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Comparison of spurs and leaves of annually and biennially bearing apple trees of 'Perkins' variety in respect to their contents of nitrogen compounds

Porównanie krótkopędów i liści drzew corocznie i przemiennie owocujących jabłoni odm. Perkins pod względem zawartości związków azotowych

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In our former investigations on several biennially bearing apple varieties (Bielińska 1954, 1956, 1957 a, b, Dzięcioł and Bielińska 1962, Włodek and Bielińska 1963) a higher nitrogen content (particularly that of the soluble fraction) was found in spurs and leaves of the trees in the "on" year. Such differences occurred, no matter whether the trees were naturally biennial bearing or only forced into that condition, provided it was done early enough (Włodek and Bielińska 1963).

The following authors: Hooker (1920), Sahulka (1962), Grochowska (1963), Hołubowicz (1963) have been comparing biennial and annual bearing varieties from the point of view of nitrogen compounds content, however, the investigated trees of both types never were of the same variety.

The authors were successful to find in 1962 a group of annually and biennially bearing trees of 'Perkins' variety in the experimental orchard at Sinołęka (Eastern Poland). The purpose of the present study was to compare the content of nitrogenous compounds in leaves from long shoots and bearing and non-bearing spurs of both: biennial and annual bearing trees of 'Perkins' variety.

MATERIAL AND METHODS

Rootstock seedlings with interstock-varieties: 'Ohm Paul', 'Yellow Transparent' and 'Transparente de Cronsels', planted in 1940, were top worked in 1949 and 1950 with scions of 'Perkins' variety. The trees grew on a medium podsolic loam developed on a medium glacial clay. Beginning from 1953 sod was introduced as cower in every second row of trees and the whole field was under grass by 1959. Heavy dosages of fertilizers (150 kg N/ha and 180 kg K_2O/ha) were applied in 1961 and 1962.

Although the total amount of precipitation (484,2 mm) was lower than the mean value for many years (515 mm), a considerable share of it fell during the month of May (113,9 mm) and June (113 mm).

Nine trees were investigated: three on each interstock in the following three groups: a) annually bearing, b) biennially bearing that produced a crop in 1962, and c) biennially bearing that were not fruiting in 1962. Apical parts of spurs comprising their last year's growth, were sampled four times: on May 17, June 22, July 20 and August 24. From biennially bearing trees being in "on" year fruiting spurs were collected and analogically from "off" trees — non fruiting ones. From annually bearing trees the samples of actually bearing and non-bearing spurs were taken separately. The samples of leaves were collected from central parts of the shoots three times: on June 22, July 20 and August 24. The spurs were freeze dried, the leaves were dessiccated in a fan-dryer at 50°C. Dry matter, total and insoluble nitrogen (by the Kjeldahl method) were then determined after extraction with boiling water for ten minutes: soluble nitrogen content was computed as a difference between total and insoluble nitrogen.

The extract was dried completely on a water bath at the temperature of approximately 70°C; the remaining dry matter was then dissolved in 2 ml of water and centrifuged. The supernatant was analysed chromatographically on strips of Whatman No. 1 filter paper prepared according to Matthias (1956). To compare the proportions of different amino acids and amides in the soluble nitrogen fraction aliquots of these fraction were applied to the strips of filter paper. Those were developed five times, in two solvents: 1) butanol: acetic acid: water (90 : 10 : 29, acc. to Fowden 1961), and 2) 70 % propanol. Detection with 1 % solution of ninhydrin in acetone and drying for 24 hrs at room temperature was followed by spraying with the solution of copper nitrate (Block 1958). Extinction of the chromatograms was measured by the aid of Pulfrich photometr equipped with the adapter „Elpho", using a device for visual measurements of the paper electrophoresis strips in transmitted light.

Additionnally, a low-voltage paper electrophoresis was done of an extract in a buffer: pyridine: acetic acid: water (6 : 20 : 1000) acc. to Matthias (1959). From 2,2 μl to 8,8 μl of extract were applied, depending on the amount of soluble nitrogen present in the sample. The extinction readings were made as described above, and the obtained results compared with the standards.

The results of nitrogen and dry matter determinations were worked out with the method of analysis of variance (R. A. Fisher), as a case of triple classification, including elimination of the individual influence of the trees. Student test "t" was used for evaluation of the significance of differences. Two levels of $p = 0{,}05$ and $0{,}01$ were applied. The corresponding values of LSD were marked with: m_1 and m_2.

RESULTS

1. Percentage of total nitrogen in dry matter of leaves from long shoots

The differences between the biennial bearing apple trees, in the "on" and "off" years were significant at all the dates (Fig. 1). At the time of first sampling the leaves from the annually bearing trees showed the total nitrogen content similar to that of the leaves of the biennially bearing trees in the "on" year. At the remaining dates leaves from the annually bearing trees contained the intermediate amount of nitrogen between those found in the leaves from trees being in "on" year and "off" year.

2. Percentage of dry matter and nitrogen contents of the current year (1962) growth of spurs

The figures 2—6 illustrated the contents of dry matter and nitrogen (total, soluble and insoluble) in the spurs. The differences between the spurs of trees being in "on" year and in "off" year were very pronounced. The results are in agreement with the previously obtained (Dzięcioł and Bielińska 1962, Włodek and Bielińska (1963). Concerning the comparison of the biennially and annually bearing trees, it was interesting to find that the contents of the above mentioned constituents are similar in the bearing spurs of the both groups of trees. Also the non-bearing spurs of the two types of trees revealed the conspicuous similarity. The span of differences in the content of dry matter and nitrogen compounds between the bearing and non-bearing spurs was a little less for the annually bearing trees than for the biennially bearing ones.

3. Share of different amino acids and amides in the pool of soluble nitrogen in spurs

The chromatographic analyses (fig. 7) were perforred for all the trees in the study. The results were represented in graphs, two of which are included herein (fig. 8 and 9). Concerning the fruiting spurs there were no clear-cut differences between the annually bearing trees and those in an "on" year. There were also no obvious differences between the non--fruiting spurs collected from annual and biennial bearers. On the contrary very conspicuous differences were found between fruiting and non fruiting spurs irrespective of the type of tree. Those differences concerned mainly arginine, glutamine and glutamic acid. In the bearing spurs there was found on higher proportion of arginine (fig. 9) and glutamine (fig. 8 and 9), and a lower proportion of glutamic acid (fig. 8 and 9). As to

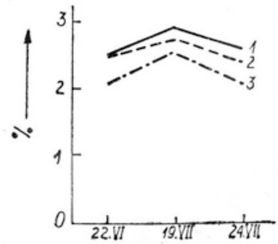


Fig. 1

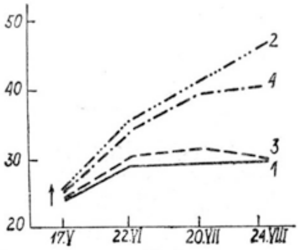
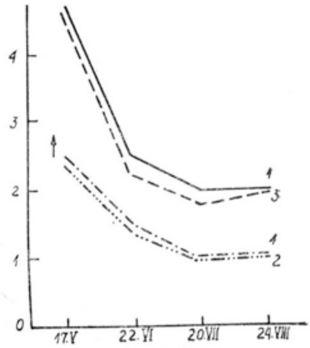


Fig. 2



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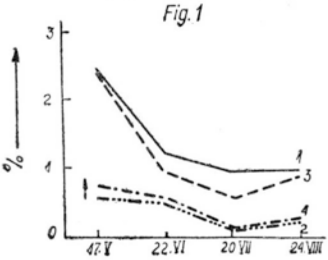

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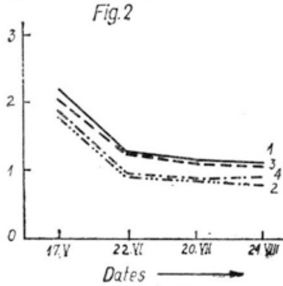

Fig. 5

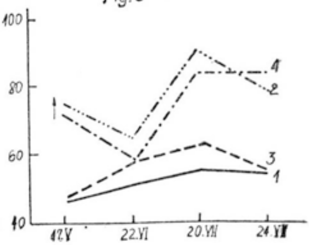

Fig. 6

Fig. 1. Total nitrogen content in leaves from long shoots (as percentage of dry matter) *

Fig. 2. Dry matter of spurs ($m_1 = 2,52$).

Fig. 3. Total nitrogen in spurs (percentage of dry matter); $m_1 = 0,264$.

Fig. 4. Soluble nitrogen in spurs (percentage of dry matter); $m_1 = 0,221$.

Fig. 5. Non-soluble nitrogen in spurs (percentage of dry matter) *.

Fig. 6. Non-soluble nitrogen expressed as per cent of total nitrogen ($m_1 = 6,33$).

Abbreviations:

Fig. 1: *1* — biennial bearing, trees in "on" year; *2* — annually bearing trees; *3* — biennial bearing, trees in "off" year.

Figs 2—6: *1* — bearing spurs of trees in "on" year; *2* — non-bearing spurs trees in "off" year; *3* — bearing spurs of annually bearing trees; *4* — non-bearing spurs of annually bearing trees.

* On the figures 1 and 5 LSD value was not given because interaction: type of tree (spurs) × date was not significat. The influence of fruiting occured independently from the term.

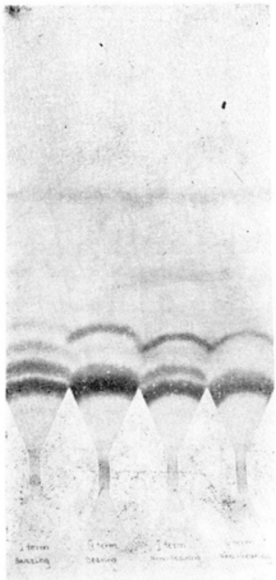

Fig. 7. Chromatograms developed five tmes in butanol: acetic acid: water. Two strips on left: bearing spurs of the same tree in first and fourth date of sampling. Two strips on right: analogically non-bearing spurs.

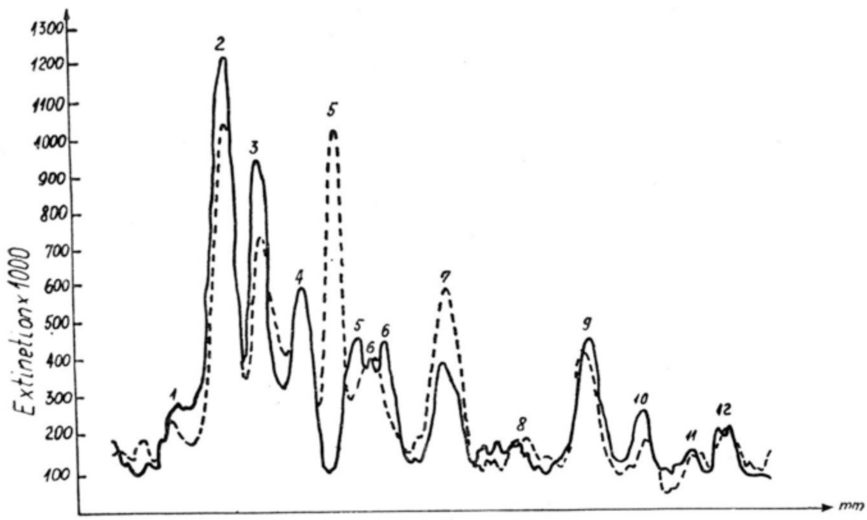

Fig. 8. Proportions of free amino acids and amides in soluble nitrogen in bearing spurs (continuous line) and non-bearing spurs (dashed line). Chromatogram was developed five times in butanol: acetic acid: water.

1 — Lysine, *2* — Arginine + Asparagine, *3* — Glutamine + Aspartic Acid, *4* — Serine, *5* — Glutamic Acid, *6* — Threonine, *7* — Alanine, *8* — Tyrosine, *9* — γ aminobutyric acid, *10* — Valine + Methionine, *11* — Phenylalanine, *12* — Leucine.

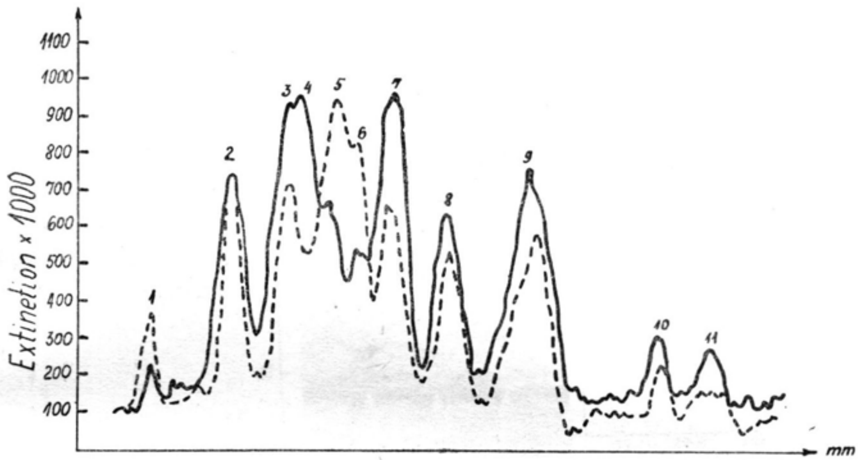

Fig. 9. Proportions of amino acids and amides in soluble nitrogen in bearing spurs (continuous line) and in non-bearing spurs (dashed line). Chromatogram was developed five times in 70 % propanol

1 — unidentified, *2* — unidentified, *3* — Aspartic acid, *4* — Arginine, *5* — Asparagine, *6* — Glutamic acid, *7* — Glutamine, *8* — Serine, *9* — Alanine + Threonine + γ aminobutyric acid, *10* — Valine + Methionine, *11* — Leucine.

the other amino acids and amides — the differences between the bearing and non-bearing spurs were smaller or absent. A trend for higher proportions of threonine, aspartic acid and an unidentified amino acid of a basic character (peak 2, fig. 9) was observed in the bearing spurs — and a lower proportions of asparagine, alanine, sometimes of γ-aminobutyric acid, and an unindentified compound having the highest R_f value in both solvents (This compound assumed the yellow color under the influence of ninhydrin, which persisted after spraying with copper nitrate). This spot was particularly pronounced on the fourth sampling date.

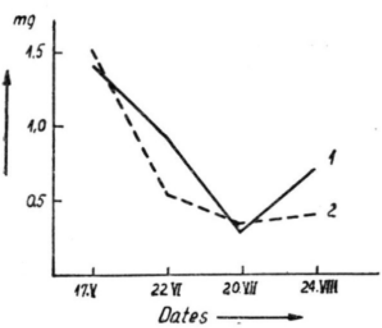

Fig. 10. Averages contents of glutamic acid in spurs (in mg/1 g air dry matter)

1 — bearing spurs;
2 — non-bearing spurs.

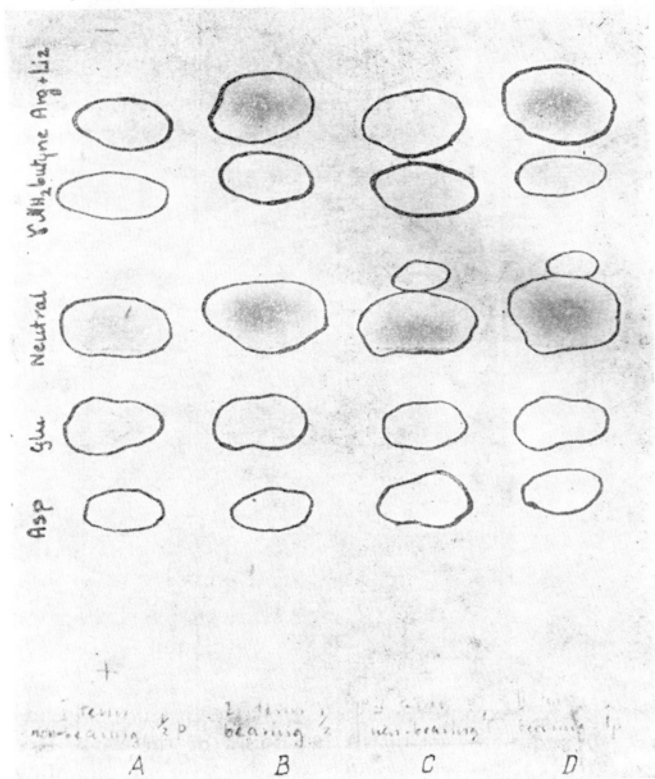

Fig. 11. Electropherograms of extract from bearing (B, D) and non-bearing (A, C) spurs of the same tree in first (A, B) and second (C, D) date of sampling

In relation to the high proportion of glutamic acid in non-bearing spurs, a question arose, pertaining to its absolute amount in the dry matter of spurs. To answer this question readings were taken on the electropherograms, where separation of glutamic acid from remaining acids is very distinct (fig. 11 and 12). It was found that the level of glutamic acid in both kinds of spurs was approximately equal (fig. 10), on May 17-th and July 20-th, and was higher in bearing spurs on June 22-nd and August 24-th. The lowest content of glutamic acid was observed on July 20-th. Similarly the content of aspartic acid, basic amino acids and amines (as expressed in absolute amounts) was on the same date lowest.

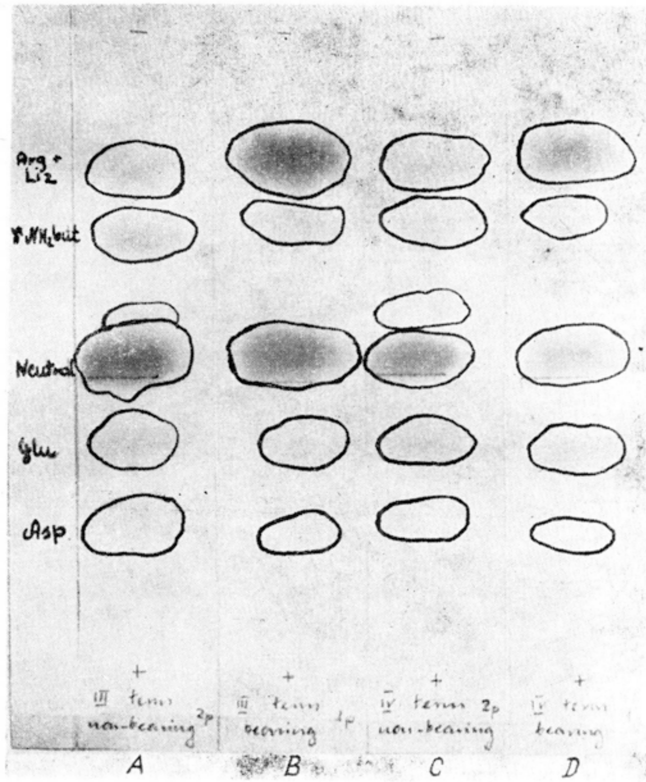

Fig. 12. Electropherograms of extract from bearing (B, D) and non-bearing (A, C) spurs of the same tree in third (A, B) and fourth (C, D) date of sampling

There appeared on electropherograms a blue — pink spot above the place occupied by neutral amino acids. It turned blue-violet when sprayed with copper nitrate. On the first sampling date it appeared only accasionally in trace amounts in the non-bearing spurs. In the sample of June 22-nd this spot was very distinct in material from both kinds of spurs. Later — its intensity was smaller; its proportion in the soluble nitrogen fraction was greater in non-bearing spurs. The location of that spot on the electropherograms and on the chromatograms developed in butanol: acetic acid: water corresponded to the β-alanine. However, its color was not in agreement with that of the standard.

DISCUSSION

Analyses of the leaves from long shoots for total nitrogen confirm the data obtained earlier (C a i n and B o y n t o n 1948, B i e l i ń s k a 1954, 1956, 1957 a, b, L a m b and G o l d e n 1959, G r o c h o w s k a 1963).

They have shown its higher content in bearing trees. Boynton and Compton (1945) and Boynton et all. (1950) were of the opinion that nitrogen level in leaves is correlated with the yield. This point of view would seemed to be confirmed by our results, since the annually bearing trees, which yielded less abundantly than the biennially bearing trees in the "on" year, showed also a lower level of total nitrogen on the later sampling dates.

The results concerning the content of nitrogen compounds in the spurs indicate that it depends mainly on the bearing of the spurs, and to a much lesser degree on the fact whether the spurs are located on annually — or biennially bearing tree. The presence of both kinds of spurs on the same annually bearing tree, affected only slightly the differences in their nitrogen content.

The technique of chromatographic readings used in this work did not allow to determine the whole number of amino acids found in the former works (Dzięcioł and Bielińska 1962, Bielińska 1963, Bielińska and Casimir 1964). It made possible, on the other hand, to compare the proportions of different amino acids and amides in the soluble fraction of nitrogen. The results thus presented could not be regarded as fully quantitative because only a short section of the extinction curve approximates sufficiently the straight line. Nevertheless they do indicate clearly enough the differences in the metabolism of nitrogen in both kinds of spurs.

The results of the present work are partially in agreement with those obtained by Sahulka (1962) who investigated the cluster bases with fruits and without them the has found in the fruiting ones an absolutely greater amount of the arginine and glutamine and their higher share in the soluble nitrogen fraction. It has to be stressed, however, that those data were obtained from a fairly heterogenous material.

The above mentioned yellow spot is probably indentical with the one formerly observed on the two-dimensional chromatograms of the crude extract (Dzięcioł and Bielińska 1962), but, considering its high R_f value, probably it is not the 4-hydroxymethylproline (Bielińska 1963).

It seems rather premature to set up any hypothesis concerning the role of different amino acids in relation to the fructification. Out of the three amino acids which occurred in different proportions in soluble nitrogen fraction of bearing and non-bearing spurs the arginine and glutamine may possibly be related to the process of fructification. In the authors opinion, however, it is not the case with glutamic acid, because its content was equal in both kinds of spurs or slightly higher in the bearing when expressed in absolute amounts of dry mater.

A high proportion of glutamic acid in relation to the other amino acids in non-bearing spurs is caused probably by the apparent lower content of all other amino acids in those spurs.

SUMMARY

The higher content of nitrogen compounds in the bearing apple spurs than in non-bearing ones was conspicuous regard-less annual or biennial fruiting habit of those trees.

In the bearing spurs the share of arginine and glutamine in the soluble nitrogen fraction was larger whereas the share of glutamic acid was lower than in the non-bearing spurs.

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STRESZCZENIE

Doświadczenie zostało przeprowadzone w 1962 roku, w Zakładzie Naukowo-Badawczym Instytutu Sadownictwa w Sinołęce i objęło 9 drzew odmiany 'Perkins': 3 corocznie i 6 przemiennie owocujących (z których połowa była owocująca, połowa nie owocowała). Drzewa były posadzone w roku 1940 na podkładkach generatywnych, w 1949 i 1950 r. przeszczepione odmianą 'Perkins'. Próbki krótkopędów pobrano czterokrotnie: 17.V, 22.VI, 20.VII i 24.VIII. Z drzew corocznie owocujących badano osobno próbki krótkopędów owocujących i nie owocujących. Próbki liści pobrano trzykrotnie, w tych samych terminach, z pominięciem pierwszego.

Przeprowadzono analizy na zawartość azotu (ogólnego oraz rozpuszczalnego i nierozpuszczalnego), suchej masy, oznaczenia chromatograficzne równoważnych ilości azotu rozpuszczalnego (celem określenia udziału poszczególnych aminokwasów) oraz elektroforezy niskonapięciowej ekstraktu.

Poziom azotu w liściach drzew corocznie owocujących był w pierwszym terminie taki sam, jak u liści drzew przemiennie owocujących w roku owocowania. W następnych terminach liście drzew corocznie owocujących zawierały pośrednią ilość azotu ogólnego w porównaniu z liśćmi drzew przemiennie owocujących, w roku owocowania i nieowocowania (wykres 1).

Zmiany zawartości azotu i suchej masy w obu rodzajach krótkopędów przebiegały podobnie u drzew corocznie i przemiennie owocujących (wykresy 2—6). Rozpiętość różnic w poziomie tych składników była mniejsza u drzew owocujących corocznie.

We frakcji rozpuszczalnej azotu krótkopędów owocujących stwierdzono wyższy udział argininy i glutaminy, a niższy kwasu glutaminowego w porównaniu do krótkopędów nie owocujących; nadto wystąpiła tendencja do wyższego udziału treoniny i kwasu asparaginowego, a niższego — alaniny i asparaginy (fot. 7, wykresy 8 i 9). W ilościach bezwzględnych poziom kwasu glutaminowego był

początkowo taki sam w obu rodzajach krótkopędów, następnie wyższy w krótkopędach owocujących (fot. 11, 12, wykres 10). Minimum zawartości kwasu glutaminowego, asparaginowego, aminokwasów zasadowych i amin obserwowano w lipcu. Zawartość aminokwasów obojętnych i kwasu γ-aminomasłowego malała stopniowo z upływem wegetacji.

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