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The development of tobacco seedlings in connection with seasons and the dynamic state of proteins.

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1. THE DYNAMIC STATE OF PROTEINS IN LEAVES

The investigations of H. B. Vickery and others (1940) on tobacco seedlings with isotopic nitrogen N^{15}, supplied in the nutrient solution, have proved that nitrogen uptaken from the exterior is readily exchanged with the nitrogen which exists in the plant proteins. The exchange of the different aminoacids does not procede equally. Aspartic and glutamic acids for instance are replaced quicker than the other ones. A similar experiment on buckwheat has proved that nitrogen contained in the leaf proteins has been exchanged in 6% with the exterior in forty seven hours.

According to Schoenheimer (1942)and Vickery (1940) proteins contained in plant and animal cells undergo an uninterrupted degradation and resynthesis. The nucleus and cytoplasmatic proteins are in a state of permanent dynamic equilibrium.

Chantrenne (1953) interprets the dynamic state of protein somewhat differently. According to him the protein molecule, which forms a "template", never hydrolyses completely, but its constituent aminoacids are exchanged independently. They are synthesised directly by the cell, from the inorganic nitrogen components. There exists in the plant cell, between the aminoacids which are in a free state and the proteins, a permanent interdependence.

Pearsall on *Narcissus pseudo-narcissus* (1949) has proved that the resynthesis of proteins occurs more intensively in young than in older tissues. This natural process of development occures in a differing quantitative relation between aminoacids in a free state and protein substances contained in the cell (Steward 1947, Walkley, Petrie 1941, Wood 1953). In this respect we can distinguish the following stages of development.

The 1st stage refers to youth. The resynthesis of proteins exceeds the hydrolyses.

The 2nd — mature state. Equilibrium between the synthesis and the hydrolyses of proteins.

The 3rd old stage begins at the moment when the hydrolyses begins to dominate the resynthesis.

In connection with the decrease of proteins the total nitrogen diminishes generally (M o t h e s 1931, S m i r n o w 1928), on the contrary the amount of aminoacids augments in the cell.

By excising a leaf from the maternal organism we interrupt the further stages of its development. In such leaves placed with their petioles in water or nutrient solution, the nitrogen metabolism is disturbed in the first place in spite of the carried on photosynthesis. The degradation of proteins exceeds their resynthesis and the amount of aminoacids increases in the cell. (C h i b n a l l 1939, V i c k e r y 1937, Y e m m 1937).

In normal circumstances disturbances occurring in the stages of development of the leaves generally promote the old age state.

Remains to be seen whether, on the contrary, one may produce conditions, which render possible a prolongation of, or a stop at a certain stage of youth. In this paper this problem has been analysed on tobacco seedlings, stopped in their development in winter time.

2. THE STAGES OF DEVELOPMENT OF TOBACCO SEEDLINGS

The stages of development of tobacco seedlings are marked by their leaves, which differ in their morphological shape.

The morphological shape of tobacco leaves are mostly stable. Well defined changes in morphology of tobacco plants, seedlings and mature plants, were observed but only in connexion with particular environments conditions, above all mineral deficiency of the soil.

M c M u r t r e y (1933) has described the symptom aggregates characteristic of mineral deficiencies of tobacco. In field culture calcium, magnesium, potassium, phosphorus deficiency produce well known deformity of tobacco (R o b e r t, S t e i n b e r g 1950). S t e i n b e r g (1947) found that diffusates from numerous bacterial strains, ordinary present in soil, are capable of causing changes in tobacco seedlings. Slightly excessive quantities of aminoacids in aseptic culture (S t e i n b e r g 1949, 1947) and other natural metabolic compounds caused also the production of specific symptom complexes in tobacco seedlings.

C a m u s G. C. and others (1950) point out that tobacco plants grown at various night temperatures show differences in growth rate, but they dont discuss morphological changes of the leaves.

As in our experiments the morphological type of youthful and first mature leaves in different stages of development of tobacco seedlings,

during the whole year, was stable, phases of growth are differentiated on the bases of morphological development of leaves.

From the beginning of March till the end of September the development of tobacco seedlings was approximately similar. On the fig. 1 and 2 is demonstrated the growth rate of 4th and 5th youthful leaves of tobacco seedlings from 6 IV to 20 V and from 9 VI to 15 VI, when the max. and min. temperature in the greenhouse was different.

The leaves of the primary phase of development of tobacco seedlings do not resemble the leaves of the mature plant.

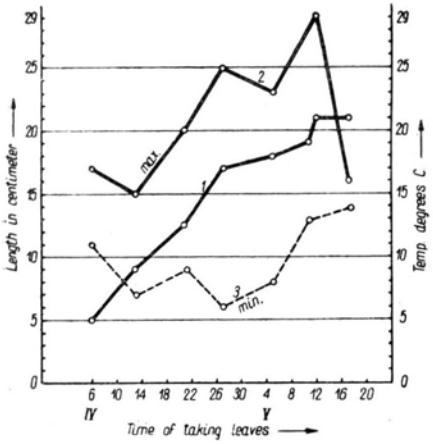

Fig. 1. The growth rate of 4th and 5th youthful leaves of tobacco seedlings from 6 IV to 20 V. 1. Leaves 2. Max. temperature. 3. Min. temperature.

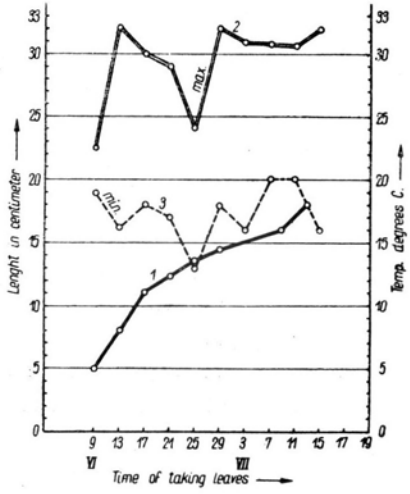

Fig. 2. The growth rate of 4th and 5th youthful leaves of tobacco seedlings from 9 VI to 15 VII. 1. Leaves 2. Max. temperature. 3. Min. temperature.

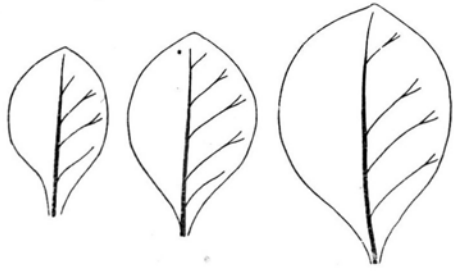

Fig. 3. The youthful leaves Ist and IInd stage of development.

The youthful leaves, about 5 or 6 in number, grow low above the ground, their length surpasses hardly their width. They have 5 or 6 side nerves. (fig 3).

The mature leaves are prolonged, sharply finished, with a big midle nerve and about 11 side nerves (fig. 4).

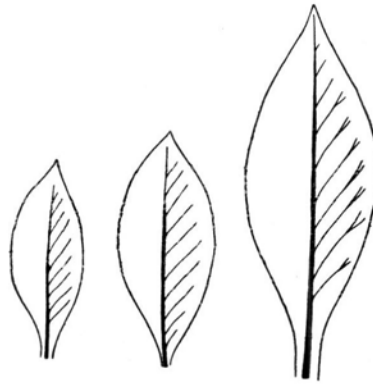

Fig. 4. The mature leaves IVth, Vth and VIth stage of development.

In spring and summer the youthful phase of the plant lasts about a month, after what the mature leaves replace the first ones. The youthful phase of tobacco plants can be divided into the following stages. The approximative length of time of these is presented for March and April, in optimal greenhouse conditions.

Ist s t a g e. Two round cotyledons without side nerves, I to 1$^1/_2$ cm long. The first youngling leaf is 3 cm long. The two following leaves from 4 to 6 cm long. The 4th leaf develops. (Duration 3—4 days) (fig. 5).

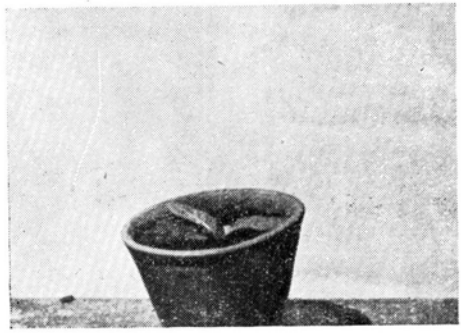

Fig. 5. Tobacco seedling, I-st period of development.

2nd s t a g e. The first youthful leaf has prolonged itself to 4 cm. The two following leaves are 7.3 — 8.2 cm long. The fourth leaf attains 4 cm. (6 days) (fig. 6).

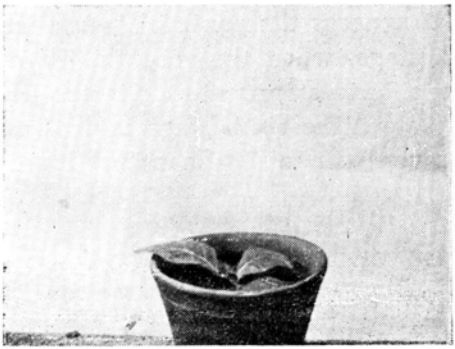

Fig. 6. Tobacco seedling, II-nd period of development.

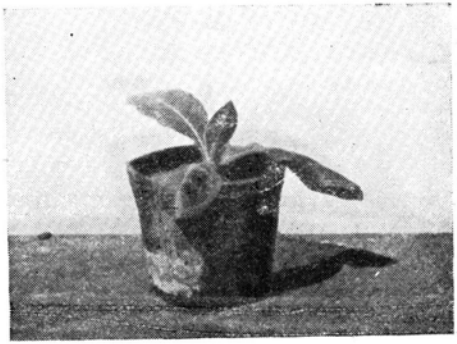

Fig. 7. Tobacco seedling, III-d period of development.

3rd stage. The second greatest youthful leaf is 9.6 — 10.2 cm. long and 6 to 6.5 cm broad. The third leaf is somewhat smaller. The fourth leaf is 7.2 cm long. The fifth youthful leaf develops (6 days) (fig. 7).

4th stage. The greatest youthful leaf is about 13 cm long. The appearence of the first mature leaf (8 days).

5th stage. Three youthful green leaves attain a length of 15 to 19 cm .The two older get yellow. The first mature leaf $10^1/_2$ cm long, has 11 side nerves. The second mature leaf $4^1/_2$ cm long. The plant continues to form a rosette above the ground (6 days) (fig. 8).

6th stage. All the youthful leaves turned yellow. The stem elongates and has 4 to 5 mature leaves (fig. 9).

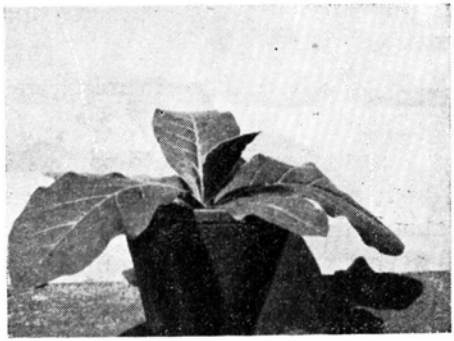

Fig. 8. Tobacco seedling, V-th period of development.

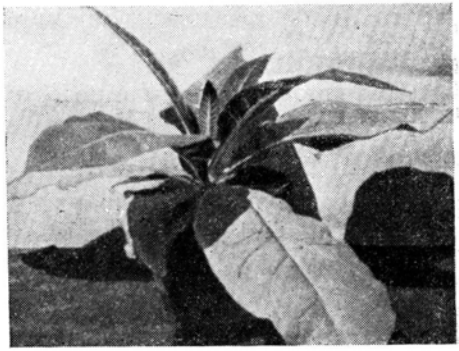

Fig. 9. Tobacco seedling, VI-th period of development.

With the fifth and sixth stage of the development of youthful leaves begins the mature phase of the plant. In the development of tobacco these stages ought to be called a transitory stage leading to maturity. In the

fourth stage, when the first mature leaf appears the youthful leaves are still in a stage of growth. In the fifth stage, when the youthful leaves have attained their full development and have stopped to grow, the development of the mature leaves accentuates. The sixth stage is decisive for the life of the plant. The youthful leaves extinguish, the mature leaves develop rapidly on the elongating stem.

3. TOTAL NITROGEN AND FREE AMINOACIDS IN THE LEAVES OF TOBACCO SEEDLINGS

The leaves of tobacco var. White Burley have been investigated without interruption, during a period of 2 years. Total nitrogen and the aminoacids, which occurs in a free state in tissues, have been determined.

In order to determine the amount of free aminoacids in the tissue, fresh leaves with 30% of water were crushed in a Waring Blendor. In the homogenate the amino N was instantly measured by the Van-Slyke nitrite method. The value for α-amino nitrogen so obtained corresponds evidently to the number of free aminoacids present without amide, ammonia nitrogen, peptide, peptone, proteose with a little amino protein nitrogen. According to Robert, Steinberg and others (1950) the free α-amino nitrogen content in leaf lamina of tobacco is approximately 76.7 to 100% of total non protein α-amino nitrogen.

The total N was determined by the micro-Kjeldahl method. According to Szmuk (1948) the chloroplastic and cytoplasmic proteins make up about 84% of the total nitrogen in tobacco seedlings. It is also possible that the α-amino nitrogen and total nitrogen may presumably serve for approximate measure of the sum total units used in the synthesis and break down of proteins.

The amount of the amino N and total N were expressed in miligrams per 1 gm of dry leaf.

When the plants had germinated they were transplanted into pots with garden earth or into washed sand, to which a full mineral nutrition was added. The investigations were done about a month after germination. The development of the plants was very similar from the beginning of March till the end of September (figs. 1, 2). Lower or higher temperature influenced only gradually a quicker or slower flow of the stages of development. The fluctuations in the amount of total nitrogen and amino N occuring in different types of leaves during the whole period were unnoticeable. Table 1 presents the mean of the analysis of youthful leaves during the above mentioned period.

The mature leaves were investigated in the sixth stage of development, when the youthful leaves had turned yellow.

According to K r e w s (1916) and H a m m e r (1940) the proteins concentrations in the successive leaves of tobacco are maintained at a relatively constant value. In our experiments total nitrogen in the youngest

Table 1

Total N and amino N in youthful leaves of different ages, mg per 1 gm of dry leaf

Stage of development length of leaves	Total N	Amino N
1-st stage leaves 4 to 6 cm long	40 — 42	4,2 — 4,9
2-d stage leaves 4,8 to 8,3 cm	39,4	5,72
3-t stage leaves 8,8 to 10,4 cm	40.2	71, — 7,3
4-th stage leaves 9—12 cm	36,1	7,4 — 7,9
5-th stage leaves 17 to 19 cm	36,4	8,23

mature leaves decreases in a small degree with the growth. On the contrary the amount of amino N increases hardly but distinctly with the growth of the mature leaves.

Table 2

Total N and amino N in mature leaves of different ages, mg per 1 gm of dry leaf

Stage of development	Total N	Amino N
leaves 6,5—7,5 cm long	30,2	6,75
leaves 8,2—9 cm	33,6	7,1—7,4
leaves 9,8—10,5 cm	28	7.8—8,1
leaves 11—11,5 cm	28	8,13
leaves 12—12,7 cm	25,2	8,4

In comparison to mature leaves the youthful leaves show a higher % of total N. Its amount accentuates itself mostly in the 1st and 3rd stage of development.

In the 1st stage, when the leaves measure 4 to 6 cm of length, the content of free aminoacids is strikingly, small, amino N amounts to 4—5 mg per 1 gm d. leaf. In the third stage when the leaves attain 10 cm of length, the amount of free amino N increases rapidly and attains 7 mg

per 1 gm d. leaf. In the following stage the amount of free aminoacids does not much increase and maintains itself on the same level a pretty long time. In leaves, which became yellow, the amount of aminoacids diminishes somewhat.

4. THE AMOUNT OF FREE AMINOACIDS IN EXCISED LEAVES OF TOBACCO

Vickery and others (1947) have proved that mature leaves of tobacco grown in the field, dipped in water aglomerate the amount of free aminoacids. After 100 hours that amount attains its maximal value. In our experiments youthful leaves in different stages of development from 6 cm to 13 cm long, as well as mature leaves in the first period of growth, were removed from plants and placed in water or solutions with ammonium or nitrate nitrogen. In all cases, after two days the excised leaves showed the same amount of total nitrogen, whereas the amount of free aminoacids regularly increased from 0.32 mg to 0.42 mg amino N per 1 gm of dry leaf.

Tobacco seedlings, grown in sand culture, supplied with ammonium or nitrate nitrogen did not differ in their contents of amino N from tobacco seedlings grown in garden soil. After excision the amount of free aminoacids augmented in their tissues in the same degree as in the plants grown in garden soil.

Steinberg Robert, and others (1950) found marked increases in free α-amino nitrogen in each instance where a mineral deficiency caused the appearance of severe symptoms. Moderate severity of deficiency symptoms were accompanied by a lesser accumulation of α-amino nitrogen.

5. THE AMOUNT OF FREE AMINOACIDS IN TOBACCO SEEDLINGS IN DEPENDENCE OF SEASONS

During a whole year, from January to December, the amount of free aminoacids was determined in youthful leaves, from 8 cm to 13 cm long, in the 3rd and 4th stage of their development, and in the developping mature leaves of about the same size. Excised leaves kept on nutrient solution on Petri dishes for two days have been investigated. The amount of free aminoacids in these leaves, as remarked above, in comparison with freshly picked leaves was somewhat higher (0.3 mg to 0.4 mg of amino N).

Fig. 10 presents the results of the experiments.

From the end of October to March a marked stoppage of growth of tobacco seedlings occured. In this winter time, December, January and at

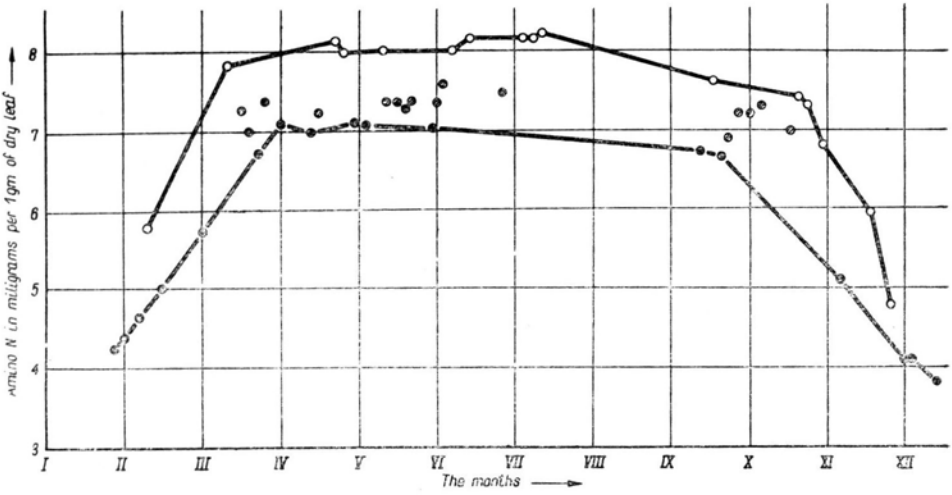

Fig. 10. Relation between the amount of amino N in leaves of tobacco seedlings in respect to month and the size of leaves. The black dots indicate about 8 cm long leaves. The empty circles indicate 12 cm. long leaves. Striped circles indicate leaves of a medium length.

the beginning of February the amount of free aminoacids in tobacco leaves was the lowest, from 3.5 mg to 4.6 mg of amino N per 1 gm of dry weight. Since the 5th od February begins a gradual increase of free aminoacids. About 20ty of March the amount of free aminoacids atains a level, which lasts until the end of October. In November the regression of free aminoacids in the leaves begins anew. In connection with this diminition of free aminoacids a stoppage of the development and growth of the leaves occurs.

6. THE MATURE LEAVES IN WINTER

Tobacco was sown in sand on the 10th July. During August the transplanted seedlings were watered with full nutrient solution. In the first days of September all the youthful leaves were developped, their length reached 14 cm.

On September 19th the first developping mature leaves 7 cm to 8 cm long contained 6.89 mg/1 gm d. w. of amino N.

On the 1st of October the youthful leaves began to get yellow. The developping mature leaves, above 8 cm long contained 7.1 mg/1 g d. w. of amino N.

On October 10th the mature leaves lengthened to 13 cm contained 7.48 mg of amino N. At the same time the stem began ot lengthen somewhat.

The end of October was a decisive moment in the development of the observed plants. The amount of free aminoacids decreased significantly.

Simultaneously the stem stopped to lengthen. The growth of the leaves was inhibited.

In November the plants were still not growing and remained in the same stage of development as in October. At the same time the amount of free aminoacids in the leaves decreased steadily and on November 30th reached the lowest level of 4.2 mg/1 g d. w. of amino N (fig. 11).

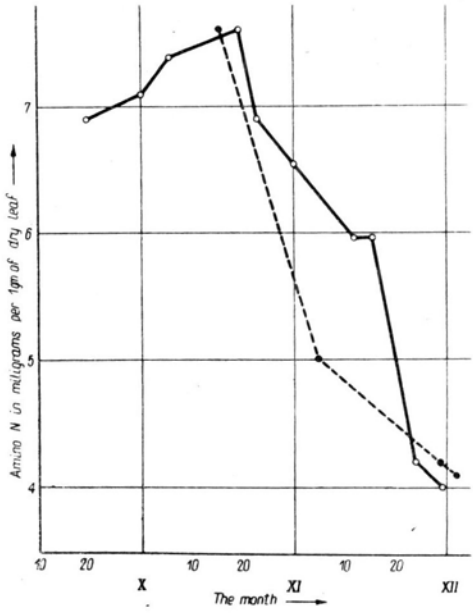

Fig. 11. The amount of amino N in mature and youthful leaves of tobacco seedlings from September to December. The black dots indicate the youthful leaves from 12 cm to 13 cm long. The empty circles indicate the mature leaves 7 cm to 13 cm long.

The plant remained in this state during December and January, did not grow and kept continually the same low level of aminoacids.

In February when the days lengthen, the plants began to deperish and did not start a new stage of development in spite of the comming spring.

The total nitrogen remained at the same level throughout the experimental time, namely 30.5 mg — 32.4 mg N per 1 gm dry weight.

7. DEVELOPPED YOUTHFUL LEAVES IN WINTER

The same change in the proportion of free aminoacids occurs in developped youthful leaves as in mature leaves.

The 1st experiment. Tobacco was sown at the end of August. In the first part of October the youthful leaves, from 10 cm to 12 cm long

contained 7.65 mg/1 g. d. w. of amino N. Later the subsequent development of the plant was very feeble.

On the 5th November the youthful leaves attained a length of 13 cm and the amino N fell to 5 mg/1 g d. w. (fig. 11).

On December 2nd, when the development of the plants was completely stopped, the amino N content in the developped youthful leaves was 4.15 mg/1 g d. w.

The 2nd experiment. Tobacco was sown in sand on September 15th and watered intensly with full nutrient solution. The youthful leaves attained 13.5 cm, when in mid-October a stoppage of development occured.

On November 30th the amount of amino N in youthful leaves 13 cm long, reached 4.22 mg/1 g. d. w.

The total N in the youthful leaves of the 3rd and 4th period of development was kept on the same level, which amounted to 35 mg per 1 g d. w.

8. YOUTHFUL LEAVES IN THE FIRST PERIOD OF DEVELOPMENT IN THE WINTER SEASON

In their first stage of development the youthful leaves, 4 to 6 cm long, have as pointed above a very small amount of amino N which amounts from 4.2 to 5 mg/1 g d. w. At this stage of development of tobacco seedlings, this amount is characteristic independently of the time it is sown (tab. 3).

Table 3

Amount of amino N in dependence of date of sowing

Date of sowing	Date of investigation	Size of leaves	Amino N
30.XII	4.II	5—6 cm	4,5 mg/1g
8.I	9.VI	4,8—8,3 cm	4,6 ,,
30.IV	15.III	6 cm	4,9 ,,

In normal conditions the first youthful stage of development lasts shortly. When the leaves grow the amount of aminoacids increases rapidly. Only in winter is the 1st youthful stage of development stopped for a few months.

Tobacco was sown on September 15th into garden soil and transplanted into pots a month later. In the second half of October they possessed 5 youthful leaves, $4^1/_2$ to $7^1/_2$ cm long.

At this time their development underwent a stop in their growth. At the end of November the amount of amino N in the leaves was 4.2 mg/1 g d. w.

On December 12th the seedlings possessed 5 developped leaves 6 cm to 7 cm long. The amount of amino N was 3.8 mg/1 g d. w.

On January 8th the appearance of the leaves was unchanged, 5 youthful leaves to 8 cm long. The amino N = 4.5 mg/1 g d. w.

On January 30th no change in the development of the plant was visible. Amino N = 3.8 mg/1 g d. w. (fig. 12).

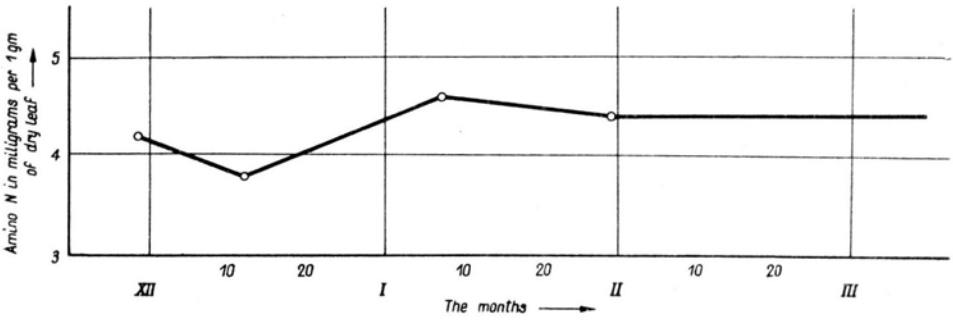

Fig. 12. The amount of amino N in „old age" youthful leaves from December to February.

In February the investigated seedlings died out, they were not capable of further development. On the contrary tobacco plants sown in the first days of January which in the beginning of February were in the first stage of youthful development and did not differ from the "old age" youthful plants kept over the winter time, entered normally into the next stages of development.

9. INTENSITY OF RESPIRATION OF TOBACCO SEEDLINGS FROM THE END OF DECEMBER UNTIL APRIL

The dynamic state of proteins, in the living organism is in strict connection with the absorption of oxygen, output of carbon dioxide and the accumulation of carbohydrates (S t i l e s 1952). A part of the carbon dioxide emitted during the respiration derives from the degradation of the proteins (G r e g o r y and S e n 1937). The stronger is the degradation and resynthesis of proteins the more intensive is the respiration (P e a r s a l l 1949).

W o o d and G r u i c k s h a n k (1944) have obtained positive correlations between respiration rate and aminoacid content, when the carbohydrate content in the plant was high.

In our experiments the respiration rate was measured in winter and at the beginning of spring by Warburg's method. The measurments were performed every 3 days.

150 mg of samples cut from tobacco leaves were placed in a darkened room in the Warburg respirometer vessel and covered with 1/15 M phosphate buffer. The vessel side arm contained 30% KOH to absorb the carbon dioxide released by respiration. The vessels were attached to Warburg manometers and shaken at the rate of 100 oscillations per minute in a water bath at 26°C. The vessels and their contents were allowed to equilibrate for a period of 60 minutes. Readings of the oxygen uptake were taken at 10 minutes intervals. Calculations were made to convert the oxygen uptake values to microliters uptake of oxygen per 150 miligrams fresh weight of the leaf samples.

The 1st experiment. Tobacco seedlings sown in September and stopped in their development were investigated from December 12th to Ferbruary 2nd. The length of the investigated leaves varied from 3.6 cm to 6 cm. The amount of absorbed O_2 by 150 mg fresh leaf tissue during

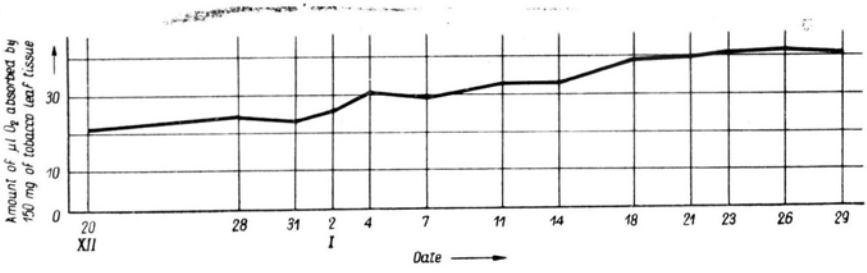

Fig. 13. Intensity of respiration youthful but „old in age" leaves from 20 December to February 2-nd (performed by M. Dwuraźna).

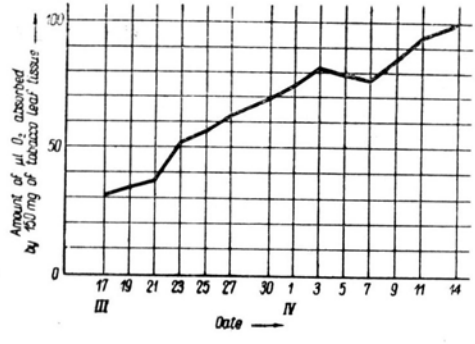

Fig. 14. Intensity of respiration of young leaves from March to April.

36 days remained more or less on the same level, 20—30 µl O_2 (fig. 13) during 60 minutes. The level of amino N was also low 3.8—4.6 mg/l g d. w. (fig. 11).

In the 2nd experiment tobacco seedlings which were sown at the beginning of January and underwent a normal stage of development were investigated.

The intensity of respiration in the first phase of development from March 17th to March 24th, when the leaves attained 6 cm of length, differed only slightly from the intensity of respiration of equally developped seedlings, which lived through the winter season in their „old age" youthful stage. The following increase in intensity of respiration begins only with the growth of the leaves. At the same time the amount of free aminoacids increases steadily. On April 27th, when the youthful leaves attained 18 cm of length and contained about 8.4 mg/1 g d. w. amino N, the respiration became very intense and manifested itself by the absorption of 90 µl O_2 per 150 mg of living tissue (fig. 14) during 60 minutes.

CONCLUSIONS

1. Independently of their age the leaves of tobacco seedlings stop their development at the end of October. At the same time the amount of free aminoacids diminishes in their tissue. At the end of November the amount of amino N attains its lowest level, which amounts to about 4 mg/1 g d. w. That state lasts the whole winter. In consequence the degradation and the resynthesis of protein are very slow.

2. The youthful leaves, which were in their 1st stage of development at the end of October remain in this state during the whole winter. The corresponding level of amino N (about 4 mg/1 g. d. w.) lasts during the winter months until February. The intensity of respiration maintains itself also at the lowest level.

3. The seedlings which in late autumn were stopped in their 1st stage of development lose the capacity of a subsequent development in February and March, whereas the tobacco, which were sown in January, begin to develop normally at the same time.

4. Youthful but „old in age" leaves in which the amino N maintains itself on a low level and the respiration is feeble have the proteins in a dynamic state in which the degradation and regeneration are slow. Such an organism loses the capacity of reaching a further stage of development even in optimal conditions.

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