendentes 4—15 cm longi glaberrimi. Foliorum rosularium exteriorum laminae suborbiculares sinu basali clauso vel angusto, interiorum orbiculari-reniformes usque reniformes sinu basali ± late aperto, 1·4—4·6 × 1·6—5·8 cm, 7—9- lobae, plicatae, glaberrimae apicibus nervorum subtus sparse pilosis exceptis, nervorum reticulo in sicco bene conspicuo; lobi in foliis exterioribus ad $^1/_7$—$^1/_5$ radii incisi, late arcuati, 2—3× latiores quam alti, in foliis interioribus ad $^1/_4$—$^1/_3$ incisi, semiorbiculares vel semielliptici, apice late rotundati, omnes ab ima basi dentati, dentibus utrinque 5—6 (7) latiusculis obtusiusculis versus apicem loborum accrescentibus, terminali vicinis non minore paulo angustiore; petioli omnes glaberrimi; stipulae magnae latae plerumque leviter rubescentes. Folia caulina

multo latiora quam longa, inferiora valde improfunde, superiora paulo profundius lobata; stipulae foliorum superiorum latae, haud profunde (ad $^1/_7$—$^1/_3$) dentatae, dentibus latis ± acutis. — Inflorescentia satis pauciflora, ramis erectis vel erecto patentibus. Florum glomeruli densiusculi; pedicelli glaberrimi, pro maxima parte floribus breviores. Flores parvuli, (2·5) 3—4 mm diam., ± virides; urceoli glaberrimi, campanulati, crassiusculi, basi breviter conico angustati, a pedicello bene delimitati, sepala latissima, subtriangulari-cordiformia, obtusiuscula usque subacuta, apice saepius brevissime sparse puberula, integra, post anthesin suberecta, urceolo (sub fructu) manifeste breviora; episepala sepalis pro maxima parte breviora et multo ($1^1/_2$—2×) angustiora, ovato-lanceolata usque lanceolata, integra, raro nonnulla denticulo uno praedita; styli pro parte conspicui. Nux urceolo non excedens. (Fig. 9).

Hab. in Tatris, in regione alpina, solo calcareo aeque ac granitico: Krzesanica 2110 m, 20. 7. 1950, lg. S. P. et B. P. (typus in Herb. Acad. Sc. Polon. Cracoviae); in iugo inter valles Mułowa et Litworowa 2050 m (iidem); vallis Litworowa 1830 m, 15. 8. 1949 (iidem); vallis «Dolina Małej Łąki» 1550—1600 m, 11. 7. 1950, lg. B. P. — Vallis Walentkowa 1800 m, 22. 7. 1925, lg. B. P.; supra Czarny Staw versus Rysy 13. 8. 1945, lg. J. Kor.; infra «Skośny Wodospad» ad lacum Morskie Oko 1410 m, 19. 7. 51, lg. Z. Paryska.

Differt ab *A. Kotulae* Pawł. coloratione aestivali minus manifesta, foliis minus profunde incisis, lobis rotundatis, ab ima basi dentatis, dentibus inaequalibus latiusculis obtusiusculis, foliis caulinis minus lobatis, stipulis eorum minus profunde incisis, floribus viridibus, sepalis fere semper integris, obtusioribus, urceolo manifeste brevioribus.

ab *A. straminea* Bus. statura minore, foliis exterioribus improfunde lobatis, foliorum lobis minus numerosis rotundatis, dentibus paucioribus obtusiusculis, foliis caulinis minus incisis, floribus densius glomerulatis.

A. demissa Bus. differt a planta nostra laminis etiam interioribus magis rotundatis, lobis subtruncatis basi incisuris integris separatis, floribus paulo maioribus, laxe glomerulatis.

A. longiuscula Bus., *A. inconcinna* Bus., *A. aggregata* Bus., *A. trunciloba* Bus. praeter alias notas iam coloratione aestivali magis manifesta a planta nostra differunt (Bus. 1, Schinz et Keller 19).

10. **Alchemilla Kotulae Pawł. n. sp.**

♃. Parva vel mediocris, gracilis et elegans, laete vel saepius ± coerulescenti-viridis. Coloratio aestivalis brunneo-purpurescens vel -rubescens. Caules 7—35 cm longi, foliis basalibus plerumque 2× longiores, adscendentes vel ± procumbentes, plerumque toti glaberrimi, raro (13% specim. examin.) in iternodio infimo valde abbreviato in vaginis acculto, vel (11%) in internodiis 2 infimis abbreviatis pilis sparsis ± adpressis obtecti. Foliorum radicalium laminae reniformes vel orbiculari-reniformes, 1·5—7·4 cm longae, 1·9—8·4 cm latae, sinu basali plerumque latiusculo vel lato, (7) 9- lobae, ± plicatae, subtus in nervorum apicibus adpresso pilosae, ceterum in utraque pagina glaberrimae; lobi ad ($^1/_4$) $^1/_3$ ($^2/_5$) radii longit. incisi, semi- vel oblongo semielliptici, saepius tamen — praecipue in foliis interioribus — subtriangulares, ima basi saltem in parte foliorum incisura parva (1·5—3 mm) integra separati, ceterum dentati; dentes utrinque 5—8 (rarissime ad 10) graciles, acuti, sat angusti, subconniventes, terminalis ambos vicinos aequans vel paululum superans, sed angustior; petioli ad 17 cm longi, omnes glaberrimi; stipulae saepius rubore rosaceo coloratae. Folia caulina sat parva, breviter petiolata, superiora saepius ad $^2/_5$—$^1/_2$ laminae, incisa, lobis ima basi integris; stipulae superiores ad $^2/_7$—$^2/_5$ in lobulos plures angustos digitato partitae. — Inflorescentia angusta, ramis paucis suberectis, florum glomerulis ± densis; pedicelli glaberrimi, floribus multo breviores. Flores parvi usque mediocres, (2·5) 3—4 (4·5) mm diam., luteovirides vel viriduli; urceoli obconico-campanulati, glaberrimi; sepala ovato-triangularia acuta, urceolo subaequilonga vel paulo breviora vel pro parte paulo longiora, post anthesin subpatentia et stylum brevem ostendentia, haud raro (30% florum examin.) singula usque omnia 4 in eodem flore apice 1—2- dentata, glaberrima, raro nonnulla setula brevissima terminata; episepala ovato-lanceolata acuta, sepalis breviora vel (rarius) subaequilonga, interdum (9% florum examin.) singula vel bina denticulo (rarissime 2 denticulis) praedita. Nux urceolo paulum excedens (Fig. 7).

Hab. in Carpatis Occidentalibus in pratis humidis prope fontes (ass. *Valerianeto-Caricetum flavae*) nec non in rupibus calcareis subhumidis a regione Fagi usque ad reg. alpinam. — Tatri Occidentales: Kominy Tylkowe 1600—1829 m, 30. 7. 1922, lg. B. P.,

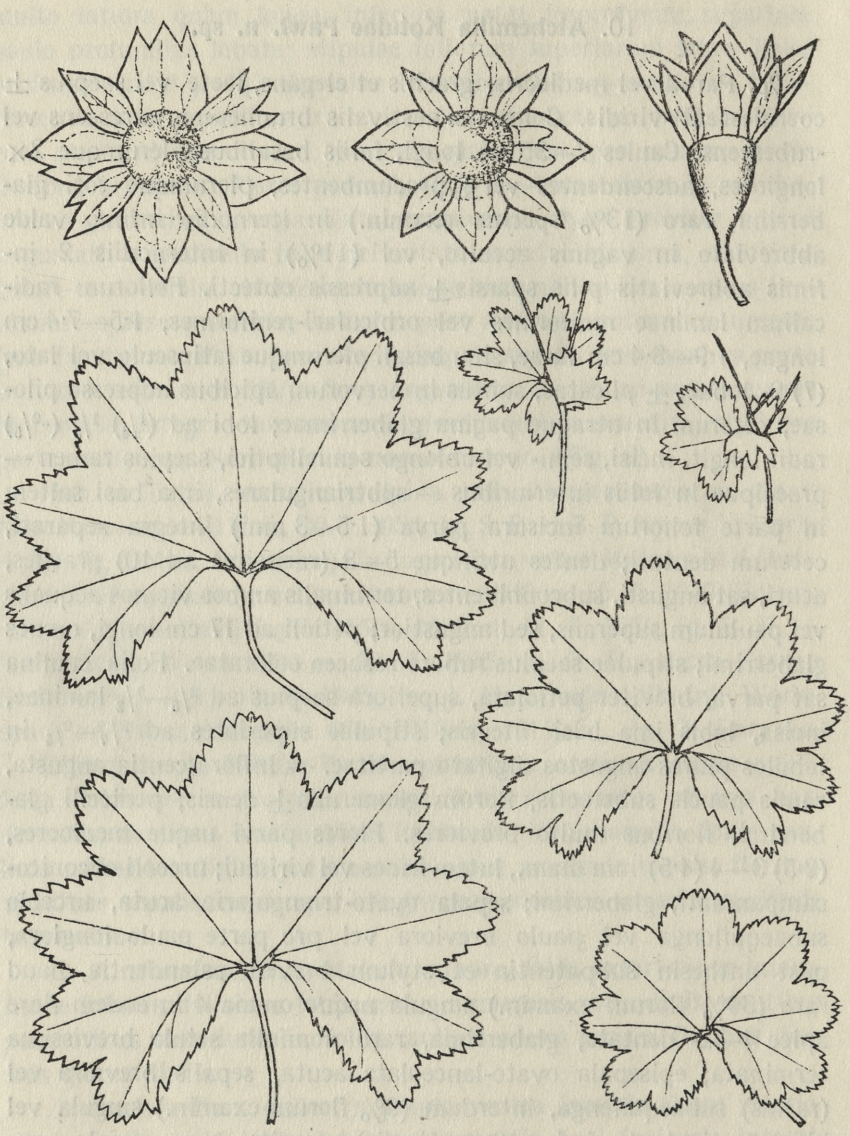

Fig. 7. *Alchemilla Kotulae* Pawł. Flores: $^{8}/_{1}$; folia 4 radicalia et 2 caulina: $^{1}/_{1}$.

16. 9. 1949, lg. S. P. et B. P.; — Kira Miętusia 940 m, 15. 7. 51, lg. S. P. et B. P.; — Gładkie Upłaziańskie 1570—1600 m, 30. 7. 51 (iid.); — Vallis «Dolina Mułowa» 1800 m, 30. 7. 51 (iid.); — Mało-

łączniak 1680—1720 m, 15. 8. 49 et 23. 7. 51 (iid.); — Giewont 19. 8. 1875, lg. W. Kulczyński; — Mały Giewont-Żleb Kirkora, 1450—1550, 31. 7. 52, lg. B. P.; — Vallis «Dolina Strążyska», 950 m, 9. 7. 52, lg. B. P.; — Mała Dolinka ad pedem m. Giewont 15. 7. 22, lg. B. P. — Regio Subtatrica: Kościelisko-Nędzówka 940 m, 17. 9. 49, lg. S. P. et B. P.; — Weszkówka 930 m, 8. 7. 51 (iid.); — Bukowina «Do Odewsia» 920 m, 10. 7. 51 (iid.); — Cyrhla ad Białką 1000—1020 m, 20. 7. 51 (iid.). — Montes Pieniny: infra «Stolarzysko» 500—550 m, 8. 6. 51, lg. B. P.; — infra Sokolica 630 m, 25. 6. 52 lg. B. P. — Montes Gorce, lg. Kor.: inter Mulowa et Obidowa 980 m, 8. 7. 49; — Obidowiec 995 m, 27. 5. 50; — Bukowina 980 m, 18. 7. 50; — Bukowina — Hala Terepakowa, 8. 7. 48; — Furcówka 835 m, 19. 7. 1951, lg. Kor., S. P. et B. P.; — infra Turbacz, 14. 6. 31, lg. A. Środoń. — Babia Góra: in lat. sept.-occ., 1300 m, 1. 7. 37, lg. B. P. — Pilsko, sub cacum., 19. 7. 38 et in turfosis «Cebula» 1330 m, 30. 7. 38, lg. Seńkowski. — Specim. exam.: 127.

A. Kotulae, in memoriam Prof. dris Boleslai Kotulae († 1898), florae Tatrorum investigatoris celeberrimi, denominata, notis nonullis: urceolis pro parte sepalis brevioribus, episepalis — quamquam pro minore parte — sepala subaequantibus, nec non nuce urceolo ± excedente seriem *Calicinae* in mentem revocat. Attamen floribus numerosis perscrutatis persuasum habeo, plantam nostram non ad illam seriem, sed ad seriem *Glabrae* pertinere.

A. Kotulae differt:

ab *A. longiuscula* Bus.: caule humili, internodiis infimis non elongatis, foliis magis reniformibus, lobis saepe subtriangularibus, dentibus parvis gracilibus, inflorescentia densa, floribus parvis, stylo prominulo. — Secundum Buser (1) et Schinz et Keller (19) *A. longiuscula* lobis circumcirca dentalis gaudet; specimen in Magnier, Flora exs. selecta 3413. editum incisuras integras inter lobos ostendit, sed lateribus loborum convexo arcuatis a nostra specie distat

ab *A. pseudincisa* Pawł. (v. infra) coloratione aestivali laetiore, lobis minus profunde incisis saepius subtriangularibus, incisuris integris inter lobos nonnisi in parte foliorum obviis et multo minus manifestis, dentibus subconniventibus, stipulis non coloratis,

inflorescentia angustiore, floribus subminoribus luteoviridibus, sepalis haud raro dentatis, nuce excedente.

ab *A. demissa* Bus.: colore, loborum forma, dentibus gracilibus, inflorescentia densa, pedicellis brevibus, floribus parvis.

11. **Alchemilla pseudincisa Pawł. n. sp.**

♃. Parva vel mediocris, saturate vel paululum coelurescenti-viridis, saepius caespites formans, propter rhizoma valde ramosum rosulas plures emittens. Coloratio aestivalis plerumque in forma macularum brunnearum vel atroviolacearum. Caules tenues, 4—20 cm longi, flexuosi, inferne ± procumbentes, superne arcuato-adscendentes, glaberrimi, rarissine (1·5% specim. examin.) in eadem planta omnes vel, pro parte in internodio infimo parce subadpresso pilosi. Foliorum radicalium laminae parvae vel mediocres, 0·8—5·1 × 1·1—7 cm, orbiculari-reniformes vel ± reniformes, sinu basali sat angusto usque sat lato, (5-) 7- (incomplete 9-) lobae, in utraque pagina glabrae, solummodo nervi subtus in dimidio superiore sparse vel sparsissime subadpresso pilosi; lobi ad $^1/_2$—$^2/_5$ radii longit. incisi, semielliptici vel oblongo semielliptici, in parte inferiore 2—5 mm longa lateribus integri et huc saepius cuneato-subangustati, supra dentati; dentes in utroque latere 5—7 (8) sat inaequales, apicem versus accrescentes, acuti vel acutiusculi, parvi graciles ± conniventes, vel — praecipue in foliis interioribus sat grossi porrecti, ad 3 mm longi, 1·5—2 mm lati, apice non penicillati; apicalis vicinis non conspicue minor; petioli ad 8 (13) cm longi, saepius omnes glaberrimi, rarius (22% specim. exam.) 1—2 ad 4, posteriores sparse subadpresso pilosi; stipulae angustae, cito brunnescentes. Folia caulina parva et pauca, media et superiora plerumque profunde (ad $^1/_2$ vel plus) partita; stipulae eorum haud magnae, superiores inaequaliter haud ultra $^1/_2$ longitudinis in lacinias acutas partitae. — Inflorescentia haud magna sed superne pro rata parte satis dilatata, ramis erecto patentibus; glomeruli densi, saepius subumbellati. Flores glaberrimi, virescentes, mediocres usque maiusculi, 3—5 mm diam.; urceoli campanulati; sepala ovato-triangularia acuta, post anthesin ± suberecta, urceolis subaequilonga; episepala eis breviora vel multo breviora et angustiora, lanceolata, acuta; discus conspicuus, saepius purpureo coloratus. Nux urceolo inclusa. (Fig. 8)

Hab. in reg. Mughi et alpina Tatrorum in lapidosis et rupestribus subhumidis, in margine *Mugheti* etc.: Gładkie Upłaziańskie 1550—1750 m, 12. 7. 1923, et 9. 8, 52 lg. B. P. et 30. 7. 50, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae); — Ciemniak 2089 m, 4.8.52 (iid.); — Dolina Mułowa 1820 m (iid.); — in iugo inter valles:

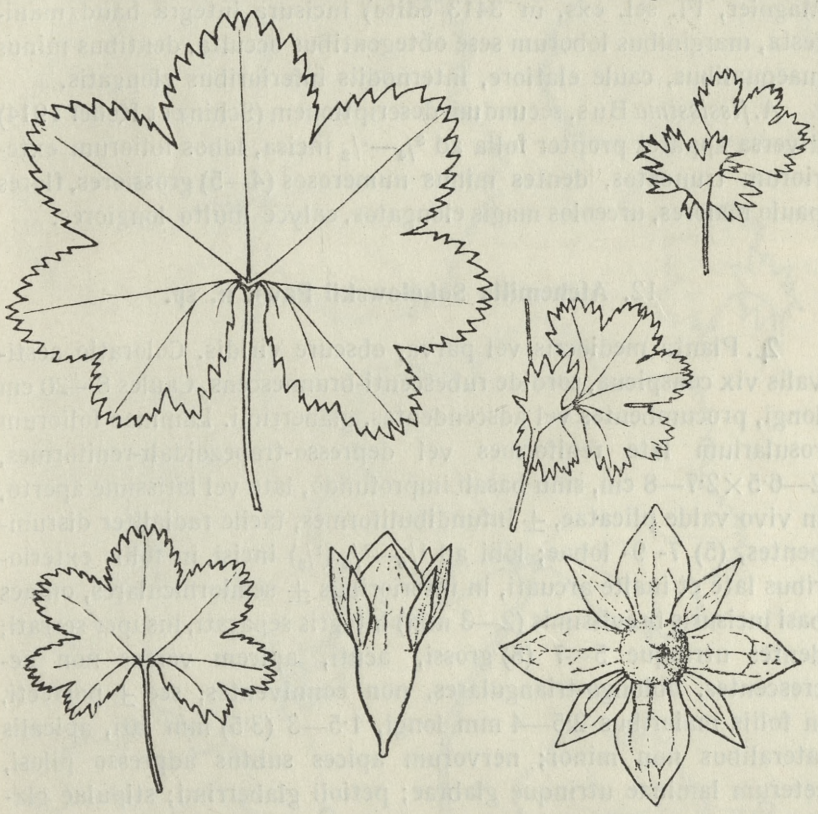

Fig. 8. *Alchemilla pseudincisa* Pawł. Flores: $^{8}/_{1}$; folia 2 radicalia et 2 (minora) caulina: $^{1}/_{1}$.

Litworowa et Mułowa 1870 m, 20. 7. 50 (iid.); — Małołączniak 1600 m, 15. 8. 49 (iid.); — Dolina Małej Łąki 1720 m, 20. 7. 50 (iid.).

Planta foliis profunde incisis et incisura integra inter lobos manifestissima ab omnibus speciebus tatricis subseriei *Glabrae* diversa, habitu et foliorum figura *A. incisae* Bus. simillima, differt tamen ab ea caulis et petiolorum glabritie, foliis pro parte grossius dentatis, coloratione aestivali magis sordida, praecipue autem flo-

ribus qui sunt typici flores subsectionis *Heliodrosium* et non subsectionis *Calycanthum*.

E speciebus alpigenis *A. longiuscula* Bus. differt a nostra foliis in sicco magis plicatis, lobis aut (sec. descriptiones — Buser 1895, Schinz et Kell. 1914) circumcirca dentatis, aut (in specim. in Ch. Magnier, Fl. sel. exs. nr 3413 edito) incisura integra haud manifesta, marginibus loborum sese obtegentibus occulta, dentibus minus inaequalibus, caule elatiore, internodiis inferioribus elongatis.

A. fississima Bus. secundum descriptionem (Schinz et Keller 1914) diversa apparet propter folia ad $^3/_4$—$^1/_2$ incisa, lobos foliorum exteriorum truncatos, dentes minus numerosos (4—5) grossiores, flores paulo maiores, urceolos magis elongatos, calyce multo longiores.

12. **Alchemilla Sokołowskii Pawł. n. sp.**

♃. Planta mediocris vel parva, obscure viridis. Coloratio aestivalis vix conspicua, sordide rubescenti-brunnescens. Caules 8—20 cm longi, procumbentes vel adscendentes, glaberrimi. Laminae foliorum rosularium late reniformes vel depresso-trapezoidali-reniformes, 2—6·5×2·7—8 cm, sinu basali improfundo, late vel latissime aperto, in vivo valde plicatae, ± infundibuliformes, facile radialiter disrumpentes, (5) 7- 9- lobae; lobi ad $^1/_6$—$^1/_4$ ($^1/_3$) incisi, in foliis exterioribus late et inalte arcuati, in interioribus ± semiorbiculares, omnes basi incisuris brevissimis (2—3 mm) integris separati, insuper serrati; dentes utrinque 5—7 (8) grossi, acuti, apicem versus non accrescentes, oblongo-triangulares, non conniventes, sed ± porrecti, in foliis maioribus 2·5—4 mm longi, 1·5—3 (3·5) mm lati, apicalis lateralibus non minor; nervorum apices subtus adpresso pilosi, ceterum laminae utrinque glabrae; petioli glaberrimi; stipulae glabrae, haud magnae, apice saepius leviter rubescentes. Folia caulina mediocria, inferiora basi ± truncata vel sinu basali latissime aperto, ad $^1/_4$—$^1/_3$ incisa, grosse acute serrata; superiora basi truncata vel latissime cuneata, suprema ad $^1/_2$ et ultra incisa, lobi latere basi integri; stipulae haud magnae, iam mediae sat profunde incisae, superiores in lobos inaequales oblongos acutissimos ad $^1/_2$ et ultra partitae. — Inflorescentiae rami breves, sat divaricati. Glomeruli laxiusculi; pedicelli glaberrimi, inferiores flores saepius aequantes vel longiores. Flores sat parvi, virides, glaberrimi, 3—4 mm diam.; urceoli 1·2—1·7 mm longi, basin versus satis subito angustati, se-

palis longiores; sepala latiuscula, acuta vel obtusiuscula, post anthesin oblique erecta; episepala eis pro parte maiore breviora, rarius

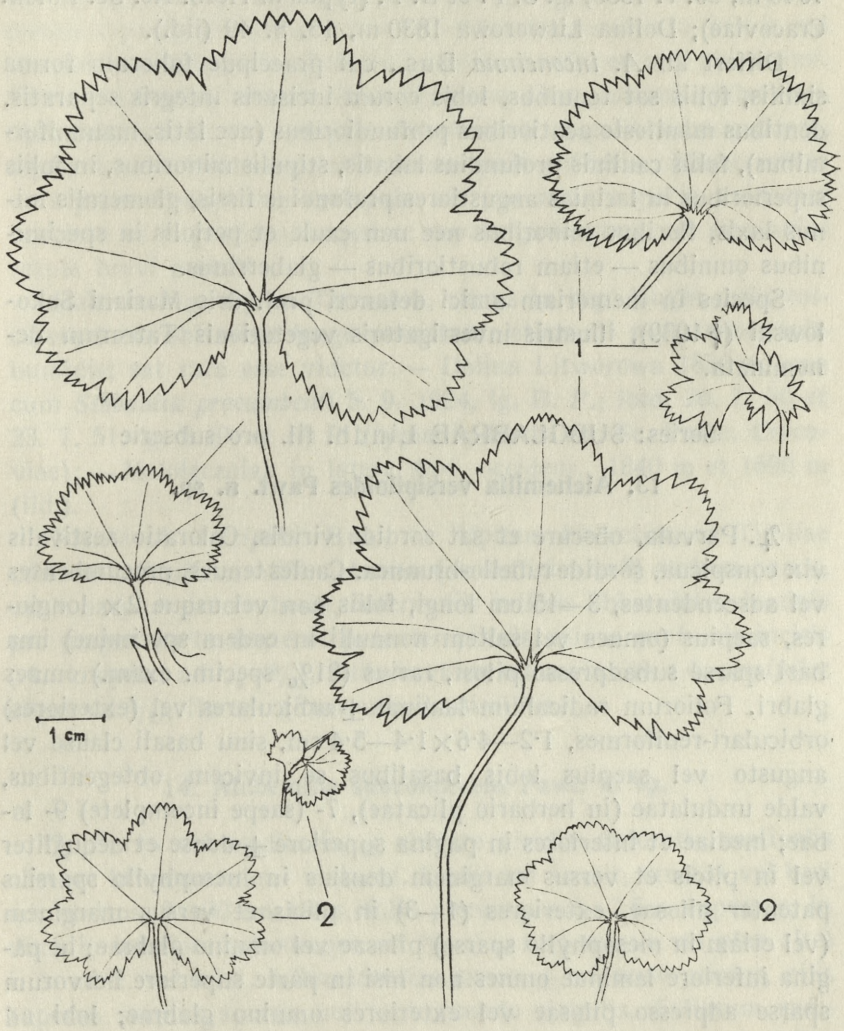

Fig. 9. 1) *Alchemilla Sokołowskii* Pawł. — folia 3 radicalia et 2 (minora) caulina. — 2) *Alchemilla Żmudae*: folia 2 radicalia et 1 (parvum) caulinum. — Omnia: $^1/_1$.

subaequilonga, multo angustiora; styli obsoleti; discus saepe rubellus. (Fig. 9,1)

Hab. in locis humidis lapidosisque, ubi nives longe non liquescunt, in parte calcarea Tatrorum Occidentalium: Dolina Małej Łąki 1540 m, 20. 7. 1950, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae); Dolina Litworowa 1830 m, 15. 8. 49 (iid.).

Differt ab *A. inconcinna* Bus., cui praecipue foliorum forma similis, foliis sat tenuibus, lobis eorum incisuris integris separatis, dentibus manifeste acutioribus profundioribus (nec latis, mammiformibus), foliis caulinis profundius lobatis, stipulis minoribus, in foliis superioribus in lacinias angustiores profundius fissis, glomerulis minus laxis, floribus minoribus nec non caule et petiolis in speciminibus omnibus — etiam robustioribus — glaberrimis.

Species in memoriam amici defuncti prof. dris Mariani Sokołowski († 1939), illustris investigatoris vegetationis Tatrorum denominata.

Series: SUBGLABRAE Lindb. fil. pro subserie

13. **Alchemilla versipiloides Pawł. n. sp.**

♃. Parvula, obscure et sat sordide viridis. Coloratio aestivalis vix conspicua, sordide rubello-brunnea. Caules tenues, procumbentes vel adscendentes, 3—15 cm longi, foliis non vel usque 2× longiores, saepius (omnes vel saltem nonnulli in eodem specimine) ima basi sparse subadpresso pilosi, rarius (21% specim. exam.) omnes glabri. Foliorum radicalium laminae ± orbiculares vel (exteriores) orbiculari-reniformes, 1·2—4·6×1·4—5·4 cm, sinu basali clauso vel angusto vel saepius lobis basalibus se invicem obtegentibus, valde undulatae (in herbario plicatae), 7- (saepe incomplete) 9- lobae; mediae et interiores in pagina superiore ± dense et aequaliter vel in plicis et versus marginem densius in mesophyllo sparsius patenter pilosae, exteriores (1—3) in plicis et versus marginem (vel etiam in mesophyllo sparse) pilosae vel omnino glabrae; in pagina inferiore laminae omnes non nisi in parte superiore nervorum sparse adpresso pilosae vel exteriores omnino glabrae; lobi ad $^1/_4$—$^1/_3$ radii incisi, semiorbiculares vel late semielliptici, marginibus sese (in herbario) ± supertegentes, ab ima basi dentati; dentes in utroque latere 5—7 parvuli vel mediocres, latiusculi, obtusi, apice minute penicillati, subaequales vel versus apicem paulo accrescentes, terminalis vicinis vix minor; petioli 2—9·5 mm longi, aut omnes glabri aut pro parte pilis erecto patentibus sparsissimis vel sat

sparsis toti vel saepius solum ima basi praediti; stipulae latiusculae, interdum vix conspicue rubore pallide violaceo coloratae, mox siccae et brunneae. — Folia caulina ad $^1/_3$—$^1/_4$ (rarius ad $^2/_5$ vel ad $^1/_5$) incisa, in pagina superiore ± disperse pilosa. Stipulae eorum improfunde dentatae. — Inflorescentia pauciflora, brevis sed pro portione latiuscula; glomeruli modice densi; pedicelli glaberrimi. Flores mediocres, viriduli, 3—4 (4·3) mm diam.; urceoli glaberrimi, obconici, sub fructu breviter campanulati, sepalis subaequilongi; sepala late ovato-triangularia, acuta, post anthesin oblique erecta et stylum obtegentia; episepala eis pro maiore parte evidenter breviora et angustiora, aeque ac sepala glaberrima vel raro apice setula brevi praedita.

Hab. in reg. alpina Tatrorum, rarius in reg. Mughi, solo calcareo, locis ubi nives longe persistunt, etiam in rupestribus subhumidis; sat rara esse videtur. — Dolina Litworowa 1830 m, una cum *Sibbaldia procumbenti* 5. 9. 1924, lg. B. P.; ibid. 20. 7. 50 et 23. 7. 51, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae); — Małołączniak in latere sept. occident., 1840 m et 1690 m (iid.).

Planta *A. versipilae* Bus., in Alpibus Helvetiae et Tiroliae crescenti, simillima, differt tamen (an sufficienter?) statura parva, caule saepius procumbenti, internodiis infimis abbreviatis, laminis pro magna parte dense et patenter pilosis, minus profunde incisis (*A. versipila*: ad $^1/_4$—$^2/_5$), foliis caulinis minus incisis (*A. versipila*: usque ad $^1/_2$), florum glomerulis densioribus.

14. **Alchemilla subconnivens Pawł. n. sp.**

♃. Parva vel mediocris, ± obscure viridis. Coloratio aestivalis obscure brunneo-rubro- violacea. Caulis sat tenuis, erectus vel basi adscendens, 8—30 cm altus, folia plerumque 2—2·5× superans, in dimidio inferiore (plerumque ad $^2/_5$—$^3/_5$, raro minus vel plus alte) i. e. ad 2. vel ad 1. inflorescentiae rarum ± adpresso pilosus, pilis superne saepius sparsis vel tantummodo singulis. Foliorum radicalium laminae orbiculari-reniformes usque suborbiculares, 0·9—6·3×1·1—7·3 mm, sinu basali angusto usque sat lato, rarius sinu clauso, rarissime lobis basalibus sese paululum supertegentibus, ± undulatae, in sicco saepe plicatae, 9- vel incomplete 9- (rarissime incomplete 11-) lobae, in pagina superiore aut pro parte (rarius omnes)

in plicis, raro praeterea in zona angusta marginali adpresso pilosae, aut omnes glabrae; pagina inferior foliorum exteriorum in nervis et in lobis basalibus (raro versus marginem quoque) adpresso pilosa, in foliis interioribus plerumque solummodo in superiore parte nervorum (ad $^1/_3$—$^1/_2$ longitudinis) ± sericeo pilosa; lobi ad ($^2/_3$) $^1/_4$—$^1/_3$ ($^3/_8$) radii longit. incisi, arcuati, semiorbiculares, semielliptici vel — in foliis interioribus saepius — triangulares vel late triangulares, basi semper incisuris integris 1·5—3·5 mm longis angustissimis separati, superne dentati; dentes in utroque latere (5) 6—8 (rarissime ad 10) minuti et graciles acutique, plerumque manifeste conniventes et apice penicillati subaequales vel versus apicem loborum paululum accrescentes, terminalis vicinis non minor; petioli ad 13 cm longi, omnes semper ± dense ± adpresso sericeo pilosi; stipulae pallidae cito brunnescentes. Folia caulina parva, media et superiora saepius ad ± $^2/_5$ (interdum usque ad $^1/_2$) incisa, lobis subdivergentibus basi integris; stipulae superiores saepius sat profunde (ad $^1/_2$ et ultra) irregulariter incisae laciniis angustis acutis. — Inflorescentia haud multiflora sat divaricata, saepe iam prope caulis basin incipiens; glomeruli laxissimi; pedicelli ± elongati glaberrimi. Flores viriduli, interdum post anthesin intus rubelli, mediocres, (2·5) 3·5—4 (4·5) mm diam.; urceoli 1·4—1·8 mm longi, obconico-campanulati, glaberrimi, sepalis subaequilongi; sepala late ovato-triangularia, acuta, episepalis longiora et multo latiora, aeque atque ea glabra, rarius nonnulla apice setulosa, post anthesin erecta.

Hab. in Tatris in rupestribus et in graminosis apertis in regione Mughi: Ciemniak, 2020—2089 m, 4 et 24. 8. 52, lg. S. P. et B. P.; — Małołączniak versus Wielka Świstówka 1400—1720 m, 16. 7. 50 et 23. 7. 51, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae); — Dolina Małej Łąki 1350 m, 11. 7. 50, lg. B. P. — Specim. exam.: 50.

Planta nostra carpatica *A. conniventi* Bus. alpigenae habitu et foliorum figura simillima est. Attamen folia *A. conniventis* secundum descriptionem et secundum specimina pauca a me visa in pagina superiore semper in plicis (saepe abundanter) nec non in zona marginali (saepe densiuscule) sericeo pilosa sunt, pili in caule et in petiolis minus adpressi, folia caulina saepius profundius (ad $^2/_3$) incisa, lobi eorum magis divaricati, inflorescentia paulo magis congesta. Itaque res meliore loco erit, si planta tatrica ut species nova describitur quam si cum *A. conniventi* connectitur.

A. minutidens Bus. (2), plantae nostrae — secundum descriptionem — simillima, ab ea tamen differre videtur colore coeruleo-viridi, coloratione aestivali vini rubore, foliis suborbicularibus planis 9—11- lobis, dentibus magis numerosis (7—10 in utroque loborum latere) ± porrectis nec conniventibus, probabiliter etiam inflorescentia, quae ut fissiformis, ceterum autem cum *A. conniventi* Bus. congrua ab auctore describitur.

A. Wichurae (Bus.) S. Stéfans., pilositate caulis foliorumque cum planta nostra congrua, differt tamen bene — secundum specimina scandinavica a me visa — colore dilutius viridi, coloratione aestivali laete violascenti-rubella, foliis radicalibus magis rotundatis, sinu plerumque angusto vel clauso et lobis basalibus saepe sese supertegentibus, foliorum caulinorum stipulis magis regulariter incisis, floribus densius glomerulatis.

15. **Alchemilla Wallischii Pawł. n. sp.**

Syn.: *A. alpestris* ssp. *montana* Pawł. olim (1928)

♃. Planta parva gracilis, sordide viridis; color aestivalis sordide rubro- vel violaceo-brunneus. Caules 7—12 cm longi ± prostrati et arcuato adscendentes, foliis radicalibus paulo usque $1^1/_2\times$ longiores, a basi ad 1. vel 2. rarum i. e. ± ad $^1/_2$ longitudinis suae parce subadpresso pilosi. Foliorum rosularium laminae reniformes, 2—4× ×3—5 cm, sinu basali late aperto saepe subrectangulari, imperfecte 9- lobae, plicatae, supra obscure sordide virides, in plicis et in zona marginali angusta vel lata (raro in mesophyllo quoque) sparse usque modice adpresso pilosae, ceterum glabrae, subtus pallidiores, in nervis principalibus (secundum totam eorum longitudinem vel parte basali excepta) interdum etiam in lobis basalibus modice adpresso pilosae ceterum glabrae; lobi ad $\pm\,^1/_3$ laminae incisi, incomplete suborbiculares vel incomplete late elliptici, basin versus contracti, ima basi incisura integra vix 2 mm longa separati, quae tamen propter loborum margines se invicem hoc loco (in statu sicco) supertegentes obsoleta apparet, insuper utrinque dentibus (4) 5—6 parvulis sed (in foliis interioribus) latiusculis et ± obtusis, paululum penicillatis, subconniventibus, subaequalibus (vel apicem versus paulum tantum accrescentibus) instructi, dente terminali paulo tantum minore; petioli 2—6 cm longi, omnes pilis subadpressis pro maxima parte sat densis obtecti. Folia caulina sat parva,

breviter petiolata, ± ad 1/3 incisa, superiora basi ± cuneata. Stipulae superiores nec non supremae (bracteiformes) obtuso paucilobae. — Inflorescentia sat parva, laxa, ramis suberectis vel erecto-subpatulis. Flores flavovirides, intus saepius purpurascentes, 3—4 mm diam., deflorati diu aperti et stylum prominulum ostendentes; urceoli elegantissimi, evidenter longiores quam crassi, 1·5—1·8 mm longi, basi sensim in pedicellum angustati et etiam sub calyce ± constricti, glaberrimi, sepalis longiores; sepala ± acuta vel obtusiuscula, in floribus infimis apice setulis paucis terminata, cetera glabra, episepalis — sape multo — longiora. Pedicelli glabri vel — in speciminibus duobus — perpauci infimi sparse pilosi, inferiores floribus aequilongi vel longiores. (Fig. 10,4)

Hab.: Tatri Alti, ad lacum Morskie Oko, alt. 1400—1500 m, in lapidosis subhumidis graniticis, 14. 8. 1927, lg. B. P.; typus in Herb. Ac. Sc. Pol. Cracoviae; ibid. 19.8. 51, lg. Z. Paryska. — Specim. exam. 7. flor. + 3 ster.

Planta in memoriam amici defuncti dris Caroli Wallisch († 1934) nominata. Ob foliorum lobos pauce et obtuse dentatos, basi ± constrictos, in pagina superiore ± pilosos nec non ob urceolorum formam valde insignis, cum nulla alia *Alchemilla* in Alpibus et in Carpatis obvia confundenda.

16. **Alchemilla czywczynensis Pawł. n. sp.**

♃. Planta sat parva, sordide vel obscure viridis. Caulis arcuato-adscendens, tenuis, folia radicalia paulo usque 2·5× superans, a basi plerumque usque ad 2. ramum i. e. ± ad 1/2 altitudinis suae densiuscule subadpresso pilosus. Foliorum rosularium laminae reniformes, ad 4·5×6·5 cm, sinu basali late aperto, 7- vel incomplete 9- lobae, plicatae, in pagina superiore in plicis et in zona marginali lata (rarius etiam inter plicas) adpresso pilosae, subtus in tota longitudine nervorum principalium adpresso sericeo pilosae, praeterea saepius in lobis basalibus nec non versus marginem et hinc inde etiam inter nervos pilosae; lobi ad 1/4—1/3 laminae incisi, late arcuati, semielliptici usque late subtriangulares, semper tamen ima basi latissimi et toto margine dentati, dentibus versus apicem lobi manifeste (bis vel ter) accrescentibus in utroque latere numero 5—9, acutiusculis vel acutis, superioribus latiusculis, terminali paulo minore; petioli 2·5—8 cm longi, omnes ± dense subadpresso pilosi.

Folia caulina parva, inferiora paulo tantum, suprema tamen usque ad $^2/_5$—$^1/_2$ incisa; stipulae eorum parvulae, inprofunde denticulatae, dentibus sat parvis obtusiusculis. — Inflorescentia angusta, saepe iam ima caulis basi incipiens; glomeruli sat pauci, densi; pedicelli glabri, pro maxima parte floribus breviores. Flores parvi, subflavido-virides, 3—4 mm diam.; urceoli campanulati, 1·5—1·8 mm longi, glaberrimi, sepalis paulo longiores vel subaequilongi; sepala oblongo-ovato-triangularia, acutiuscula, una cum episepalis glaberrima vel nonnulla apice paucisetulosa, post anthesin erecto-patula; episepala plerumque multo breviora et multo angustiora; styli haud prominuli.

Hab.: Carpati Orientales, Montes Czywczynenses (pron.: Tchyftchynenses); in graminosis subalpinis: in cacum. Czywczyn 1580 m, 10. 8. 1887, lg. E. Wołoszczak (typus in Herb. Ac. Sc. Polon. Cracoviae); — Lozdun, ca. 1550 m, 21. 7. 1933, lg. B. P.

A. appressipila Juzep. (Sibiria Or. et Mongolia) et *A. subrectipila* Juzep. (Caucasus) differunt a planta nostra colore laete vel dilute viridi, foliis caulinis bene evolutis, floribus laxe glomerulatis. *A. appressipila* differt praeterea foliis plurilobis, in tota pagina superiore densiuscule pilosis, dentibus subaequalibus, floribus minoribus; *A. subrectipila* statura maiore, foliis planis, dentibus magis numerosis, floribus virescentibus. — *A. psilocaula* Juzep. (Caucasus) differt caule solummodo in internodio 1. piloso, stipulis superioribus grandidentatis; inflorescentia sat lata et multiflora, floribus laxe glomerulatis. — *A. flavescens* Bus. (Sibiria Or., Mongolia) differt caule saepius solummodo in internodiis 1—2 pilosis, foliis plurilobis, dentibus foliorum interiorum obtusis fere mammiformibus, foliis caulinis bene evolutis, urceolis elongato-obconicis, sepalis obtusiusculis (v. Juzepczuk 7).

17. **Alchemilla turkulensis Pawł. n. sp.**

2_4. Specimina mediocria, obscure coerulescenti-viridia, serius sordide subflavescentia. Caules graciles, adscendentes, in tota fere longitudine (suprema parte $^1/_{10}$ excepta) sat dense subadpresso pilosi. Foliorum rosularium laminae orbiculari-reniformes, 2·5—8×3—9 cm, sinu basali ima basi angusto, versus ambitum folii latius aperto, 9- 11- lobae, subplicatae, in pagina superiore obscurae, glaberrimae vel nonnullae in plicis pilis perpaucis praeditae, subtus pallidiores,

in tota longitudine nervorum principalium, plerumque etiam in lobis basilaribus dense adpresso sericeo pilosae, ceterum glabrae vel pro parte inter nervos pilis perpaucis ornatae; lobi ad $^1/_3$—$^2/_5$ incisi, semielliptici usque (in foliis interioribus) elongato semiellipticotriangulares, utrinque dentibus 6—9 subaequalibus (terminali paulo minore), in foliis exterioribus angustis acutis, in interioribus sat grossis latiusculis acutiusculis praediti, ima basi incisura integra perbrevi (2—3 mm) separati; petioli omnes dense, sericeo subadpresso pilosi, ad 10 cm longi; stipulae non coloratae. Folia caulina bene evoluta, inferiora ad $^1/_3$, superiora ad $^2/_5$—$^2/_3$ incisa; stipulae superiores ad $^2/_5$—$^1/_2$ in lacinias valde inaequales latiusculas acutiusculas partitae. — Glomeruli florum sat densi. Pedicelli glaberrimi floribus ± breviores. Flores sordide virides, 3·5—4·5 mm diam.; urceoli glabri vel pro parte pilis perpaucis praediti, sepalis florendi tempore saepe breviores; sepala ovato-oblonga, acutiuscula, glabra vel in floribus inferioribus setula terminata; episepala eis ± aequilonga vel paulo breviora, conspicue angustiora. (Fig. 10, 3.)

Hab. in Carpatis Orientalibus, in montibus Czarnohora: infra cacumen Turkuł VII. 1908, leg. Exc. Inst. Biol.-Bot. Leopoliensis; speciei typus in Herb. Univ. Jagell. Cracov.; Pożyżewska, 18. 7. 1928, lg. J. Madalski.

A. georgica Juzep. (Caucasus), quem non vidi, differt — e descriptione — a planta nostra colore laete viridi, foliis minoribus reniformibus, lobis semiellipticis usque late triangularibus (itaque ut videtur, brevioribus et latioribus quam in *A. turkulensi*), dentibus utrinque solummodo 5—7, pagina foliorum superiore in plicis et versus marginem (rarissime in tota superficie) disperse pilosa.

A. Murbeckiana Bus., quae plantae nostrae primo aspectu valde similis apparet, differt tamen ab ea manifestissime caule non nisi ad $^1/_2$ ad summum ad $^3/_4$ longitudinis piloso, praeterea etiam dentibus acutioribus, floribus maioribus, urceolis et sepalis semper glabris.

A. glomerulans Bus. praeter alias notas optime distinguitur foliorum facie superiore pilosa nec non loborum figura.

Possibile mihi quoque videtur plantam meam non ad seriem *Subglabrae* sed ad seriem *Flatae* subsectionis *Calycanthum* pertinere. Quod tamen diiudicare nequeo, quia nonnisi 3 specimina vidi — omnia in statu florenti urceolis igitur nondum maturis.

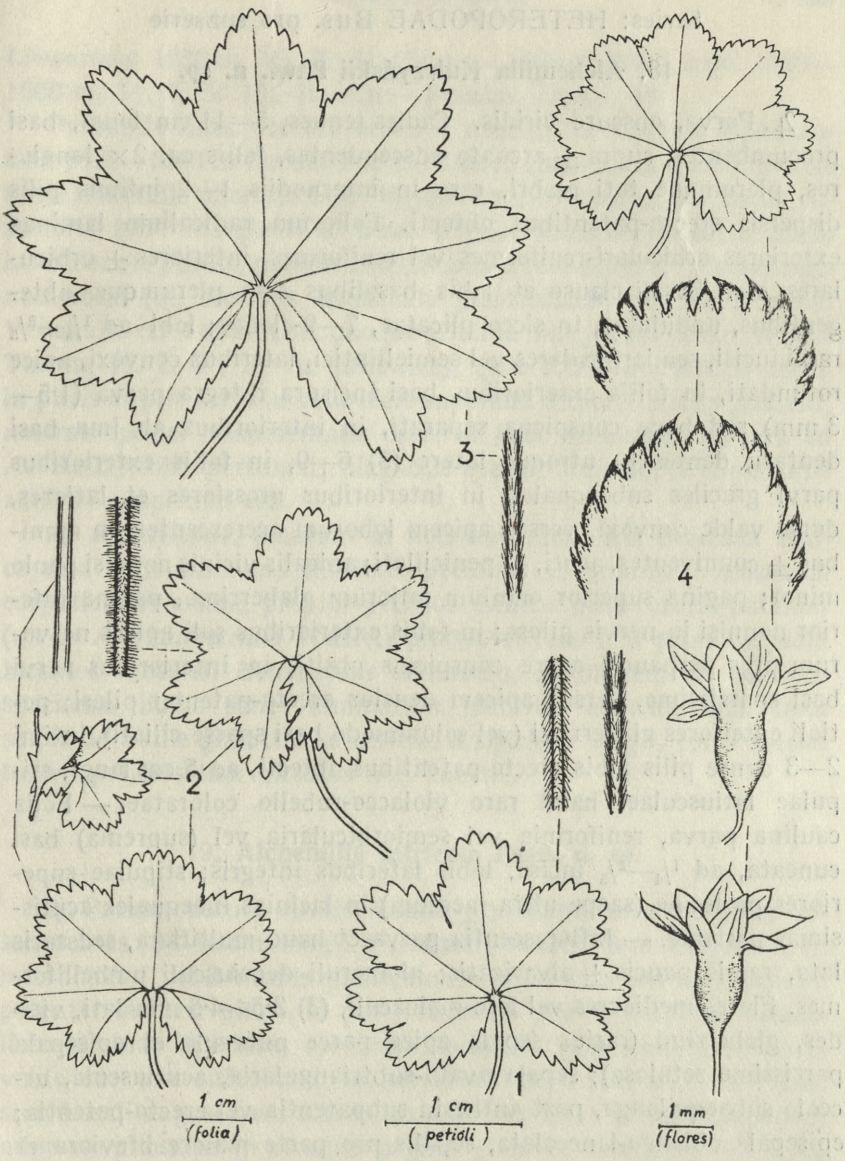

Fig. 10. 1) *Alchemilla erythropodoides* Pawł. — folium radicale et petiolus retripilosus. — 2) *Alchemilla tatricola* Pawł. — folium radicale exterius et petiolus eius glaber; fol. radic. interius et petiolus pilosus; fol. caulinum. — 3) *Alchemilla turkulensis* Pawł. — folium radicale et petiolus subadpresso pilosus. — 4) *Alchemilla Wallischii* Pawł. — folium radicale, lobi duo, petiolus subadpressopilosus; flores.

Series: HETEROPODAE Bus. pro subserie

18. **Alchemilla Kulczyńskii Pawł. n. sp.**

♃. Parva, obscure viridis. Caules tenues, 5—11 cm longi, basi procumbentes, supra ± arcuato adscendentes, foliis ca. 2× longiores, plerumque toti glabri, raro in internodiis 1—2 infimis pilis dispersis erecto-patentibus obtecti. Foliorum radicalium laminae exteriores orbiculari-reniformes vel reniformes, interiores ± orbiculares, sinu basali clauso et lobis basalibus sese plerumque obtegentibus, undulatae, in sicco plicatae, 7—9- lobae; lobi ad $^1/_3$—$^2/_5$ radii incisi, semiorbiculares vel semielliptici, lateribus convexi, apice rotundati, in foliis exterioribus basi incisura integra parva (1·5—3 mm) sed bene conspicua separati, in interioribus ab ima basi dentati; dentes in utroque latere (5) 6—9, in foliis exterioribus parvi graciles subaequales, in interioribus grossiores et latiores, dorso valde convexi, versus apicem loborum accrescentes, in omnibus ± conniventes, acuti, ± penicillati; apicalis vicinis nonnisi paulo minor; pagina superior omnium foliorum glaberrima, pagina inferior nonnisi in nervis pilosa; in foliis exterioribus soli apices nervorum pilis perpaucis aegre conspicuis obsiti, in interioribus nervi basi sparsissime, versus apicem densius erecto-patenter pilosi; petioli exteriores glaberrimi (vel solummodo basi sparse ciliati), intimi 2—3 dense pilis albis erecto-patentibus obtecti, ad 5 cm longi; stipulae latiusculae, haud raro violaceo-rubello coloratae. — Folia caulina parva, reniformia vel semiorbicularia vel (suprema) basi cuneata, ad $^1/_4$—$^2/_5$ incisa, lobis lateribus integris; stipulae superiores profunde (saepe ultra medium) in lacinias inaequales acutissimas partitae. — Inflorescentia parva et haud multiflora, sed satis lata, ramis paucis ± divaricatis; glomeruli densiusculi umbelliformes. Flores mediocres vel fere maiusculi, (3) 3·5—4·5 mm lati, virides, glaberrimi (rarius sepala apice parce puberula et episepala parcissime setulosa); sepala ovato-subtriangularia, acutiuscula, urceolo subaequilonga, post anthesin subpatentia vel erecto-patentia; episepala oblongo-lanceolata, sepalis pro parte maiore breviora et 1·5—2× angustiora, acuta. (Fig. 11)

Hab. in Reg. Mughi et alpina montium Tatrorum in rupestribus et graminosis subhumidis: Dolina Mułowa versus Twardy Upłaz 1800 m, 30. 7. 1951, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae); — Gładkie Upłaziańskie 1570—1600 m (iid.); — Dolina

Litworowa 1830 m, 23. 7. 51 (iid.); — Dolina Małej Łąki 1550—1600 m, 11. 7. 50 (lg. B. P.). — Specim. exam.: 18.

Probabile mihi videtur etiam 1 specim. in Gładkie Upłaziańskie 1620—1640 m lectum huc pertinere, quamquam a descriptione notis nonnullis aberrat: foliis rigidioribus, interioribus in faciei superioris zona marginali disperse pilosis, subtus pro parte etiam inter nervos pilosis, petiolis interioribus erecto-patenter pilosis magis numerosis (6), floribus laxiuscule glomerulatis, ad 5 mm diam. Verisimile est 4 specimina quoque (Dolina Małej Łąki versus Wielka Turnia, 16. 7. 51, lg. A. Jasiewicz), quae foliis interioribus etiam in plicis et pro parte in tota facie superiore disperse pilosis gaudent, nostrae speciei adnumeranda esse. Si haec specimina re vera ad *A. Kulczyńskii* pertinent, diagnosis speciei notis supra enumeratis additis complenda est.

A. Kulczyńskii, plantam in honorem prof. dris Stanislai Kulczynski denominatam, pilositate peculiari ab omnibus *Alchemillis* carpaticis diversam, propter petiolos intimos dense erecto-patenter (modo *A. glaucescentis* Wallr.) pilosos et ideo iam primo aspectu hirsutos subseriei *Heteropodae* adnumero, quamquam ab omnibus speciebus huius seriei laminarum glabritie aberrat. Qua nota aeque ac caulis glabritie ad seriem. *Subglabrae* vergit, cuius species tamen petiolos tam hirsutos non habent.

19. **Alchemilla tatricola Pawł. n. sp.**

♃. Parva, obscure viridis. Caules ± procumbentes vel apice adscendentes, 3—14 cm longi, ± flexuosi, tenues, fere filiformes, in internodiis 1 vel 2 infimis glabri, supra usque ad 2. vel ad 1. ramum patenter molliter pilosi, in parte suprema glabri. Foliorum radicalium laminae in eodem specimine forma et praecipue pubescentia valde inter sese differentes: exteriores orbiculari-reniformes, 7- lobae, sinu basali angusto vel clauso, praeter nervos in dimidio superiore adpresso pilosulos in utraque pagina glabrae; interiores — tenues, orbiculares, usque ad 6 cm longae et latae, sinu basali clauso et lobis basalibus sese plerumque late obtegentibus, in vivo valde undulatae, in sicco valde plicatae, pro parte in facie superiore solummodo in zona marginali et in plicis, subtus in zona marginali et secus nervos, pro parte autem praeterea utrique in tota lamina quamquam sparsius et disperse ± patenter pilosae, 7- usque incom-

plete 9- lobae; lobi ad $^2/_5$ usque fere ad $^1/_2$ radii incisi, in foliis interioribus elongato semielliptici, lateribus convexi, apice ± rotundati, ab ima basi dentati, dentibus in utroque latere 5—7 (8) grossiusculis obtusis subaequalibus vel (saepius) 1—3 infimis conspicue minoribus, terminali non minore vel paulo minore; lobus medianus vel lobi 3 mediani saepe ceteris multo maiores; lobi foliorum exte-

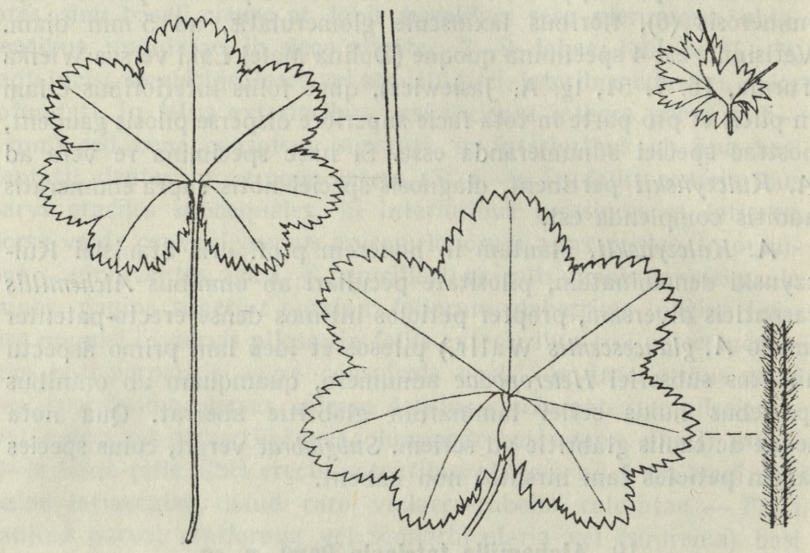

Fig. 11. *Alchemilla Kulczyńskii* Pawł. Folia radicalia: exterius (ad sinistram) $^1/_1$ (cum petiolo $^2/_1$) et interius (ad dextram) cum petiolo $^2/_1$ et folium caulinum (supra ad dextram). — Pili nonnisi in petiolorum parte ($^2/_1$) delineati.

riorum semiorbiculares vel late semielliptici, apice saepius ± truncati, basi interdum incisuris integris minutis separati, dentibus minoribus subaequalibus utrinque 4—6; petioli 1—3 exteriores glabri, posteriores dense molliter patenter pilosi, ad 7·5 cm longi; stipulae albidae vel pallide virides. — Folia caulina sat bene evoluta, ad $^1/_3$ usque (superiores) ultra $^1/_2$ incisa, obtuse dentata, in facie superiore plerumque ad summum in zona marginali, subtus praeterea in nervis et in lobis basalibus sparse pilosa; stipulae eorum amplae, haud profunde (usque ad $^1/_4$) incisae, dentibus obtusiusculis vel subacutis. — Inflorescentia sat depaupertata ramis brevibus sub angulo acuto vel acutissimo abeuntibus, omnibus vel infimo excepto

glabris; glomeruli densiusculi; pedicelli glaberrimi. Flores viriduli, mediocres, (3) 3·5—4 (4·5) mm lati; urceoli glaberrimi, breviter obconici, sub fructu campanulati, basi cito contracti; discus distincte flavus; sepala lata, triangularia, acuta, glabra, rarius apice setula praedita, urceolis breviora vel subaequilonga, post anthesin ± patentia; episepala eis plerumque conspicue minora et multo (ca. 2×) angustiora, acuta, glabra vel apice setula vel setulis paucis praedita. Styli haud prominuli. (Fig. 10,2)

Hab. in Reg. alpina nec non Mughi montium Tatrorum in rupestribus et graminosis subhumidis calcareis, praecipue ubi nives longe persistunt: Dolina Litworowa 1830 m, 20. 7. 1951, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae); — Dolina Mułowa 1800 m, 30. 7. 51 (iid.); — Gładkie Upłaziańskie 1540—1600 m, 30. 7. 51 (iid.); — Małołączniak 1720 m, 23. 7. 51 (iid.) ;— Dolina Małej Łąki 1540—1600 m, 11. et 20. 7. 50 (iid.). — Specim. exam.: 49.

A. undulata Bus., Alpium incola, quae similiter atque *A. tatricola* foliis interioribus orbicularibus valde undulatis, profunde — in specim. ab O. Buser lecto (Mt. Salève, Baenitz, Herb. Eur. nr 8294) usque ad $^4/_9$ — incisis lobisque basalibus sese obtegentibus gaudet, differt caulibus robustioribus, foliis perfecte 9- lobis, interioribus in utraque pagina densius et uniformiter pilosis, lobis p. p. subtriangularibus, foliis caulinis utrinque densius uniformiter pilosis, inflorescentia magis expansa, urceolis magis elongatis, sepalis post anthesin erectis.

A. diversipila Juzep. (e Caucaso) differt — secundum descriptionem — foliis perfecte 9-lobis evidenter minus profunde incisis, dentibus acutiusculis, foliis caulinis minus profunde incisis, floribus minoribus, urceolis anguste campanulatis.

20. **Alchemilla Szaferi Pawł. n. sp.**

♃. Planta parva vel mediocris, sordide viridis; coloratio aestivalis sero apparens, haud intensa, sordide rubroviolaceo-brunnea. Caules 5—22 cm longi, prostrati vel adscendentes, crassiusculi, foliis basalibus 1·5—3× longiores, in internodio 1. subglabri vel sparse adpresse pilosi, ceterum usque ad inflorescentiae ramos superiores densiuscule pilosi, pilis horizontaliter patulis vel — in parte inferiore — erecto-patulis. Foliorum rosularium 1—2 infimorum petioli subglabri (pilis nonnisi perpaucis praediti) vel interdum

omnino glabri; laminae eorum solummodo subtus in parte superiore nervorum principalium pilosae, ceterum glabrae, lobis humilibus, late arcuatis, vix ad $^1/_6$—$^1/_4$ radii laminae incisis. — Reliquorum foliorum rosularium petioli (ad 7 cm longi) densissime pilosi, pilis (praecipue in parte inferiore) erecto-patentibus; laminae reniformes vel orbiculari-reniformes, 2·5—5×3—6 cm, crassiusculae, undulatae, sinu basali plerumque late aperto, rarius angusto, 7—9-lobae, utrinque densiuscule (in pagina superiore subadpresse, in inferiore subpatule) pilosae; lobi $^1/_4$—$^1/_3$ radii laminae attingentes, semiorbiculares usque oblonge semielliptici, circumcirca dentati, dentibus obtusiusculis subaequalibus utrinque 4—6, dente terminali nonnisi paulo minore; stipulae brunneae vel ± rubellae. — Folia caulina parva, breviter petiolata; infimum solummodo subtus in nervis pilosum; reliqua ± pilosa, ad $^1/_4$ incisa. — Inflorescentia satis divaricata, ramis patenter pilosis, ramulis glabris; glomeruli densiusculi; pedicelli glabri, plerumque floribus breviores. Flores mediocres, 3—4·5 mm lati, flavido-virides; urceoli omnes glabri globoso-campanulati, 1·2—1·7 mm longi; sepala ovato-triangularia, acutiuscula vel obtusiuscula, urceolis subaequilonga, omnia glabra, post anthesin diu horizontaliter patentia; episepala eis semper evidenter breviora et multo angustiora.

Hab. in Carpatis Orientalibus in montibus Czarnohora, locis humidis secundum rivos in regione Mughi: Breskuł 1500—1600 m, 2. 9. 1925, lg. B. P. (speciei typus in Herb. Acad. Sc. Polon. Cracoviae); — Zaroślak, in convalle inferiore, 1. 9. 25, lg. B. P.; -infra cac. Turkuł, 8. 1908, lg. A. Żmuda (sub. nom.: «*A. heteropoda* var. *tenuis*»). — Specim. exam.: 11.

Planta in honorem magistri mei illustrissimi prof. dris Ladislai Szafer denominata.

A. compta Bus. differt e descriptione: caule folia vix superante, etiam supra erecto-patenter piloso, internodio basali et petiolis infimis glaberrimis, foliis tenuibus, 9- lobis, lobis profundius (usque ad ad $^2/_5$) incisis, utrinque 5—7 dentatis, petiolis interioribus adpresso pilosis, stipulis non rubentibus, inflorescentia angusta, floribus minoribus, inferioribus ± pilosis.

A. fontinalis Juzep., species pamiro-alaica, differt a planta nostra caulibus ± erectis, pilis in caule etiam superne erecto- patentibus, foliis planis, lobis pro parte fere breviter triangularibus, dentibus utrinque 5—7 paulo accrescentibus, pilis in petiolis pro

parte laxe accumbentibus, inflorescentia angusta, floribus laxiuscule glomerulatis minoribus et praecipue urceolis pilosis; in descriptione ipsa tam rossica (7, p. 369—370) quam latina (7, p. 628) urceoli «glabri» indicantur, sed sine dubio per errorem, nam in clavi analytica nec non ad finem diagnosis latinae expressis verbis «pilosi» appellantur.

Reliquae species seriei *Heteropodae* longius a nostra distant.

Series: HIRSUTAE Lindb. f. pro subserie

21. **Alchemilla Braun-Blanquetii Pawł. n. sp.**

♃. Mediocris, obscure viridis. Caulis 10—30 cm altus, tenuis, ± erectus vel basi adscendens, flexuosus, folia non vel usque bis superans, in inferiore $^1/_4$—$^1/_2$ parte (rarissime ad $^2/_3$) pilis pro parte minore fere rectanguliter, pro maiore tamen erecto patentibus, ad $^1/_4$—$^2/_5$ caulis densis, supra sparsis obtectus, in parte superiore omnino glaber. Foliorum radicalium laminae ± reniformes vel suborbiculares, ad 6·2 cm longae, ad 7·2 cm latae, sinu basali modice aperto usque clauso vel lobis sese pro parte obtegentibus, undulatae, 7—9- lobae; lobi ad $^1/_6$—$^1/_4$ ($^1/_3$) radii incisi, humiliter arcuati vel semiorbiculares vel in foliis supremis semielliptici vel semiorbiculari-subtriangulares, circumcirca dentati; dentes in utroque latere 5—8 (rarissime 9), acuti vel acutiusculi (in foliis infimis pro parte obtusiusculi), ± porrecti, terminali incluso ± aequales; foliorum pagina superior uniformiter densiuscule pilosa vel in foliis supremis pro parte in zona marginali, in plicis et in lobis basalibus abundantius, ceterum sparse vel sparsissime pilosa; pagina inferior in apice nervorum sparsissime usque sat abundanter sericeo-adpresso vel erecto-patenter pilosa, ceterum glabra vel (in foliis inferioribus) in lobis quoque basalibus, rarius etiam in zona marginali pilosa; petioli ad 16 cm longi, omnes dense (raro nonnulli sparse) pilis pro maiore parte erecto-patentibus obtecti, apice tamen praecipue petioli foliorum superiorum saepius ± glabri; stipulae non coloratae. — Folia caulina sat bene evoluta, ad $^1/_6$—$^1/_4$ radii incisa, eodem modo ac folia radicalia pilosa; stipulae eorum amplae, sat profunde in lacinias divaricatas inaequales ± acutas partitae. — Inflorescentia ± depauperata, inferne ramis paucis brevibus ± erectis paucifloribus instructa, apice paululum dilatata, glaberrima; glomeruli ± laxi; pedicelli glaberrimi. Flores virides, mediocres usque

maiusculi, 3—4 mm longi, 3—5 mm lati; urceoli glaberrimi, manifeste crassi, late obconici vel late obconico-campanulati, basi cito contracti, sepalis (sub fructu) subaequilongi; sepala latissime ovato-triangularia, acuta, glabra vel sparsissime puberula, post anthesin ± erecta; episepala eis pro parte multo maiore breviora et conspicue (1·5—3×) angustiora, glaberrima vel apice setula praedita. (Fig. 12)

Tatri Occident.: Małołączniak versus Wielka Świstówka, in graminosis, solo calcareo, 1600—1700 m, 23. 7. 1951, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae). — Specim. exam.: 11.

Planta in honorem illustrissimi botanici dris Josiae Braun-Blanquet, magistri et amici mei, denominata, ab omnibus Alchemillis carpaticis e subserie *Hirsutae* caule solummodo in parte basali piloso optime distincta. A speciebus alpigenis similem pilorum dispositionem ostendentibus (*A. diversiloba* Bus., *A. filicaulis* Bus., *A. hirtipes* Bus., *A. multidens* Bus., *A. strigosula* Bus.) aeque atque ab omnibus a Juzepczuk ad circulum *Exuentes* Juzep. adnumeratis aberrat pilis erecto-patentibus. E speciebus, mihi tantum e descriptione notis, quae a Juzepczuk ad circulum *Nemorales* Juzep. numerantur et quae caulem inferne erecto-patenter pilosum, superne glabrum habent.

A. Lindbergiana Juzep. differt a specie nostra foliis 9—11- lobis, dentibus magis numerosis, pagina inferiore foliorum mediorum et superiorum tota disperse pilosa, floribus minoribus;

A. Sedelmeyeriana Juzep. differt foliis 9- incompl. 11- lobis, in utraque pagina densiuscule pilosis, floribus parvis;

A. laeticolor Juzep. differt lobis incisura integra separatis, dentibus magis nemerosis inaequalibus, foliorum pagina superiore glabra vel minus pilosa quam pag. inferior, floribus minoribus flavidis, urceolis p. p. parce pilosis.

Praeterea tres species supra enumeratae nervis subtus in tota longitudine pilosis excellunt; petioli eorum probabiliter toti pilosi sunt, de hac enim nota auctor nullam mentionem facit, dum petiolos aliquot aliarum specierum expressis verbis sub lamina glabros esse indicat.

Quae cum ita sint, *A. Braun-Blanquetii* a circulo *Nemorales* Juzep. valde discrepat et potius circulo *Exuentes* Juzep. adnumeranda est.

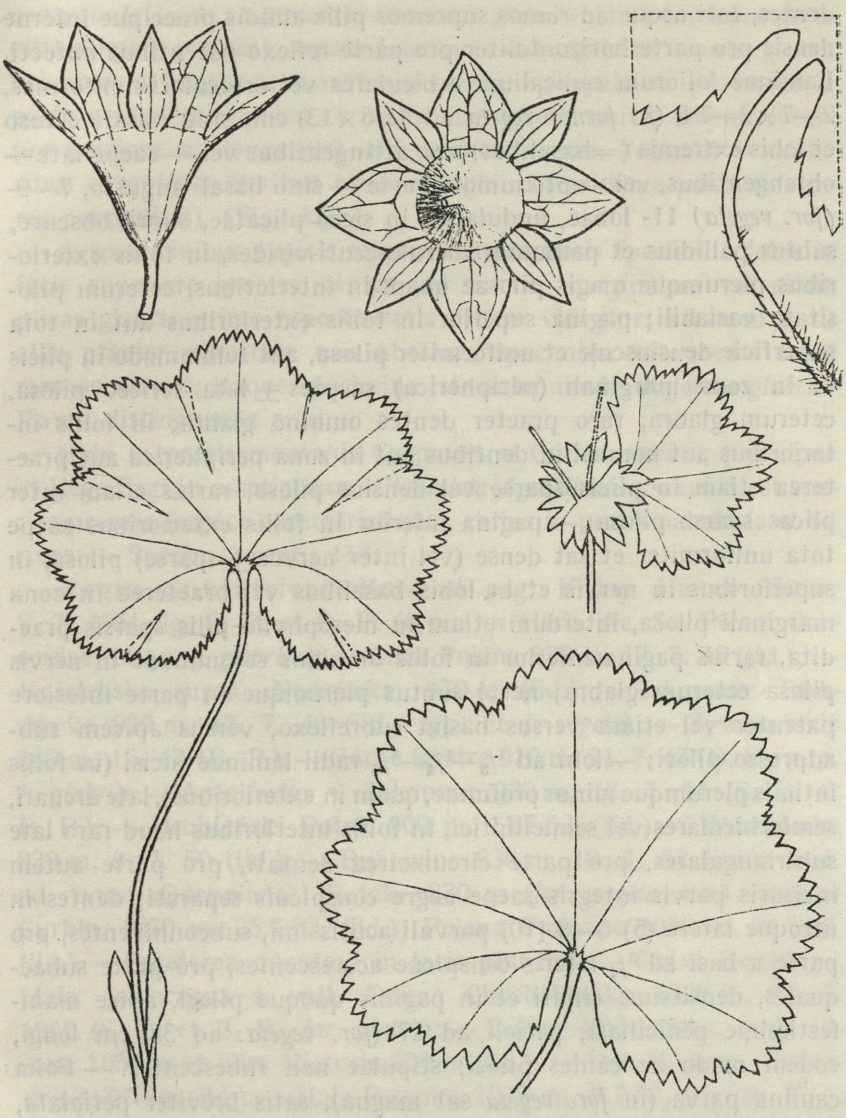

Fig. 12. *Alchemilla Braun-Blanquetii* Pawł. Flores: $^{8}/_{1}$; folia radicalia et caulinum: $^{1}/_{1}$; petioli pars apicalis: $^{2}/_{1}$.

22. **Alchemilla Walasii Pawł. n. sp.**

4. Planta mediocris, rarius *(for. vegeta)* satis magna, satis obscure viridis. Caules 6—30 (in *for. vegeta* ad 60) cm longi, adscen-

dentes, toti usque ad ramos supremos pilis albidis praecipue inferne densis pro parte horizontaliter pro parte reflexo patentibus obtecti. Laminae foliorum radicalium orbiculares vel orbiculari-reniformes, 2—7×2—7·6 (in *forma vegeta* ad 11·6×13) cm, sinu basali clauso et lobis extremis (= basalibus) sese attingentibus vel — saepe late — obtengentibus, vel — pro minore parte — sinu basali angusto, 7—9-*(for. vegeta)* 11- lobae, undulatae, in sicco plicatae, supra obscure, subtus pallidius et paululum coerulescenti-virides, in foliis exterioribus plerumque magis pilosae quam in interioribus, ceterum pilositate variabili; pagina superior in foliis exterioribus aut in tota superficie densiuscule et uniformiter pilosa, aut solummodo in plicis et in zona marginali (peripherica) saepius ± lata sericeo pilosa, ceterum glabra, raro praeter dentes omnino glabra; in foliis interioribus aut nonnisi in dentibus aut in zona peripherica aut praeterea etiam in plicis sparse vel densius pilosa, rarius etiam inter plicas sparse pilosa; — pagina inferior in foliis exterioribus saepe tota uniformiter et sat dense (vel inter nervos ± sparse) pilosa, in superioribus in nervis et in lobis basalibus vel praeterea in zona marginali pilosa, interdum etiam in mesophyllo pilis sparsis praedita, rarius pagina inferior in foliis omnibus solummodo in nervis pilosa ceterum glabra; nervi subtus plerumque in parte inferiore patenter vel etiam versus basim subreflexo, versus apicem subadpresso pilosi; — lobi ad $^1/_3$—$^1/_4$—$^1/_5$ radii laminae incisi (in foliis intimis plerumque minus profunde, quam in exterioribus), late arcuati, semiorbiculares vel semielliptici, in foliis interioribus haud raro late subtriangulares, pro parte circumcirca dentati, pro parte autem incisuris parvis integris saepe aegre conspicuis separati; dentes in utroque latere (5) 6—8 (10) parvuli acutissimi, subconniventes, pro parte a basi ad $^3/_4$ lateris conspicue accrescentes, pro parte subaequales, densissime ciliati et in paginis quoque pilosi, apice manifestissime penicillati; petioli ad 27 (*for. vegeta*: ad 38) cm longi, eodem modo ac caules pilosi; stipulae non rubescentes. — Folia caulina parva (in *for. vegeta* sat magna), satis breviter petiolata, ad $^1/_4$—$^1/_3$ (rarius ad $^2/_5$) incisa, lobis circumcirca dentatis vel basi incisura integra parva separatis. – Inflorescentia folia non vel vix paulo (ad summum 1·5×) superans, angusta, pauci ramosa et satis pauciflora; rami breves, erecti vel erecto patuli; glomeruli densiusculi usque laxiusculi. Flores virides, mediocres, 2·5—3·5 (4) mm longi, 3—4·5 (raro ad 5) mm diam.; urceoli 1·3—2·3 mm longi, breviter

campanulati vel campanulato-obconici, glabri vel inferiores pro parte pilis aliquot praediti; sepala late ovato-triangularia, acuta, glabra vel apice setulosa, post anthesin erecta; episepala eis pro maxima parte evidenter breviora et multo angustiora, acuta, saepius setula pro portione sat longa (0·3—0·5 mm) terminata. Pedicelli — axillaribus exceptis — floribus breviores, glabri (rarissime pauci inferiores parciciliati). (Fig. 13)

A speciminibus typicis saepius magis pilosis specimina foliis in facie superiore praeter dentes vel zonam marginalem vel etiam plicas glabris primo aspectu satis differe videntur; attamen notis aliis praeter pilositate non aberant; specimina intermedia haud raro occurrunt; itaque specimina ista solummodo ut *forma glabrior* Pawł. distinguenda sunt.

Hab. in pratis montanis Carpatorum Occidentalium, praecipue in Reg. Fagi in assotiatione «*Gladioleto-Agrostidetum*», cuius est species propria (espèce caractéristique), rarius in pratis paludosis et ad rivos. — Specim. exam.: 114.

Regio Subtatrica: «Mur» ad pag. Kośny Hamer 850 m, 9. 7. 1951, lg. S. P. et B. P. (typus in Herb. Ac. Sc. Polon. Cracoviae; etiam *f. glabrior*); — Siwa Polana 920 m, 17. 7. 46 (iid.); — Kościelisko supra «Nędzówka» 970 m (iid.); — Zakopane: Buńdówka 920 m, 17. 7. 46 (iid., typ. et *f. vegeta*); — Żywczańskie 880 m, 15. 47 (B. P.); — Górne Bystre 910 m, 21. 7. 47 (B. P., p. p. *f. glabr.*); — Antałówka s. Zakopane 930 m, 18. 7. 51 (S. P. et B. P.); — Hrubiański Potok 900 m, 11.7.52, (iid.); — Weszkówka 930 m, 9. 7. 50 (iid.); — Bukowina 930 m, 10. 7. 51 (iid.); ibid. ad rivum «Odewsiański Potok» 830 m (*for. vegeta*, iid.). Polana Cyrhla, 1070 m, 25.7.52, (iid.); Polana Poroniec 1050 m, 28.7.52, (iid.). — Praeterea adnotavi in locis pluribus. — Tatri Occiden.: Hala Jaworzyna s. vall. Dolina Chochołowska 1320 m, 14. 7. 1950 (S. P. et B. P., *for. glabr.*); — Dolina Kościeliska: Hala Pisana 1050 m et Kira Miętusia 950 m (iid.); — Dol. Miętusia: Zahradzisko 970 m (iid.); — Mała Polanka 1230 m, 9.7.52, leg. B. P. — «Hala w Jaworzynce» 1090 m, 11. 7. 51, S. P. et B. P. — Wawrzeczkowa Cyrhla 950 m, 11.7.52, (iid.). Hala Skupniowa 1230 m, 6. 7. 50 (iid., p. p. *for. glabrior*).

Orawa: Podszkle — VII 1925 — lg. R. Kobendza.

Montes Gorce (lg. Kor.): Hala Czoło infra cac. Turbacz, 10. 7. 1950; — Turbaczyk 1020 m, 14. 7. 50; — ?Dolina Kowańców; —

Bukowina, infra Kotlarka et in «Hala Mulowa», 18. 7. 50; — Gorc, vallis rivi Jamna 1015 m, 21. 7. 51; — Polana Ustępne 980 m, 21. 7. 51 *(for. glabrior)*; — Lubań 945 m, 16. 7. 51; — Lubań —

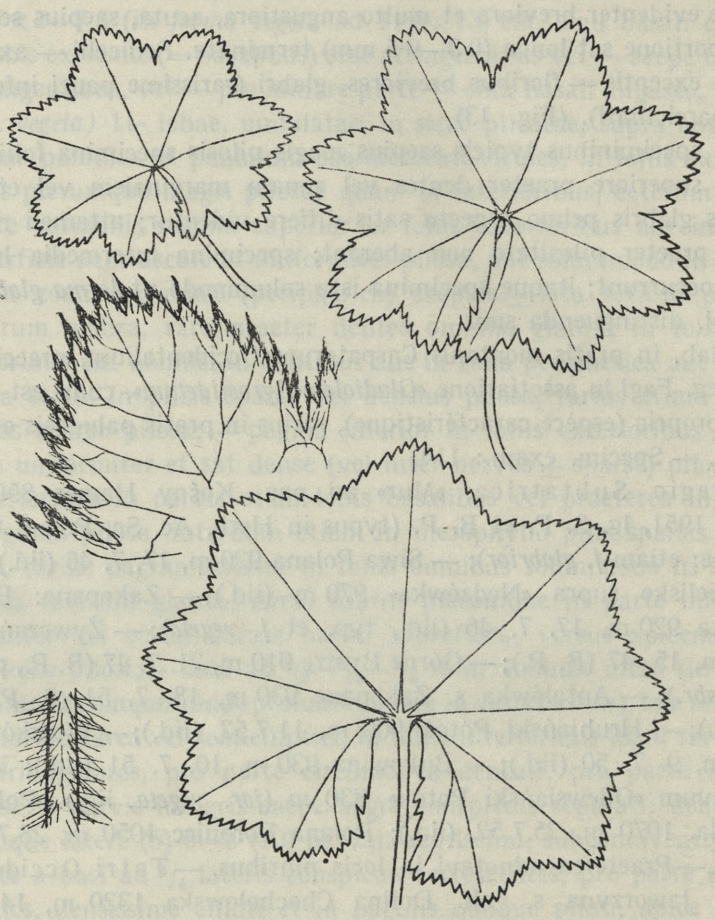

Fig. 13. *Alchemilla Walasii* Pawł. Folia radicalia: $^1/_1$; lobus folii et pars caulis (pilis retroflexis obtecta): $^2/_1$.

Mraźnica 945 m, 10. 7. 51; — vallis fl. Kamienica inter Białe et Rzeki 655 m, 20. 7. 51.

Montes «Beskid Wyspowy»: Białe infra cac. Mogielnica 610 m, 7. 6. 51 (Kor.).

Planta in honorem amici prof. dris Johannis Walas denominata, ad circulum specierum a Juzepczuk *Retropilosae* appellatum ad-

numeranda. A speciebus huc pertinentibus, quae ei simillimae apparent, differt *A. Walasii* notis sequentibus:

ab *A. retropilosa* Juzep. (Asia Centr.): foliis valde undulatis pro magna parte orbicularibus, sinu basali clauso vel lobis sese supertegentibus, dentibus parvis acutissimis, foliis plerumque minus pilosis nec non pilositate paulo minus manifeste retroflexa;

ab *A. cyrtopleura* Juzep. (Asia Centr.): caule non rubescente, usque ad ramos supremos piloso, foliorum forma, dentibus acutissimis non valde inaequilateralibus, pilositate foliorum interiorum minore, stipulis pallidis, floribus viridibus;

ab *A. sarmatica* Juzep.: colore obscure viridi, foliis saepe minus pilosis, lobis in foliis interioribus saepe subtriangularibus, incisura integra inter lobos parva vel nulla, dentibus acutissimis pro parte versus apicem accrescentibus, floribus viridibus submaioribus, sepalis acutis;

ab *A. Litwinowii* Juzep.: foliis saepius multo magis pilosis, incisura integra inter lobos parva vel nulla, dentibus magis numerosis, acutissimis, apicem versus saepe manifeste accrescentibus, stipulis et caulibus in sole non purpurascentibus, urceolis glabris vel tantum pro minore parte pilosis, sepalis acutis;

ab omnibus speciebus supra enumeratis foliis interioribus saepius minus profunde incisis quam folia exteriora, a 3 speciebus posterioribus etiam caule folia non superante vel paulo tantum superante.

23. **Alchemilla Ivonis Pawł. n. sp.**

♃. Parva, sordide viridis. Caulis tenuis, procumbens, supra adscendens, flexuosus, foliis 2—2·5× longior, usque ad supremas ramificationes pilis brevibus pro parte horizontaliter patentibus pro parte ± retroflexis praecipue inferne dense obtectus. Foliorum radicalium laminae parvae, ± reniformes, sinu basali angusto vel lato, plerumque 7- lobae, siccae planae vel paululum plicatae, subtus sordide coerulescenti-virides, utrinque dense ± patenter pilosae vel infimae saepius in facie superiore solummodo in zona marginali et ad plicas, subtus ad nervos et in lobis basalibus pilosae; lobi ad $^1/_4$—$^1/_3$ radii incisi, suborbiculares vel saepius subtrapezoidales, apice ± truncati, ab ima basi dentati; dentes in utroque latere 5—7 parvi obtusiusculi subaequales, terminalis paulo minor; petioli dense

pro parte patenter pro parte retroflexo pilosi; stipulae pallide virides, cito brunnescentes. — Folia caulina parva, basi inprofunde cordata vel ± truncata; stipulae eorum haud magnae, superiores ad $^1/_3$—$^2/_5$ incisae. — Inflorescentia parva et pauciflora, brevis et angusta, ramis perpaucis brevissimis sub angulo acuto abeuntibus. Flores dense glomerulati, parvi, 2·4—3·5 mm lati, flavido-virides; pedicelli glabri; urceoli 1·3—1·8 mm longi, glabri vel inferiores pilis singulis praediti; sepala ovato-triangularia, ± acuta vel acutiuscula, cum episepalis saepius margine setulis aliquot praedita, urceolis breviora; episepala eis (interdum multo) breviora, obtusiuscula vel obtusa.

Caulis in specim. meis ca. 10 cm long., fol. radic.: ad 2·7× ×3·5 cm, petioli ad 4 cm.

Hab.: Bulgaria, montes Pirin: Damianica, in pascuis, solo granitico, ca. 2050 m, 22. 8. 48, lg. B. P. — Specim. exam.: 5.

Planta in honorem amici prof. dris Ivo Horvat denominata, circulo *Retropilosae* Juzep. inserenda. Proxima *A. breviloba* Lindb. fil. differt foliis 7—9- lobis, omnibus utrinque dense pilosis, lobis incisuris integris sat profundis separatis, stipulis vini colore.

Series: PUBESCENTES Bus.

24. **Alchemilla pirinica Pawł. n. sp.**

♃. Planta parva, griseoviridis. Caules arcuato adscendentes, sat rigidi, 7—13 cm alti, toti pilis mollibus horizontaliter patentibus densissime obtecti, paululum violaceo-rubescentes. Foliorum radicalium laminae parvae, ad 2·3×2·5 cm, orbiculares vel orbiculari-reniformes, sinu basali — saltem basi eius — clauso vel angustissimo, exsiccatae planae, 7- (vel valde incomplete et vix conspicue 9-) lobae, supra dense, subtus densissime et in statu iuvenili sericeo pilosae; lobi ad $^2/_5$—$^1/_2$ radii longitudinis incisi, basin versus subcuneato angustati et spatio 2—4 mm integri, superne semi-orbiculares vel late semielliptici et utrinque dentibus (3) 4—5 (6) obtusis et terminali incluso subaequalibus, manifeste longioribus quam latis, apice valde penicillatis praediti; petioli ad 4·5 cm longi, caule ca. 2—3× breviores, sat longe horizontali-patenter pilosi. — Folia caulina parva et male evoluta, stipulae in lacinias paucas obtusas partitae. — Inflorescentia angusta, ramis 1—3 sub angulo acuto abeuntibus, glomerulis paucis densis ± congestis. Pedicelli

inferiores floribus longiores, superiores breviores, omnes densissime patenter pilosi. Flores mediocres, 3·3—4·6 mm diam., intus flavidi, postea saepius ± rubelli, etiam post anthesin late aperti; urceoli globoso-campanulati, densissime patenter pilosi; sepala oblongo-triangularia, acuta, dorso dense pilosa, urceolis subaequilonga; episepala ca. $^3/_4$—$^5/_6$ longitudinis eorum attingentia, multo angustiora, acuta, dorso pilosa.

Hab.: Bulgaria, montes Pirin: vallis Damianica, in rupestribus graniticis alt. 2120 m, 22. 7. 1948, lg. B. P. (typ. in Herb. Ac. Sc. Polon. Cracoviae).

A. caucasica Bus., quae plantae nostrae similis esse videtur, differt secundum descriptionem (Juzepczuk 7): pilis in caule et in petiolis pro parte erecto-patentibus, caulibus et petiolis manifeste rubro coloratis, foliis reniformibus, lobis subtruncatis, dentibus minus numerosis (utrinque 2—4), floribus minoribus (3—3·5 mm latis).

A. lanuginosa Rothm. (15) differt sufficienter foliis solummodo ad $^1/_3$ incisis, incisura inter lobos brevi ypsiloniformi, dentibus inaequalibus, minus numerosis, floribus minoribus.

25. **Alchemilla erythropodoides Pawł. n. sp.**

♃. Griseoviridis; coloratio aestivalis obsoleta, subbrunnea. Caulis 9—12 cm altus, plerumque sordide violaceo-rubescens, tota longitudine densissime pilis pro parte horizontaliter, pro parte ± reflexo patentibus tectus. Foliorum basilarium laminae suborbiculares vel rotundato-reniformes, ad 4×4·5 cm, sinu basali aperto, ± planae, 7- lobae, utrinque densissime pilosae, in statu evoluto non sericeae; lobi ad $^2/_5$—$^1/_2$ radii longitud. incisi, ambitu fere rhomboideo-elliptici i. e. apicem et basim versus paulo angustati, ima basi spatio 2—4 mm longo integri, ceterum grosse, fere inciso dentati; dentes utrinque 4—6, obtusiusculi, fere pectinati, terminalis haud minor; petioli caulis modo pilosi, non rubescentes vel vix rubescentes. — Folia caulina parvula, inferiora paulum incisa, superiora profunde 3—5-loba, lobis lateribus integris, apice 5—7- dentatis; stipulae eorum improfunde incisae, dentibus paucis latiusculis obtusiusculis. — Inflorescentiae rami suberecti; glomeruli pauci, sed multiflori, densissimi, subglobosi; pedicelli omnes dense patenter pilosi, floribus breviores. Flores mediocres, 3·5—4·8 mm diam. Urceoli globoso

campanulati, opaci, densissime patenter pilosi; sepala lata, obtusa vel acutiuscula, densiuscule pilosa, etiam post anthesim ± patentia, episepala eis multo minora et angustiora, extus pilosa. (Fig. 10,1,).

Hab. in graminosis calcareis in parte occidentali Tatrorum: mons Kopa inter cacumina: Ostra et Babki, alt. 1637 m, 27. 7. 1927, lg. B. P. (typ. in Herb. Ac. Sc. Polon.).

A. erythropodae Juzepczuk e Caucaso descriptae et usque ad Bulgariam et Macedoniam dispersae (Rothmaler 16) e descriptione valde affinis. Quoniam tamen planta nostra notis aliquot sat gravibus aberrat, scil.: foliis profundius lobatis, dentibus paulo magis numerosis, petiolis non rubroviolaceis, foliis caulinis saepius plurilobatis et pluridentalis, floribus densissime glomerulatis, mea opinione separanda est ut species propria.

Observationes de nonnullis speciebus Alchemillae

I. DE FLORIBUS

Examinavi exactius plus quam 1700 flores 39 specierum et proposui mihi nonnullas eorum proprietates numeris exprimere.

a) Florum diametros seu latitudo — aestimata semper mensuris florem horizontaliter expansorum — inter 2·2 et 7·2 mm nutat. Latitudo maior quam 5 mm nonnisi in subsect. *Calycanthum* saepius, in subsect. autem *Heliodrosium* rarissime occurrit. *A. Achtarowii* flores omnium Alchemillarum examinatarum maximos habet. Flores diametro minore quam 3 mm in sola *A. pastorali* haud raro (30%) inveniuntur. In subsect. *Heliodrosium* floribus maioribus haud raro ultra 4·5 mm latis, excellunt: *A. Stanislaae*, *A. pseudincisa*, *A. reniformis*, *A. Braun-Blanquetii* et *A. micans*.

b) Rationes comparativae longitudinis episepalorum et sepalorum multo maioris sunt momenti. Quorum rationum 5 categoriae distinguuntur: Si differentia longitudinis summatim omnium 4 episepalorum et omnium 4 sepalorum eiusdem floris 1 mm vel plus (in floribus minoribus, ad 3·5 mm latis: 0·8 mm vel plus) efficit, episepala sepalis «multo longiora» vel «multo breviora» appellantur; si differentia 0 ad 0·2 mm est, episepala sepalis «subaequilonga» vocantur; si differentia inter 0·2 et 1 (vel 0·8) mm includitur, episepala sepalis «longiora» vel «breviora» dicuntur. — Mensurae a me confectae in tabula I. exponuntur.

Apud species subsectionis *Calycanthum*, sola *A. fissa* excepta, episepala in floribus omnibus vel in maxima eorum parte (90% vel plus) sepalis subaequilonga vel longiora sunt. *A. Achtarowii* et *A. holocycla* locum extremum tenent, quia episepala eorum in florum parte maxima (75% vel plus) sepalis multo sunt longiora. *A. Zapałowiczii* tamen hac nota paululum se inclinat ad subsectionem *Heliodrosium*. *A. fissa* ab omnibus speciebus affinibus maxime discrepat: relatio, de qua agitur, apud eam tantopere variabilis est quantopere apud nullam aliam speciem, attamen flores episepalis brevioribus (pro parte multo brevioribus) quam sepala numero praevalent similiter atque in subsect. *Heliodrosium*. Niholominus *A. fissa* sine ulla dubitatione ad *Calycanthum* pertinet, quia sepala eius urceolis multo sunt longiora.

In subsectione *Heliodrosium* loco duarum serierum et 4 subserierum (Rothmaler 13) 5 series ± aequivalentes distinguo. E quibus series *Pubescentes* subsectioni *Calycanthum* maxime opposita apparet: apud 4 species examinatas episepala in omnibus floribus sepalis sunt breviora vel (50—100% flor. examin.) multo breviora. Series *Hirsutae* ut aliis notis ita episepalorum et sepalorum relatione proxime ad *Pubescentes* accedit, quamquam duae species: *A. Walasii* et *A. Braun-Blanquetii* relatione, de qua agitur, satis aberrant. Similis est positio *A. Kulczyńskii* e serie *Heteropodae* nec non *A. versipiloidis* et *A. reniformis* e serie *Subglabrae*; sed *A. reniformis* hac nota iam paululum ad subsect. *Calycanthum* vergit. Species seriei *Glabrae* pro maiore parte similem positionem habent; apud *A. Stanislaae* flores episepalis sepalisque subaequilongis numero praevalent, fere ut in subsectione *Calycanthum*, a qua tamen haec species urceolis elongatis sepala manifeste superantibus longissime abest. Itaque rectum mihi videtur, seriem *Glabrae* subsectioni *Calycanthum* proxime ponere, seriem *Pubescentes* autem loco maxime distante i. e. ad finem subsectionis *Heliodrosium* inserere.

c) Denticulatio episepalorum (v. tab. II.) multo frequentior apparet in subsect. *Calycanthum*. Flores, quorum saltem 1 episepalum denticulatum est, in 1 specie huc pertinente soli occurrunt (*A. Achtarowii*: 100%), in 3 speciebus multo praevalent (*A. jumrukczalica*: 88%, etc.), in 6 speciebus haud sunt rari (28—38%); apud 2 species *(A. incisa, A. Zapałowiczii)* numerus eorum est 12%; solummodo in 1 specie examinata *(A. fissa)* flores tales rari

sunt (4%), in una *(A. cuspidens)* a me prorsus non sunt animadversi. In subsect. *Heliodrosium A. Stanislaae* hac nota subsectionem *Calycanthum* appropinquat (flores saltem 1 episepalo dentato: 39%); apud 5 species flores, de quibus agitur, 8—12% efficiunt; apud 9 species flores tales non sunt observati.

Numerus episepalorum denticulatorum apud plurimas species subsectionis *Calycanthum* 8% vel plus exhibet (*A. Achtarowii* 92%, *A. jumrukczalica* 60% etc.); apud species e subsect. *Heliodrosium* episepala dentata aut omnino desunt (9 spec. exam.) aut nonnisi perpauca apparent (ad summum 4%); apud solam *A. Stanislaae* numerus maior (12·7%) adnotatus est.

Episepalorum denticulatio originem eorum in mentem revocat. Episepala enim in genere *Alchemilla* simili modo atque omnino in familia *Rosacearum* probabiliter bracteae sunt transformatae. Quae quidem bracteae certe plerumque partitae vel dentatae erant. Itaque haec species, in quibus episepala denticulata frequentur apparent, notam quandam vetustam prae se ferunt. Quod imprimis de *A. Achtarowii* valet.

d) Sepala dentata multo rarius inveniuntur quam episepala dentata. Quae res comprobatur numeris, qui sequuntur:

Species	Flores saltem 1 sepalo dentato	Sepala dentat
A. Kotulae Pawł.	30%	17%
A. Zapałowiczii Pawł.	20%	5%
A. subtatrica Pawł.	12%	7%
A. Stanislaae Pawł.	12·2%	2·7%
A. Achtarowii Pawł.	9%	3%
7 species exam.	1—3%	0·3—2%
27 ,, ,,	0	0

II. DE SPECIEBUS CARPATICIS

E parte boreali montium Carpatorum nonnisi 16 species *Alchemillarum* adhuc notae erant, indicationibus nonnullis falsis vel valde incertis omissis. E quibus speciebus nulla endemica erat. Post investigationes ab autore perfectas ex eodem territorio nunc species circa 40 notae sunt (B. Pawłowski 10); e quibus 20 species sunt novae supra descriptae verisimiliter pro maiore parte endemicae. Qui numeri non sunt ultimi immutabiles, observationibus enim ulterioribus additis certe accrescent. Iam herbariis ab autore exami-

natis specimina aliquot continentur, quae verosimillime species sunt novae, quamquam materia ad eas describendas haud sufficit.

Inde sequitur, ut montes Carpati aream differentiationis generis *Alchemilla* sectionis *Brevicaulon* (subsect. *Chirophyllum* Rothm. exclusa) determinent. Neque vero haec area est principalis, ut Alpes et Caucasus, sed area est secundaria, quae nihilominus characteres quosdam proprios ostendit. Exempli gratia in Alpibus species aliquot (ca. 10%) occurrunt foliis rosulariis profunde — plus quam ad $^1/_2$ laminae — incisis, cum in Carpatis borealibus formae tales omnino desint. Species autem foliis valde imprcfunde — vix ad $^1/_4$—$^1/_{10}$ incisis — in Carpatis pro portione magis sunt numerosae quam in Alpibus.

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TABULA I.

subsectio	series	Alchemilla sectio Brevicaulon	Florum examinatorum numerus	Flores, quorum episepala sepalis sunt: multo longiora %	longiora %	subaequilonga %	breviora %	multo breviora %
Calycanthum	Elatae	Achtarowii Pawł.	33	**75**	18	6	—	—
		holocycla Rothm.	11	(**81**)	(18)	—	—	—
		jumrukczalica Pawł.	25	—	**48**	**52**	—	—
		mollis (Bus.) Rothm.	33	6	**48**	**42**	3	—
		gorcensis Pawł.	50	—	**52**	**42**	6	—
		armeniaca Rothm.	10	—	(20)	(**70**)	(10)	—
		peristerica Pawł.	25	—	20	**76**	4	—
		persica Rothm.	10	—	(30)	(**60**)	(10)	—
		Zapałowiczii Pawł.	25	8	16	**60**	16	—
	Calicinae	firma Bus.	50	—	**66**	30	4	—
		Eugenii Pawł.	33	15	**33**	**48**	3	—
		incisa Bus.	50	2	26	**70**	12	—
		cuspidens Bus.	20	5	15	**70**	10	—
		fissa Günth. et Schumm.	50	6	4	22	**52**	16
Heliodrosium	Glabrae	Stanislaae Pawł.	33	—	9	**66**	24	—
		subtatrica Pawł.	82	—	—	**46**	**53**	—
		Żmudae Pawł.	50	—	2	22	**70**	6
		Kotulae Pawł.	121	—	2	27	**66**	5
		Sokołowskii Pawł.	33	—	—	21	**75**	3
		pseudincisa Pawł.	70	—	1	4	**64**	30
	Subglabrae	reniformis Bus.	50	—	4	**40**	**56**	—
		versipiloides Pawł.	53	—	—	24	**63**	12
		subconnivens Pawł.	50	—	—	10	**70**	20
		glabra Neygenf.	230	—	—	8	**72**	20
		czywczynensis Pawł.	20	—	—	5	35	**60**
		Wallischii Pawł.	14	—	—	—	(**50**)	(**50**)
	Heteropodae	Kulczyńskii Pawł.	33	3	—	30	**60**	6
		tatricola Pawł.	33	—	—	12	**66**	21
		Szaferi Pawł.	33	—	—	—	**78**	21
	Hirsutae	Braun-Blanquetii Pawł.	33	—	—	30	**56**	12
		Walasii Pawł.	50	—	—	22	**76**	2
		micans Bus.	50	—	—	—	**42**	**58**
		pastoralis Bus.	50	—	—	—	**54**	**48**
		sarmatica Juzep.	25	—	—	—	**40**	**60**
	Pubescentes	pirinica Pawł.	22	—	—	9	**90**	—
		flabellata Bus.	50	—	—	—	**50**	**50**
		glaucescens Wallr.	50	—	—	—	24	**76**
		erythropodoides Pawł.	20	—	—	—	15	**85**
		colorata Bus.	33	—	—	—	—	**100**

TABULA II.

Alchemilla sectio Brevicaulon			Florum examinatorum numerus	Flores in quibus inveniuntur episepala dentata numero:					Epiſepalorum examinatorum numerus	Numerus episepalorum in quibus apparent dentes numero:					
subsectio	series			0 %	1 %	2 %	3 %	4 %		0 %	1 %	2 %	3 %	4 %	5 %
Calycanthum	Elatae	Achtarowii Pawł.	33	—	—	6	15	**78**	132	8	17	**32**	**23**	16	2
		jumrukczalica Pawł.	25	12	12	**24**	**36**	16	100	**40**	**40**	20	—	—	—
		armeniaca Rothm.	10	(20)	(**40**)	(20)	(20)	—	40	**65**	32	—	2	—	—
		holocycla Rothm.	11	(18)	(**45**)	(**36**)	—	—	44	**76**	21	2	—	—	—
		mollis (Bus.) Rothm.	33	**63**	21	15	—	—	132	**88**	10	2	—	—	—
		persica Rothm.	10	(**70**)	(20)	(10)	—	—	40	**90**	10	—	—	—	—
		gorcensis Pawł.	50	**72**	16	6	4	2	200	**88**	9	2·5	0·5	—	—
		peristerica Pawł.	25	**72**	24	4	—	—	100	**92**	7	—	1	—	—
		Zapałowiczii Pawł.	25	**88**	12	—	—	—	100	**97**	3	—	—	—	—
	Calicinae	firma Bus.	50	**62**	36	2	—	—	200	**90**	10	—	—	—	—
		Eugenii Pawł.	33	**63**	33	3	—	—	132	**90**	10	—	—	—	—
		incisa Bus.	50	**88**	8	4	—	—	200	**96**	4	—	—	—	—
		fissa Günth. et Schumm.	50	**96**	4	—	—	—	200	**99**	0·5	0·5	—	—	—
		cuspidens Bus.	20	**100**	—	—	—	—	80	**100**	—	—	—	—	—
Heliodrosium	Glabrae	Stanislaae Pawł.	33	**60**	30	6	3	—	132	**87**	12	0 75	—	—	—
		Kotulae Pawł.	280	**91**	6	2·5	0·3	—	1120	**97**	3	0·4	—	—	—
		Żmudae Pawł.	50	**92**	6	2	—	—	200	**97**	2	—	—	—	—
		pseudincisa Pawł.	70	**94**	4	1	—	—	280	**98**	1·3	0·3	—	—	—
		Sokołowskii Pawł.	33	**96**	3	—	—	—	132	**99**	0 7	—	—	—	—
		subtatrica Pawł.	82	**96**	3	—	—	—	328	**99**	0·3	0·6	—	—	—

Heliodrosium														
Subglabrae	reniformis Bus.	50	**92**	8	—	—	—	200	**98**	2	—	—	—	—
	versipiloides Pawł.	33	**94**	6	—	—	—	132	**98**	1	—	—	—	—
	Wallischii Pawł.	14	**(91)**	(7)	—	—	—	50	**98**	2	—	—	—	—
	subconnivens Pawł.	50	**98**	2	—	—	—	200	**99·5**	0·5	—	—	—	—
	glabra Neygenf.	230	**99**	0·4	—	—	—	920	**99·9**	0·1	—	—	—	—
	czywczynensis Pawł.	20	**100**	—	—	—	—	80	**100**	—	—	—	—	—
Heteropodae	tatricola Pawł.	33	**87**	12	—	—	—	132	**97**	3	—	—	—	—
	Szaferi Pawł.	33	**100**	—	—	—	—	132	**100**	—	—	—	—	—
	Kulczyńskii Pawł.	33	**100**	—	—	—	—	132	**100**	—	—	—	—	—
Hirsutae	Braun-Blanquetii Pawł.	33	**91**	9	—	—	—	132	**96**	4	—	—	—	—
	micans Bus.	50	**94**	4	2	—	—	200	**98**	2	—	—	—	—
	Walasii Pawł.	50	**98**	2	—	—	—	200	**99·5**	0·5	—	—	—	—
	sarmatica Juzep.	25	**100**	—	—	—	—	100	**100**	—	—	—	—	—
	pastoralis Bus.	50	**100**	—	—	—	—	200	**100**	—	—	—	—	—
Pubescentes	flabellata Bus.	50	**96**	2	2	—	—	200	**98·5**	1·5	—	—	—	—
	erythopodoides Pawł.	20	**100**	—	—	—	—	80	**100**	—	—	—	—	—
	pirinica Pawł.	22	**100**	—	—	—	—	88	**100**	—	—	—	—	—
	colorata Bus.	33	**100**	—	—	—	—	132	**100**	—	—	—	—	—
	glaucescens Wallr.	50	**100**	—	—	—	—	200	**100**	—	—	—	—	—

Badania nad szczepieniem ziemniaków. II. Szczepienie Solanum Rybini na pomidorze. — Potato grafting experiments. II. Grafting of Solanum Rybini onto tomato stocks

Mémoire

de **E. MALINOWSKI, H. BAŃKOWSKA** and **I. OSKIERKA**

présenté le 5 Novembre 1951 par M. E. Malinowski m. t.
et M. M. Korczewski m. c.
(Plate 17)

Our experiments concern a self-incompatible strain of *Solanum Rybini*. We grafted this species onto tomato stocks in order to get seeds after self-pollination.

Tomatoes for stocks were sown on 21 st. and 23 rd. of March 1951. They were transplanted into pots on April 7 th. On April 24 th we began to graft *S. Rybini* scions. Grafting was made onto tomato stocks having 2 leaves. Potato sprouts for grafting were obtained from bulbs planted on 29 th. of March and on 5 th of April.

We grafted *S. Rybini* onto three kinds of tomato stocks, namely: 1) Tomato stocks without lateral shoots, 2) Tomato stocks with 1—2 young lateral shoots. As the young shoots grew older and attained a certain height they were cut off and replaced by new shoots growing out from younger buds. 3) Tomato stocks with old, flowering lateral shoots. These shoots grew old and they flowered and produced fruits. For stocks the variety Golden Jubilee was used.

In this way we distinguished three categories of potato scions accordingly as they were grafted onto one of the three above mentioned kinds of tomato stocks. The first category of potato scions began flowering on June 6 th and in the other categories the first flowers unfolded on 11 th of June. At the beginning we counted in each

plant all unfolded flowers every day but afterwards every week or at 10 day intervals. The results of o r experiments are shown in Tables I—VII.

Our double plants composed of potato scions and tomato stocks were grown in pots and in the field. In pots we grew 18 such plants, 6 of each category and in the field 29 plants.

The number of inflorescences and the number of unfolded flowers proved larger in *S. Rybini* grafted onto tomato stock than in the control plants. But not all categories of tomato stocks were equal as to their influence upon the potato scions. We obtained the best results with the stocks of category I, viz without any lateral shoots.

In 6 potato scions grafted onto tomato stocks of category I grown in pots we obtained 337 inflorescences and 1323 flowers or on the average 52·9 inflorescences and 220·5 flowers in each plant (Table I). In 6 other potato scions grafted onto tomato stocks of category II we observed 141 inflorescences and 534 flowers or 23·5 inflorescences and 89·0 flowers on an average. For tomato stocks of category III the respective numbers were as follows: 59 inflorescences and 248 flowers in 6 plants or 9·9 inflorescences and 41·3 flowers on an average (Table II).

TABLE I

Frequency distribution of the number of inflorescences of *Solanum Rybini* grown in pots

Tomato stocks	Number of inflorescences								Number of plants	Number of inflores-cences	Mean
	0	1—10	11—20	21—30	31—40	41—50	51—60	61—70			
Category I					1	1	1	3	6	337	52·9
Category II		1	1	2	2				6	141	23·5
Category III	1	2	3						6	59	9·9

Analogous results were obtained for plants grown in the field. We grew in the field 9 plants of category I, viz. 9 potato scions grafted onto tomato stocks of category I, 10 of category II and 10 of category III. In he plants of category I we

TABLE II

Frequency distribution of the number of flowers of *Solanum Rybini* grown in pots

Tomato stocks	Number of flowers												Number of plants	Number of flowers	Mean
	0	1—25	26—50	51—75	76—100	101—125	126—150	151—175	176—200	201—225	226—250	250—275			
Category I								1	1	—	3	1	6	1323	220·5
Category II		1	—	1	2	—	2						6	534	89·0
Category III	1	—	3	2									6	248	41·3

TABLE III

Frequency distribution of the number of inflorescences of *Solanum Rybini* grown in the field

Tomato stocks	Number of inflorescences											Number of plants	Number of inflores-cences	Mean
	1—10	11—20	21—30	31—40	41—50	51—60	61—70	71—80	81—90	91—100	101—110			
Category I					4	3	1	1				9	486	54
Category II	1	3	1	1		—	—	—	—	—	1	10	358	35·8
Category III	2	4	3	1	3							10	150	15

TABLE IV

Frequency distribution of the number of flowers of *Solanum Rybini* grown in the field

Tomato stocks	Number of flowers																	Number of plants	Number of flowers	Mean
	1—25	26—50	51—75	76—100	101—125	126—150	151—175	176—200	201—225	226—250	251—275	276—300	301—325	326—350	351—375	376—400	401—425	426—450			
Category I							4	2	1	—	1	1							9	1826	202 9
Category II		1	3	—	1	—	2	1	1	—	—	—	—	—	—	—	—	1	10	1428	142·8
Category III	3	1	2	3	—	1													10	622	62·2

observed 486 inflorescences and 1826 flowers (or 54·0 inflorescences and 202·9 flowers on an average (Table III), in category II

358 inflorescences and 1428 flowers (or 35·8 inflorescences and 142·8 flowers on an average), in category III 150 inflorescences and 622 flowers (or 15 inflorescences and 62·2 flowers on an average. Table IV).

Twenty control plants grown in the field produced markedly less inflorescences and flowers than the potatoes grafted onto tomato stocks of the I-st and II-nd category. As to the plants of the category III (viz. potatoes grafted onto tomato stocks with old lat-

TABLE V

Frequency distribution of the number of inflorescences of *Solanum Rybini* control plants grown in the field

Line	Number of inflorescences					Number of plants	Number of inflores-cences	Mean
	1—10	11—20	21—30	31—40	41—50			
087	3	5	1	—	1	10	171	17·1
1311	2	6	1	1		10	169	16·9

TABLE VI

Frequency distribution of the number of flowers of *Solanum Rybini* control plants grown in the field

Line No	Number of flowers									Number of plants	Number of flowers	Mean
	1—25	26—50	51—75	76—100	101—125	126—150	151—175	176—200	201—225			
087		3	3	2	1	—	—	1		10	805	80·5
1311	1	1	5	1	1	—	—	—	1	10	755	75 5

eral shoots) they produced slightly fewer flowers than the control plants (Tables V and VI). Control plants of *Sol. Rybini* were grown from tubers planted directly in the field.

We isolated with paper bags 40 inflorescences in all three categories of plants and under such controlled conditions we obtained fruits and seeds only in the potato scions grafted onto tomato stocks of the I st. category. In the plants of the I t. category we ob-

tained after self-pollination 1 fruit in the field and 1 in pots. Categories II and III did not set fruits at all. The control plants of *S. Rybini* did not produce any fruit either.

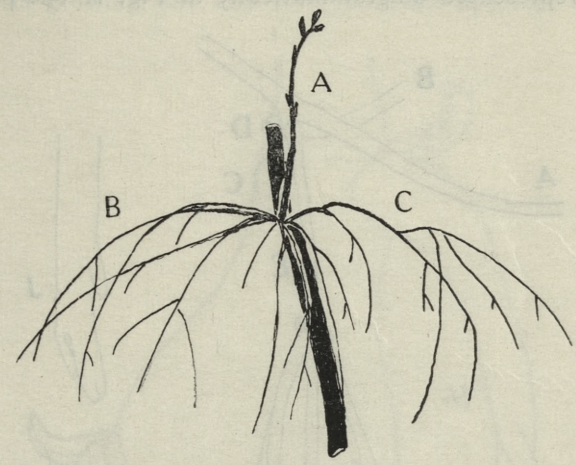

Fig. 1. A cluster of aerial stolons (B, C) growing out at the base of a lateral shoot (A) of *Solanum Rybini* scion grafted onto tomato stock.

At the end of the season there appear on the potato scions more or less numerous clusters of aerial stolons. Such clusters are most

TABLE VII

Frequency distribution of the number of clusters of stolons of *Solanum Rybini* grafted onto tomato stocks

Tomato stocks	Number of clusters														Number of plants	Number of clusters	Mean
	0	1	2	3	4	5	6	7	8	9	10	11	12	13			
Category I					1	—	1	1	1	—	1	—	1		6	47	7·8
Category II					3	—	1	1	—	—	—	—	—	1	6	38	6·3
Category III	4	2													6	2	0·3

numerous in the case of the I st category of tomato stocks, less numerous in the second and they are few in the third category (Table VII).

The aerial stolons appear always at the base of newly formed lateral shoots, which turn upward and become green. The stolons

themselves turn downward and they are brown. There appear usually many stolons at the base of one lateral shoot and they form clusters, mentioned above. One such cluster of downward directed stolons is represented diagrammatically in Fig. 1. The photograph

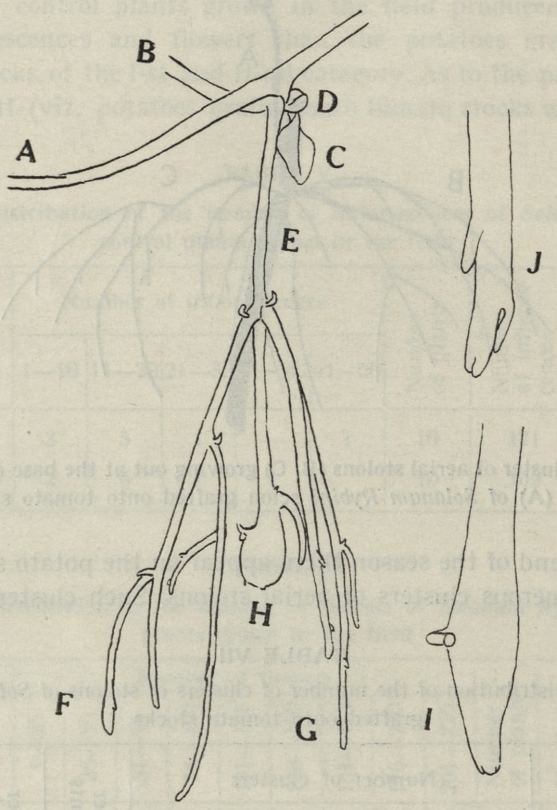

Fig. 2. A — main axis of an inflorescence. B — lateral shoot developing from the axil of the leaf C. E — aerial stolon growing downward and originating in the axil of the leaf D at the base of the lateral shoot B. F — ramification of the stolon E with swollen end. G — ramification with not swollen top. H — ramification with small tuber. I — magnified drawing of the top of the ramification F. J — magnified drawing of the top of the ramification G.

(Fig. 4) shows 2 such clusters a few cm above the region of union between stock and scion. We see in Fig. 4, a and c, two green lateral shoots at the bases of which grow downward the clusters of stolons.

Similar clusters of stolons grow out also from upper potato branches and even from inflorescences, which in the greenhouse are

often hanging down. One of such instances is shown in Fig. 2. We see in this figure the main axis of the inflorescence bent downward (Fig. 2 A). From it, at the axil of a leaf (Fig. 2 C), a lateral shoot (Fig. 2 B) is growing out and it is directed upward. This shoot

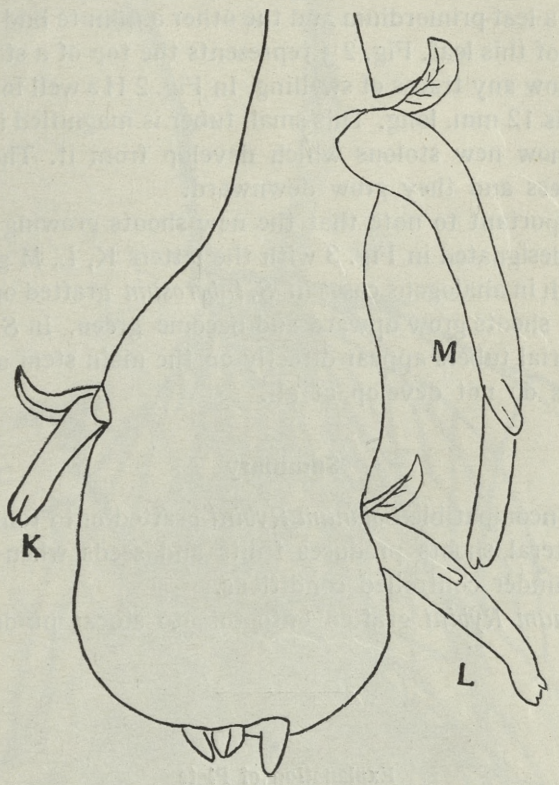

Fig. 3. Magnified drawing of the tuber shown in Fig. 2 H. K, L, M — three young aerial stolons growing downward.

is green and it bears young normal leaves. At the base of this shoot we see a small leaf (Fig. 2 D) and from the axil of this leaf a brown stolon grows out which ramifies afterwards. The main stolon (Fig. 2 E) and its ramifications are growing downward (Fig. 2 F and G). The length of the cluster from D to G equals 8 cm. The growing top of each ramification is green for a distance of about 3 mm in length. Next portion of the stolon for a distance of about 6 mm is colourless, while the rest of the stolon is brown. There are of course imperceptible

tible gradations in colour between the three just mentioned parts. In late autumn the tops of the stolons swell and tubers develop gradually. We see the beginning of such a swelling in Fig. 2, F. This swollen part represented in Fig. 2 F is magnified in Fig. 2 I. At the left side of this drawing two protuberances are seen, one of which is a leaf primordium and the other a minute bud developing in the axil of this leaf. Fig. 2 J represents the top of a stolon, which does not show any traces of swelling. In Fig. 2 H a well formed tuber is seen. It is 12 mm. long. This small tuber is magnified in Fig. 3 in order to show new stolons which develop from it. These stolons are colourless and they grow downward.

It is important to note that the new shoots growing out of the tuber and designated in Fig. 3 with the letters K, L, M grow downward though in analogous cases in *S. tuberosum* grafted onto tomato stocks such shoots grow upward and become green. In *S. tuberosum* however aerial tubers appear directly on the main stem of the scion and stolons do not develop at all.

Summary

1. Self-incompatible *Solanum Rybini* grafted onto tomato stocks without lateral shoots produces fruits and seeds when it is self-pollinated under controlled conditions.

2. *Solanum Rybini* grafted onto tomato stocks produces aerial stolons.

Explanation of Plate

Fig. 4. Two clusters of aerial stolons of *Solanum Rybini* scion grafted onto tomato stock. *a* and *c* — lateral shoots of the main axis of *S. Rybini*. *b* — base of the lateral shoot from whith aerial stolons develop.

Fig. 4.

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contenues dans le Bulletin International de l'Académie Polonaise des Sciences et des Lettres (Classe des Sciences Mathématiques et Naturelles. Série B: Sciences Naturelles (I).

Année 1951.

Le nombre inscrit à la suite de chaque Mémoire indique la page.

BULLETIN INTERNATIONAL DE L'ACADÉMIE POLONAISE DES SCIENCES ET DES LETTRES

CLASSE DES SCIENCES MATHÉMATIQUES ET NATURELLES

SÉRIE *B*: SCIENCES NATURELLES

Cena zł 32·—

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