

The content of soluble and insoluble nitrogen compounds in spurs of biennial bearing apple trees

Zawartość rozpuszczalnych i nierozpuszczalnych związków azotowych
w krótkopędach jabłoni przemiennie owocujących

U. DZIĘCIOŁ AND M. BIELIŃSKA-CZARNECKA

INTRODUCTION

In their investigations on biennial bearing apple trees Potter and Phillips (1930), Ursulenko (1955), Bielińska (1956, 1957) found a close relationship between the fruiting and the content of insoluble nitrogen compounds in fruit-bearing organs of the apple trees.

In the present studies the previous (Bielińska 1956, 1957) investigations were continued with particular emphasis on different methods of evaluating the data of nitrogenous fractions. Soluble and insoluble nitrogen was related to dry matter and total nitrogen content of the spurs. Also the ratio of soluble to insoluble nitrogen was calculated.

REVIEW OF THE LITERATURE

Potter and Phillips, in 1930, were the first investigators who found a close relationship between the form of organic nitrogen compounds in fruit bearing organs and flower bud formation in apple trees. Their experiment was carried out on 30-year old apple trees of the Baldwin variety. Samples of present year's growth of spurs were taken in July and in August. In the following spring there was counted the percent of the flower bud set on the same trees. A relationship between the amount of insoluble nitrogen in the spurs and the flower bud set was found. The highest coefficient of correlation (0,607) was obtained when the insoluble nitrogen was related to 100 spurs, the lower (0,456) when related to fresh matter, the lowest one (0,438) when related to dry matter; however, as regards statistics, the relationship was significant in all three cases.

Some years later, in 1939, Ursulenko (1955) studying the spurs

of heavy, light and not flowering trees of Antonovka variety found a relationship between the amount of protein nitrogen (as compared with total nitrogen) and the flower bud formation. In the spurs of the trees with light or no blossom the amount of protein nitrogen (higher in the case of no blossom) was increasing from outset of blossoming till the flower bud formation; at the same time the amine nitrogen was decreasing. In the spurs of heavily blossoming trees the amount of protein nitrogen was decreasing till the time of June drop, and afterwards slightly increased, remaining however the lowest of three kinds of spurs investigated; the amine nitrogen was increasing from the beginning of the period of blossom till the time of flower bud differentiation. Ursulenko's opinion was that flower bud differentiation takes place when 70—80 per cent of nitrogen occurs as protein nitrogen. He explains a decrease in the protein synthesis in fruit bearing spurs as due to the utilization of carbohydrates for flowering and then for fruit development.

Another investigator, however, Monastyrskij (according to Rubin 1958) did not confirm that relation between the amount of protein nitrogen (as compared with total nitrogen in spurs) and the flower bud formation. He found the setting of the buds at the higher amount of protein nitrogen as well as at the lower one; yet, he found, the buds were not setting at a relatively very high amount (about 90 per cent) of protein nitrogen.

Kolomijec (1955) found a higher amount of protein as well as of total nitrogen in a dry matter of bearing spurs in the period from June till the end of July, afterwards those amounts were aequal. Kolomijec considered, however, that a proper level of concentration of cell sap in the buds is the condition of flower buds differentiation.

Similar to Ursulenko's results were obtained by Bielińska (1956, 1957). The investigated present year's growth of spurs in apple of Grosser Rheinischer Bohnapfel, Wealthy, Antonovka, and Fameuse. The samples were taken on three following dates: in the period of flower bud differentiation, in the rest period and in the period of buds swelling. In those investigations she found a higher amount of protein nitrogen (as compared to total nitrogen) in non-bearing spurs, setting flower-buds for the following year. In bearing spurs there was a higher amount of soluble nitrogen as related to total nitrogen and to dry matter.

Some authors (besides above mentioned Hooker 1920, Kraybill and comp 1925, Rubin 1958, and others) found higher amount of total nitrogen in spurs of bearing trees as compared to non-bearing ones. There were particularly big differences in spring — in the period of maximum amount of nitrogen, or at the beginning of summer — in the period of intensive vegetative growth.

Changes occurring in the amount of total nitrogen in different tissues of a tree during the year are generally known. Much less is known of changes in the amount of soluble and insoluble nitrogen. Kobel has written in his handbook published in 1954: „Über die Schwankungen im Gehalt an Eiweissstoffen, die unsere Obstbäume im Verlauf des Jahres aufweisen, sind wir nur ungenügend unterrichtet" (We do not know enough of the fluctuations during a year in the content of protein in fruit trees). At the same time he quotes the results of Traub's investigations, who had found that the amount of amine nitrogen was the highest in the period of an active growth and the lowest in the period of dormancy; protein nitrogen behaved reversely.

In recent years Oland (1954, 1956, 1959) studied the changes in the amount of soluble and insoluble nitrogen in one-year-old and two-year-old apple trees grown in sand cultures. He investigated one-year-old shoots, stems, roots (and also leaves) 3—6 times during the vegetative period. In most cases he observed spring maximum and autumn minimum in the amount of soluble nitrogen. Fluctuations of the amount of insoluble nitrogen, particularly in stem, were relatively low. In some cases he observed decline of the amount of insoluble nitrogen in the time of spring maximum of the amount of soluble nitrogen. The amount of insoluble nitrogen in roots and in one-year-old shoots was the lowest in autumn. Besides, Oland determined chromatographically amino acids and amides of the soluble fraction of nitrogen. He found about twenty amino acids and amides (two spots remained unidentified). The author considers that soluble nitrogen, and mainly free amino acids and amides, play an important role as nitrogenous reserves in apple trees.

Bollard (1953) investigated tracheal sap of apple trees. He identified somewhat less number of amino acids and amides than Oland, but some of them were different.

Lately Sahulka and Silova (1960) investigated the quantitative changes of ten free amino acids and amides in leaves of apple trees before and during the time of flower bud differentiation. They observed decreasing amount of aspartic acid and increasing quantity of γ -amino butyric acid prior to flower bud differentiation. Arginine was decreasing in leaves at the time of flower bud differentiation. The amount of other amino acids did not undergo any substantial changes.

MATERIALS AND METHODS

The experiment was started in spring of 1957 and conducted on ten-year-old apple trees of the following varieties: Reder's Goldreinette, Boiken and Macoun, grown in the experimental orchard of the Research

Institute of Pomology at Dąbrowice (central Poland). Reder's Goldreinette is a typical biennial bearing variety; Boiken bears biennially but fits to be forced to annual bearing; Macoun bears biennially with a tendency to annual bearing.

In some of the trees (with small quantity of flowers) or in halves of the tree-crowns there were picked off the flowers to obtain the trees or halves of them non-bearing in the given year. The experiment consisted of the following trees of each variety: two bearing trees, two non-bearing and two trees bearing only in a half of their crowns. This experiment was carried out till autumn 1958.

Samples of present year's growth of spurs were collected four times a year: in the period of flower bud differentiation, at the harvest in the dormant season and in spring — at the time of bud swelling, namely on: July 31 — August 2, September 19—21 of 1957; January 3—7, April 24—25, July 2—4, September 24—26 of 1958. Sixty and forty spurs were collected on each sampling date from non-bearing and bearing trees respectively. Immediately after collection the samples were cut finely with a knife and subsamples were weighed for the following determinations: dry matter, total nitrogen and soluble or insoluble nitrogen. The ethanol extract of soluble nitrogen was chromatographed to determine amino acids and amides.

The total nitrogen was determined by Kjeldahl's method, using 0,05 hydrochloric acid in receiver and Tashiro mixture as indicator. The ethanol extract was prepared in the following way: two gm of fresh material was ground in the mortar with 80 per cent ethanol and glass sand. The extract was transferred into centrifuge tubes, and the remainder in mortar was washed out with ethanol five times. The 50 cc. extract was centrifuged for five minutes at 3000 rpm. Supernatant was poured off, and the sediment was mixed with a new portion of ethanol and again centrifuged; that was repeated twice.

For determining the soluble nitrogen by Kjeldahl's method the combined extracts were at first evaporated in Kjeldahl's flasks. The amount of insoluble nitrogen was calculated from the difference between the amount of total and soluble nitrogen.

For chromatographic determination of amino acids and amides the ethanol extract was evaporated on the water bath at 80° C, then transferred into the flasks of 25 c.c. and filled with redistilled water. 200—700 μ l of this solution was transferred on the chromatographic paper Whatman No 3 corresponding to 40 μ g of amine nitrogen.

Since the above described method was very laborious, in 1958, instead of using centrifuge, the sediment together with ethanol extract was transferred on the weighed filter paper and washed with 80 per cent ethanol to a constant volume. The filtered extract was evaporated at 60° C and then was dissolved in two c.c. water. 20—50 μ l of the aliquot were transferred on the chromatographic paper for determination of amino acids and amides. The sediment remaining on the filter paper was used to determine the content of insoluble nitrogen. The soluble nitrogen was calculated from the difference between the total and the insoluble nitrogen. On five paralld samples repeated three times it was verified that the results obtained by these two methods were almost identical and the differences remained in a range of the technical error.

In order to verify the method used, the protein and the soluble nitrogen was also determined in samples extracted with 20 per cent three-chloro-acetic acid. The mean results obtained in this extract were lower for protein nitrogen by over nine per cent, what indicated in effect nearly twice as much the amount of soluble nitrogen.

The determining of the free amino acids and amides was made by two-dimentional ascending chromatography on Whatman's No 3 filter paper of the size 28 \times 23 cm. The first solvent was n-butanol-acetic acid-water (4 : 1 : 1), and the second solvent was phenol water (4 : 1) in ammonia atmosphere. The spots were developed with 0,2 per cent solution of ninhydrin in acetone. Occasionally 0,4 per cent solution of isatin in acetone was used for developing the spots (Block 1958, Opieńska 1957).

The data obtained were analysed statistically using R. A. Fisher's method of analysis of variance for a relative arrangement. The significance of the differences was evaluated with T. Student's test. In the investigation there were accepted two levels of confidence: $\alpha_1 = 0,05$, $\alpha_2 = 0,01$. The corresponding values of the confidence interval were denoted: m_1 and m_2 .

In calculations of total nitrogen and soluble nitrogen content, separate statistical analysis was conducted for the trees bearing on their full crowns and for the trees bearing on one half of their crowns. No significant differences were found i.e. the halves of the crowns behaved similarly as the whole trees. Because of that for the purpose of further statistical analysis the halves of the crowns were treated as the whole trees.

Weather conditions in both years of the experiment were similar except that in 1957 it was somewhat warmer and wetter than in 1958.

RESULTS

Dry matter

On Fig. 1 determinations of the amount of dry matter of spurs are given. These results indicate quite similar course of changes in the amount of dry matter in all three varieties during a year. The minimum of the amount of dry matter in spurs of the bearing as well as non-

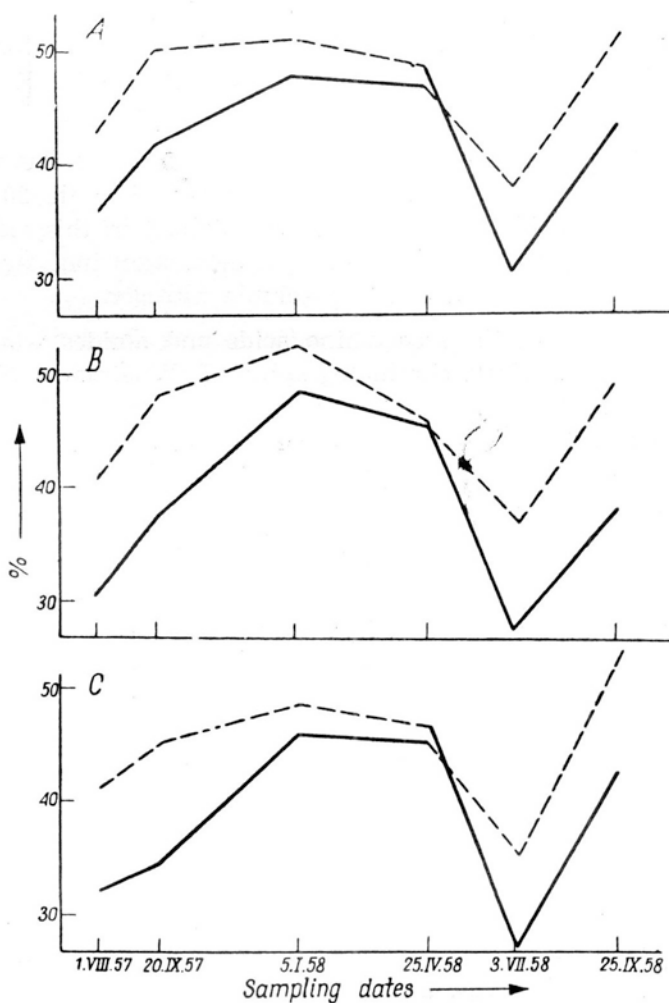


Fig. 1. Dry weight of the spurs of varieties: A — Macoun var.; B — Reder's Goldreinet var.; C — Boiken var.

———— bearing; - - - - - non-bearing

In all cases, except for Fig. 2, the spurs which were bearing in 1957, became non-bearing in 1958, and vice versa.

-bearing apple trees, was in the samples collected in July, the maximum — in the winter. The non-bearing spurs contained more dry matter than bearing spurs, except spring time (significance of interaction: kind of spurs \times date) when the amount of dry matter equalized in both kinds of spurs. With regard to the amount of dry matter the differences between the bearing and non-bearing spurs, and between the dates, were significant at 0,01 level. Besides, the Macoun variety contained significantly higher average amount of dry matter (43,28 per cent) than other varieties (41,73 per cent and 41,34 per cent; $m_1 = 1,063$, $m_2 = 1,457$).

Total nitrogen

The average amount of total nitrogen in the bearing spurs was higher (1,98 per cent) than in non-bearing ones (1,37 per cent). These differences were the highest in the samples collected in the summer (significance

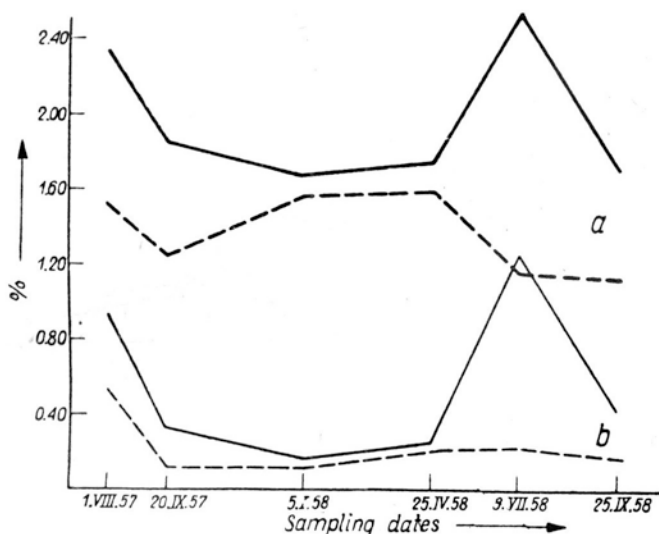


Fig. 2. Total nitrogen (a) and soluble nitrogen (b) contents in the apple spurs. (Percent of dry weight). Interactions kind of spurs \times date
 — bearing; - - - - - non-bearing

of interaction: kind of spurs \times date). The maximum of the nitrogen content was in the bearing spurs at that time. (Tab. 1, Fig. 2 and 3). The smallest differences were in the period from January to April (they remained significant then for the Boiken variety sampled in both that dates as well as for the Reder's Goldreinette sampled in January. Tab. 1).

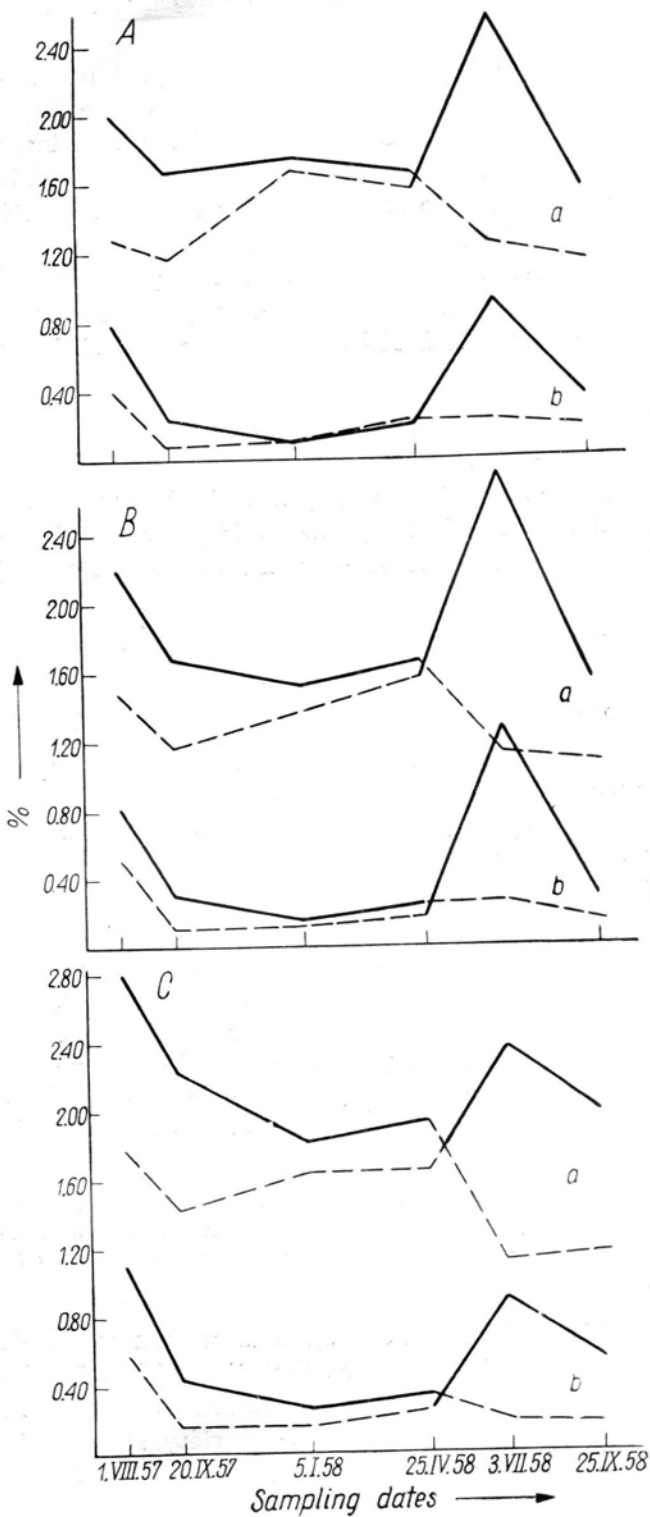


TABLE 1

Average contents of total nitrogen in the apple spurs (percent of dry weight)

Variety	Kind of spurs	Dates of sampling						Average
		31.VII— 2.VIII. 1957	19—21 IX. 1957	3—7. I. 1958	24—25. IV. 1958	2—4. VII. 1958	24—26. IX. 1958	
Macoun	Bearing	2,02	1,65	1,74	1,66	2,56	1,56	1,60
	Non-bearing	1,27	1,15	1,65	1,55	1,26	1,15	
Reder's Gold- reinette	Bearing	2,19	1,68	1,52	1,66	2,75	1,55	1,59
	Non-bearing	1,48	1,16	1,37	1,56	1,12	1,06	
Boiken	Bearing	2,80	2,21	1,82	1,94	2,36	1,98	1,83
	Non-bearing	1,79	1,43	1,64	1,65	1,12	1,17	
Average		1,93	1,55	1,63	1,67	1,86	1,41	

Conf. interval for dates of sampling $m_1 = 0,062$; $m_2 = 0,082$
 „ „ for varieties $m_1 = 0,024$; $m_2 = 0,034$
 „ „ for interaction date \times variety \times kind of spurs
 $m_1 = 0,152$; $m_2 = 0,200$

The differences appeared clearly between the investigated varieties as regard to the amount of total nitrogen in the spurs. In general the Boiken variety contained the highest amount of nitrogen (Tab. 1). Nevertheless the curves for the amount of nitrogen varied for particular varieties, depending upon kind of spurs and date of sampling (significance of interaction; kind of spurs \times date).

Insoluble nitrogen

The percentage of insoluble nitrogen in total nitrogen was significantly higher in non-bearing spurs, except the spring time, when those values equalized or were very near (depending upon the variety — Fig. 4; Tab. 2). For the Macoun that equalization already occurred in the winter period and lasted to the spring; the differences in the amounts of insoluble nitrogen (compared with total nitrogen) were relatively

Fig 3. Total nitrogen (a) and soluble nitrogen (b) contents in spurs. (Percent of dry weight): A — Macoun var.; B — var. Reder's Goldreinette var.; C — Boiken var.
 ————— bearing; - - - - - non-bearing

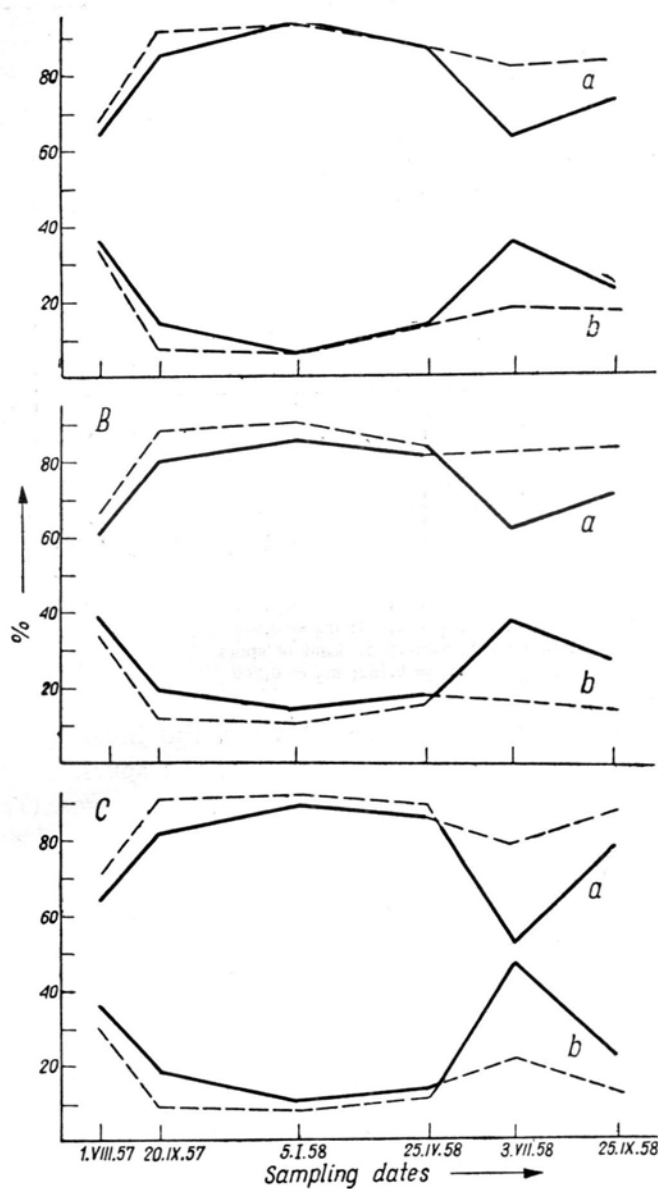


Fig. 4. Insoluble (a) and soluble nitrogen (b) contents in spurs. (Percent of total nitrogen). A — Macoun var.; B — Boiken var.; C — Reder's Goldreinette var.
 ————— bearing; - - - - - non-bearing

TABLE 2

Percent of insoluble nitrogen to total nitrogen in the apple spurs

Kind of spurs	Date of sampling						Conf. interval
	31.VII— 2.VIII. 1957	19—21. IX. 1957	3—7. I. 1958	24—25. IV. 1958	2—4. VII. 1958	24—26. IX. 1958	
Bearing	62,18	82,56	89,80	84,96	60,18	75,95	$m_1=3,57$
Non-bearing	67,38	90,28	92,10	86,71	81,05	85,05	$m_2=4,70$

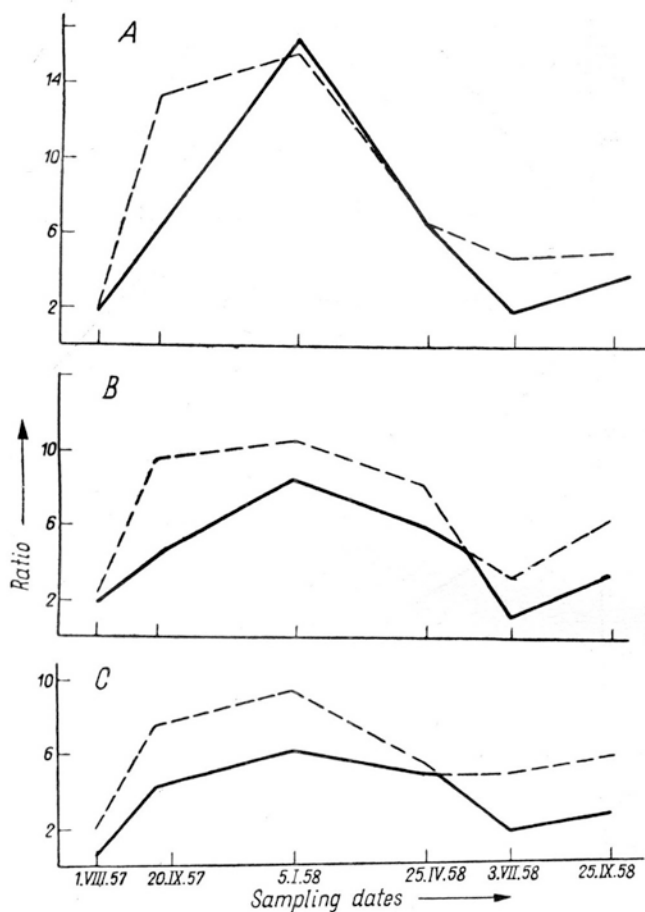


Fig. 5. The ratio of insoluble nitrogen to soluble nitrogen in the spurs. A — Macoun var.; B — Reder's Goldreinette var.; C — Boiken var.
 — bearing; - - - - - non-bearing

small between bearing and non-bearing spurs of that variety in other dates too.

Yet more distinct picture is for the ratio of the insoluble nitrogen to the soluble nitrogen (Fig. 5). The differences between bearing and non-bearing spurs as well as the winter maximum of the amount of protein nitrogen in comparison with the soluble nitrogen appeared clearly. The highest ratio of protein nitrogen to soluble nitrogen occurs in the spurs of Macoun variety.

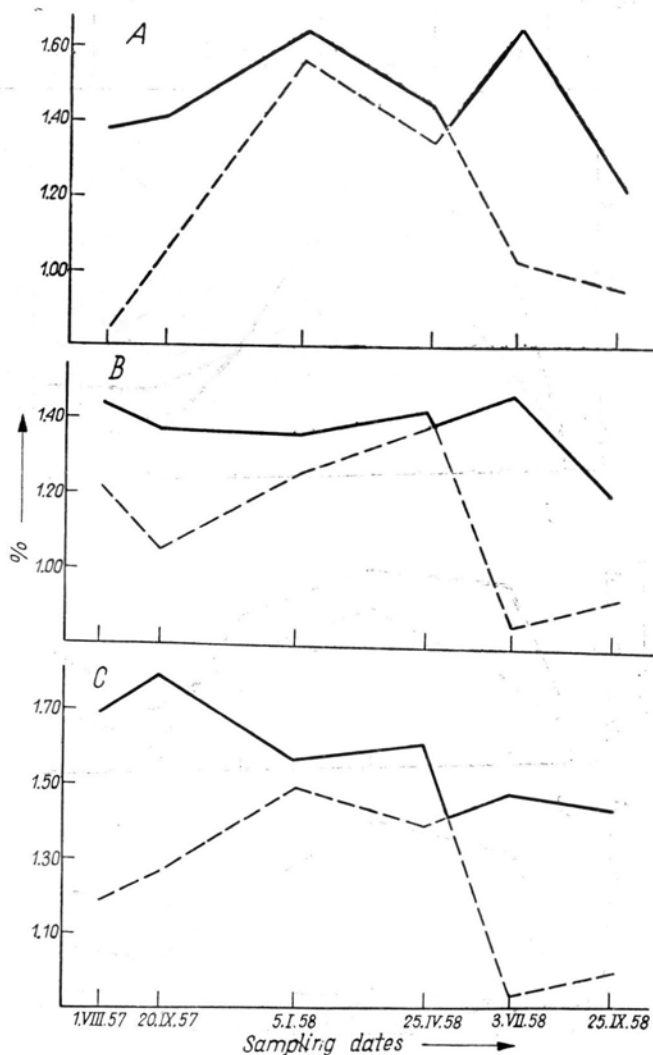


Fig. 6. Insoluble nitrogen contents in the spurs (percent of dry weight). A — Macoun var.; B — Reder's Goldreinette var., C — Boiken var.
— bearing; - - - non-bearing

On the other hand the percentage of insoluble nitrogen in dry matter was significantly higher in the bearing spurs (1,48 per cent) in comparison with the non-bearing spurs (1,16 per cent; $m_2 = 0,06$). It is not, however, save to conclude as to any regularity of the curves in the scheme for particular varieties (Fig. 6).

Soluble nitrogen

The amount of soluble nitrogen was higher in bearing spurs as compared with the dry matter (Fig. 3; Tab. 3) as well as with the total nitrogen. At the sampling dates the differences of the soluble nitrogen amount were the highest in the period of early summer maximum; in the 1958 they appeared larger than in 1957. The smallest differences appeared in the period from January to April when they for all the varieties turned out to be not significant with reference to the dry matter (Fig. 2; Tab. 3).

TABLE 3
Soluble nitrogen content in the apple spurs
(percent of dry weight)

Variety	Kind of spurs	Dates of sampling						Aver. for kinds of spurs	Aver for varieties
		31.VII-2.VIII. 1957	19-21. IX. 1957	3-7. I. 1958	24-25. IV. 1958	2-4. VII. 1958	24-26. IX. 1958		
Macoun	Bearing	0,785	0,237	0,097	0,222	0,910	0,347	0,363	0,262
	Non-bearing	0,415	0,087	0,097	0,217	0,225	0,195	0,163	
Reder's Gold-reinette	Bearing	0,820	0,315	0,162	0,237	1,270	0,347	0,466	0,312
	Non-bearing	0,520	0,110	0,115	0,170	0,257	0,137	0,158	
Boiken	Bearing	1,015	0,427	0,260	0,340	0,897	0,552	0,496	0,344
	Non-bearing	0,600	0,172	0,160	0,260	0,195	0,177	0,193	
Average		0,744	0,225	0,149	0,240	0,625	0,293		

Conf. interval for dates of sampling $m_1 = 0,042$; $m_2 = 0,056$
 „ „ for varieties $m_1 = 0,042$; $m_2 = 0,060$
 „ „ for interaction variety \times kind of spurs
 $m_1 = 0,056$; $m_2 = 0,074$
 „ „ for interaction date \times variety \times kind of spurs
 $m_1 = 0,104$; $m_2 = 0,138$

Although the amount of soluble nitrogen was strongly increased at fruiting in all tested varieties (particularly in the period of early summer), yet it was not in each of them by the same rate (Tab. 3). The Macoun variety was found to be the most slow in this case.

Chromatographic determinations of free amino acids and amides
in soluble nitrogen fraction

In spurs of the tested varieties the following amino acids and amides were found: aspartic acid, glutamic acid, asparagine, glutamine, alanine, arginine and γ -amino butyric acid as well as smaller quantities of serine, glycine (traces), threonine, citrulline + valine, proline, leucine and unidentified spot with an $R_f = 0,82$ in the solvent butanol: AA: water and an $R_f = 0,67$ in the solvent phenol: water; that spot gives yellow colour with ninhydrin and decolorize the sodium azide-iodine reagent (characteristic of compounds containing sulphur) (Block 1958, Opieńska 1957).

The resolution of amino acids was not always satisfactory because of checking substances in the extract.

The qualitative analysis did not show any differences in composition of amino acids and amides between the bearing and non-bearing spurs.

DISCUSSION AND CONCLUSIONS

1. The amount of determined soluble nitrogen depends on the method of extraction. It is confirmed by the obtained results at the comparison between the extraction with 80 per cent ethanol and with 20 per cent three-chloro-acetic acid. The lower amount of soluble nitrogen obtained in the ethanol extract is quite comparable with the results published by Oland (1959). He compared different extractive reagents (70 per cent ethanol, water, water solutions of HCl, NaOH and NaCl), and obtained also the smallest amount of soluble nitrogen in ethanol extract than in water solution extracts. The lower amount of soluble nitrogen in ethanol extract should be ascribed mainly to the insufficient extraction of the basic nitrogenous compounds and first of all of arginine (Oland). Besides, it is possible that when TCAA is used some peptides and polipeptides go into the extract.

2. The behaviour of the tree-crown halves and that of the whole crowns were alike with regard to the physiological processes connected with fruiting (the amount of dry matter and nitrogen). This stays in agreement with the observations made in previous years (Bielińska 1956, 1957), and indicates the local character of these processes.

3. The fact that the differences between bearing and non-bearing spurs with regard to the amount of dry matter and total and soluble nitrogen were larger in 1958 than in 1957, was perhaps due to the somewhat earlier summer sampling in 1958 than in 1957. Perhaps, in 1958, the date of sampling was nearer to the period of maximum differences in the amount of dry matter and nitrogen compounds between bearing and non-bearing spurs. It may be too that some role was played by the fact that more flowers had to be picked in 1957 than in 1958 in order to obtain non-bearing trees. The exception was Macoun, which being inclined to annual bearing, needed almost as much picking in 1958 as in 1957. Besides, the physiological response connected with fruiting of trees of this variety was mostly weaker than with the other varieties.

4. The higher amount of dry matter in non-bearing spurs persisting to the spring date (in which a crossing of the curves corresponding to bearing and non-bearing spurs appeared Fig.1) does not quite agree with the data obtained by Hooker. He found that crossing to occur earlier, e.g. between November and January. This discrepancy was probably due to the differences in climatic conditions under which both experiments were carried out.

5. The data which show higher amount of protein nitrogen in the dry matter of bearing spurs, than of the non-bearing ones, are contradictory to the respective results obtained by Potter and Phillips. However they are partly in agreement with the data obtained by K o ł o m i j e c, who found a higher amount of protein nitrogen in dry matter of bearing spurs during the period from the beginning of June to the end of July. Similar relation was observed in our investigations also in autumn and winter (Fig. 6). The same relation can be found in Bielińska's results — 1956, 1957 — calculating them to the amount of dry matter*.

6. The comparison of the curves of total nitrogen contents in dry matter with those representing protein nitrogen to soluble ratio (Fig. 3 and 5) indicates that the relation of two forms of nitrogen is changed during the year independently of the fluctuations in the amount of total nitrogen and in the contents of different forms of nitrogen. During the

* In the English summary of the Bielińska's „Studies on Nitrogen and Carbohydrates Content in the Leaves and Shoots of Biennial Bearing Apple Trees” published in the „Polish Agricultural Annual” (Roczniki Nauk Rolniczych, vol. 75-A-3: 518, 1957 there was mistakenly printed: „The percent of protein nitrogen in relation to total nitrogen and to dry matter is higher in non-bearing trees”. It should read: „The percent of protein nitrogen in relation to total nitrogen is higher in non-bearing trees”, as it was given in the Russian summary.

period from winter to spring, when the content of total nitrogen remains almost unchanged, the amount of soluble nitrogen in both kinds of spurs increases as the amount of protein nitrogen decreases. (The changes are not so distinct when the amounts of different forms of nitrogen in the dry matter are considered, because of the small amount of soluble nitrogen during the period from autumn to spring).

During the period from summer to winter the ratio of insoluble to soluble nitrogen greatly increases in both kinds of spurs in spite of the decrease of total nitrogen content in bearing spurs and unchanged or slightly decreased level in non-bearing ones.

7. The regular course of the curves representing dry matter, total nitrogen, soluble nitrogen, and protein nitrogen in percent of total nitrogen, soluble nitrogen as to dry matter, and protein nitrogen to soluble nitrogen ratio in the spurs of bearing and non-bearing trees indicate clearly a relation of those compounds to the process of fruiting. It would be justified, on the other hand, to conclude that the content of protein nitrogen in the dry matter of spurs does not remain in any direct relation to the fruiting. Consequently it should be assumed that the role of protein nitrogen in its connection with fruiting may be considered only in relation to total nitrogen (i.e., strictly speaking, to soluble nitrogen).

An opinion often is met that synthesis of proteins in bearing organs of trees is decreased as a result of carbohydrates being used up for the development of fruit. Considering, however, that the amount of both soluble and insoluble (much more of soluble than of insoluble) nitrogen in dry matter increases in those organs during the years of bearing, the using up of carbohydrates by the development of fruit may have only a relative significance as an agent influencing the changes in protein nitrogen soluble nitrogen balance.

When comparing the curves of total and soluble nitrogen (Fig. 3) one can observe that they are almost parallel in the bearing spurs in the period from the spring to the early summer maximum of the nitrogen. The increase of total nitrogen is almost exclusively a result of the increase of soluble nitrogen at this time. Then the amount of the soluble nitrogen in non-bearing spurs remains almost constant, and the amount of total nitrogen falls down (and thus the decreasing of the amount of protein nitrogen in non-bearing spurs). It may seem that the protein reserves are used up in the spurs of non-bearing trees for the growth and development in the spring, whereas an accumulation of nitrogen compounds (mainly of soluble ones) occurs in the spurs of bearing trees. This mobilization comprises not only the spurs, but also other young parts of bearing trees, as leaves (a higher amount of total nitrogen

in the leaves of bearing trees — Boynton and Cain 1948, Bielińska 1956, 1957), and even though to a smaller degree, shoots (a higher amount of total nitrogen in shoots was found by Bielińska (1956, 1957) in two biennial bearing varieties: Grosser Reinischer Bohnapfel and Wealthy).

Summarizing, a pronounced relation was found between the soluble nitrogen, the mobile nitrogen form, and the fruiting of trees.

SUMMARY

The larger amount of total, soluble and insoluble nitrogen in percent of dry matter, as well as of soluble nitrogen in percent of total nitrogen was found in bearing as compared with non-bearing spurs of the apple trees. The contrary relationship was found for dry matter, protein nitrogen in percent of total nitrogen and the ratio of protein to soluble nitrogen. The maximal differences in the bearing and non-bearing spurs at the investigated dates were proved in early summer period.

The close interrelationship between the metabolism of nitrogenous compounds and the fruiting of the apple trees is suggested.

*Research Institute of Pomology
Skierniewice, Poland*

(Entered: 29.4.1961.)

STRESZCZENIE

Doświadczenie prowadzono od wiosny 1957 do jesieni 1958 r. w sadzie doświadczalnym I. S. w Dąbrowicach na dziesięcioletnich drzewach jabłoni odmian: Żłotka Redera, Boiken, Macoun. W obrębie każdej odmiany do doświadczenia wchodziły 2 drzewa owocujące, 2 nie owocujące i 2 owocujące połową korony. Próbkę krótkopędów pobierano w 4 terminach w ciągu roku: w okresie różnicowania pąków kwiatowych, jesienią w okresie zbioru owoców, zimą w okresie spoczynku i wiosną w czasie nabrzmiewania pąków.

Stwierdzono wyższą zawartość suchej masy w krótkopędach nie owocujących niż w owocujących, z wyjątkiem terminu wiosennego, kiedy następowało zrównanie zawartości w obu rodzajach krótkopędów. W badanych terminach maksimum zawartości suchej masy w krótkopędach występowało zimą, minimum latem (fig. 1).

Krótkopędy owocujące zawierały więcej azotu ogólnego i rozpuszczalnego (zarówno w stosunku do azotu ogólnego, jak i suchej masy) niż

krótkopędy nie owocujące. Różnice te w naszych warunkach występowały najsilniej w okresie wczesnoletnim (fig. 2, 3, 4).

W krótkopędach owocujących przebieg krzywych zawartości azotu ogólnego i rozpuszczalnego w okresie od wiosny do lata był prawie równoległy. Od zimy od wiosny różnice między krótkopędami owocującymi i nie owocującymi były pod tym względem nieznaczne.

Zawartość azotu białkowego w stosunku do ogólnego była wyższa w krótkopędach nie owocujących, natomiast w stosunku do suchej masy — w krótkopędach owocujących (fig. 4, 6). Przy tym przebieg krzywych zawartości azotu białkowego w suchej masie był nieregularny.

Niezależnie od wahań w zawartości azotu ogólnego i różnych form azotu, stosunek dwóch form azotu ulegał zmianom w ciągu roku. Maksimum zawartości azotu białkowego w stosunku do rozpuszczalnego występowało zimą, minimum — latem. Stosunek ten był wyższy w krótkopędach nie owocujących (fig. 5).

Na podstawie otrzymanych wyników sądzić można, że zawartość azotu rozpuszczalnego jest dość ściśle związana z owocowaniem drzew. Rola azotu białkowego w związku z owocowaniem może być rozpatrywana jedynie w jego stosunku do azotu rozpuszczalnego — w krótkopędach nie owocujących, tworzących pąki kwiatowe na rok następny, następuje przesunięcie procesów w kierunku syntezy białek (jakkolwiek zawartość w nich azotu ogólnego i białkowego w ‰ s.m. była ogólnie biorąc niższa).

Oznaczono ponadto metodą chromatograficzną skład jakościowy wodnych aminokwasów i amidów frakcji rozpuszczalnej azotu. W obu rodzajach krótkopędów stwierdzono występowanie następujących aminokwasów: kwasu asparaginowego, kwasu glutaminowego, asparaginy, glutaminy, alaniny, argininy i kwasu γ -aminomasłowego oraz w mniejszych ilościach seryny, glicyny (ślady), treoniny, cytruliny, metioniny + waliny, proliny, leucyny oraz nie zidentyfikowanej plamy o $R_f = 0,82$ w butanolu i $R_f = 0,67$ w fenolu, barwiącej się żółto z ninhydriną.

Mgr. Kazimierzowi Szczepańskiemu autorki składają serdeczne podziękowanie za wskazówki i pomoc w opracowaniu statystycznym.

REFERENCES

1. Bielińska M., 1956, Chemical investigations on biennial bearing of apple trees, Bull. Acad. Polon. Sci. Ser. sci. biol. 4: 179—181.
2. Bielińska M., 1957, Studia nad zawartością azotu i węglowodanów w liściach i pędach jabłoni przemiennie owocujących, Roczn. Nauk. Roln. 75-A-C: 433—520.

3. Bielińska M., 1957, Zawartość azotu i węglowodanów w liściach i pędach jabłoni przemiennie owocujących, *Prace I. S.* 2: 223—232.
4. Block R. J., Burrum E. L., Zweig G., 1958, *A manual of paper chromatography and paper electrophoresis*, New York, Ac. Press.
5. Bollard E. C., 1953, Nitrogen metabolism of apple trees, *Nature* 171:571—572.
6. Cain J. C. and Boynton D., 1948, Some Effects of Season, fruit crop and nitrogen fertilization on the mineral composition of McIntosh apple leaves, *Proc. Amer. Hort. Sci.* 51:13—22.
7. Hooker H. D., 1920, Seasonal changes in the chemical composition of the apple spurs, *Mo. Agr. Exp. Sta. Res. Bull.* 40: 1—54.
8. Kobel F., 1954, *Lehrbuch des Obstbaus auf physiologischer Grundlage*, Berlin—Göttingen—Heidelberg, Springer Verlag.
9. Kołomijec I. A., 1955, Przyczyny pieriodicznosti płodnoszenija jabłoni. Jeżegodnoje płodnoszenje jabłoni, 32—52, Moskwa, Sielchozgiz.
10. Kraybill H. R. and others, 1925, Some chemical constituents of fruit spurs associated with blossom bud formation in the Baldwin apple, *N. H. Agr. Exp. Sta. Techn. Bull.* 29:1—41.
11. Oland K., 1954, Nitrogenous constituents of apple maidens grown under different. nitrogen treatments, *Physiologia Plantarum*, 7: 463—474.
12. Oland K. and Yemm E. W., 1956, Nitrogenous reserves of apple trees, *Nature*, 178: 219.
13. Oland K., 1959, Nitrogenous reserves of apple trees, *Physiologia Plantarum*, 12: 594—648.
14. Opieńska-Blauth J., Waksmundzki A., Kański M., 1957, *Chromatografia*, Praca zbiorowa, Warszawa, PWN.
15. Potter G. F. i Phillips T. G., 1930, Composition and fruit bud formation in non-bearing spurs of the Baldwin apple, *N. H. Agr. Exp. Sta. Techn. Bull.* 42: 1—41.
16. Rubin S. S., 1958, *Udobrenie płodowych i jagodnych kultur*, Moskwa, Sielchozgiz.
17. Sahulka J., Silova A., 1960, Free amino acid relations in apple leaves before and during differentiation of flower buds, *Biologia Plantarum* 2(1):70—75.
18. Ursulenko P. K., 1955, *Biologičeskije osnovy agrotechniki i jeżegodnogo płodnoszenija jabłoni*, Jeżegodnoje płodnoszenije jabłoni: 3—19; Moskwa, Sielchozgiz.