

**EFFECT EXERTED ON SOIL PROPERTIES BY APPLE-TREE CULTIVATION FOR MANY YEARS AND BY REPLANTATION.
PART I. BIOCHEMICAL SOIL PROPERTIES**

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Abstracts. Studies were carried out in an experimental orchard in the years 2008–2009. Apple-trees of ‘Topaz’ cultivar were planted in five “soil localities”: 1 – directly in grubbed up rows of an apple-tree orchard, 2 – in herbicide fallow belts of grubbed up apple-tree orchard, 3 – in turf belts of grubbed up apple-tree orchard, 4 – in a locality after a four-year break in apple-tree cultivation, without any preparatory treatments and 5 – in a soil after previous agricultural use – virgin soil. During the studies realization, an evaluation of the soil biochemical properties was carried out. This evaluation was done on the basis of activity of enzymes: dehydrogenases, proteases, acid phosphatase and urease, IAA content, total content of organic carbon and of organic carbon soluble in water. Our studies have shown a significant influence of the soil locality and of the term of soil sampling for the identification of the biochemical properties of soil expressed by the enzymatic activity. The activity of three studied enzymes was significantly lower in the locality 1 in comparison with the soil in a locality 5. Also in case of indole-3-acetic acid (IAA), its highest value was found in the soil of combination 1 while the highest value of the IAA acid was found in combination 3. The previous method of soil utilization also exerted a significant influence on the content of organic matter in the soil. The content of total organic carbon and organic carbon soluble in water were significantly lower in the soil from the orchard planted directly after previous agricultural use. Enzymatic activity and content of organic carbon were the highest in the autumn period, while the lowest value was shown in summer.

Key words: replantation, activity of enzymes, indole-3-acetic acid, total organic carbon and organic carbon soluble in water

INTRODUCTION

Frequent changes in tree plantation induce many fruit producers to apply replantations which may have a negative influence on the growth and yielding of trees [Pacholak and Rutkowski 2000]. Renewal of an orchard and particularly plantation of the same species of plants (e.g. apple-tree after apple-trees) can lead to the occurrence of the replantation disease evoked by soil fatigue [Utkhede and Smith 1994]. Many fruit growers have realized this fact when they applied new plantations shortly after grubbed up of the previously grown trees. They have observed a poor growth of trees and a low yielding of the new plantation [Pacholak and Rutkowski 2000]. Sometimes, there followed a withering or even dying out of the newly planted trees [Rebandel 1987, Pacholak et. al. 1995].

An indicator of change taking place in the soil under the influence of natural and anthropogenic factors is the activity of enzymes. Enzymatic tests are regarded as representing some of the more sensitive indicators of ecosystem functioning [Bielińska and Węgorok 2005]. According to Kucharski and Niewolak [1996], soil enzymes belonging to oxydoreductases (dehydrogenases) and hydrolases (proteases, ureases, phosphatases) play the most important role. The sources of enzymes occurring in the soil are microelements, plant root and their residues, as well as the specific soil flora [Gołębiowska and Grzyb-Miklewska 1991].

In soil there occur not only enzymes, but also biologically active compounds which exert a significant influence on the development and functioning of plants. This group includes plant hormones [Szajdak 2004]. Among hormones, the greatest importance is attributed to auxins e.g. indole-3-acetic acid (IAA) [Pokojska-Burdziej and Strzelczyk 1998]. The free form IAA is characterized by a biological activity, while in the combined form, this activity is not present.

Soil respiration exerts a significant influence on the correlation of organic matter content and on the majority of biochemical and microbiological processes. In turn, the respiration is affected by cultivation treatments, introduction of fertilizers into the soil, protection methods against diseases, pests and weed (pesticide), as well as by contaminations (heavy metals, diesel oil, sewages).

In natural conditions, plant roots contribute significantly to the amount of the emitted carbon dioxide which may amount even to 10–50% of the total CO₂ emission from organic soils [Brzezińska 2006].

The aim of the investigations carried out in the years 2008–2009 was to determine the effect of soil (locality) use on the biochemical properties of soil in an apple orchard after replantation.

MATERIAL AND METHODS

Studies carried out in the years 2008–2009 in the Experimental Station of the Department of Pomology University of Life Sciences in Poznań, on the area of the Agricultural and Fruit-Growing Farm in Przybroda. The experiment was established on a grey-brown podzolic soil created of boulder clay sandy loam. The arable soil layer consisted

of boulder clay sandy loam strongly sandyfied, containing 17–20% of floatable parts. The groundwater level was at the depth of 120–180 cm.

The investigated material was represented by apple-trees of 'Topaz' cultivar inoculated on M.26 rootstock, planted in spring 2000 in a space distribution of 3.5×1.5 m ($1900 \text{ trees} \cdot \text{ha}^{-1}$) on three quarters:

Quarter I – from the year 1949 until the termination of our studies (2009), apple-trees were cultivated. In that period, three replantations of trees were carried out. In 1979, the second replantation was performed without any additional preparation of soil. Apple-trees of 'Cortland' cultivar, on M.26 rootstock were planted in the spacing of 5×3 m ($667 \text{ trees} \cdot \text{ha}^{-1}$) in a one-row system. In spring 2000, the third replantation was carried out with the application of 'Topaz' cultivar. The introduced decrease of the spacing permitted to obtain the following combinations of soil localities:

- combination 1 – directly in the rows of the grubbed up trees,
- combination 2 – in the belts of the herbicide fallow of the grubbed up trees,
- combination 3 – in the belts of turf between the grubbed up trees.

Quarter II – similarly as in quarter I, apple-trees cultivation had been carried out since 1949. The second replantation was done in 1979 using 'Cortland' cultivar apple-trees (as described above), which were grubbed up in autumn 1996. In that quarter, no other plants were cultivated and no additional treatments were carried out to prepare it under the orchard. The third replantation was performed in spring 2000, which permitted to separate a locality with a 4-year break in apple-tree cultivation and this locality constituted – combination 4.

Quarter III – it was locality, where no orchard plants had been grown before (it was used for agricultural purposes). The first orchard was established here in spring 2000 as – combination 5.

All combinations of soil localities were established in two blocks and in each block, 40 trees were planted. In each block, 20 trees were selected for observation purposes. All agricultural treatments were carried out according to the recommendations for this species.

Enzymatic activity (EA) of the soil was determined in three terms: in the spring season, samples were taken two weeks after tree blooming, in summer – after the termination of the intensive tree growth and in autumn – one week after fruit harvest.

Protease activity (PA) – measured by spectrophotometric method was carried out according to Ladd and Buttler [1972] using 1% sodium caseinate solution as substrate after one hour incubation time at 50°C and 578 nm wave length. Enzymatic activity is expressed in mg of tyrosine $\cdot \text{h}^{-1} \cdot \text{kg}^{-1}$ d.m. soil.

Dehydrogenase activity (DhA) was determined by colorimetric method according to Thalmann [1968] with the use of 1% solution of triphenyltetrazolium chloride (TTC) as substrate after a 24-hour incubation at 30°C and 485 nm wave length, which showed its activity in $\text{cm}^3 \text{H}_2 \cdot 24 \text{h}^{-1} \cdot \text{kg}^{-1}$ d.m. soil.

Acid phosphatase activity (APhA) was determined spectrophotometrically by the method of Tabatabai and Bremner [1969] using sodium p-nitrophenylphosphoran solution as substrate, after 1 hour incubation at 37°C and 4000 nm wave length expressing its activity in mg of p-nitrophenol $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$.

Urease activity (UA) – was measured colorimetrically by the method of Hoffmann and Teicher [1961] using 10% urea solution after 3-hour incubation at 37°C and 630 nm wave length expressing its activity in μmol of urea $\cdot \text{h}^{-1} \cdot \text{g}^{-1}$ d.m soil.

Parallely with the determination of the enzymatic activity, in the same term, the following values were determined in the soil:

- content of total organic carbon by Tiurin method – C_{org} in $\text{g} \cdot \text{kg}^{-1}$ [Mocek et al. 2000],
- content of organic carbon soluble in water $C_{\text{org sol}}$ in $\text{g} \cdot \text{kg}^{-1}$ [Smolander and Kintunen 2002],
- content of indolo-3-acetic acid, by fluorescent method IAA in $\mu\text{g} \cdot \text{kg}^{-1}$ [Szajdak 2004].

RESULTS AND DISCUSSION

Our studies have shown an influence of locality on the biochemical properties of soil from replanted apple orchard. It has been confirmed among others by the activity of four investigated enzymes (dehydrogenases, proteases, acid phosphatase and urease). Their highest activity was found in the soil of orchard planted in a locality after previous agricultural use while the lowest one was in the soil of the orchard established directly in rows after grubbed up trees (tab. 1–4). The applications of a 4-year break in apple-tree cultivation, similarly as the plantation of trees in the belts of turf in the grubbed up orchard, contributed to the increased activity of the studied enzymes. Similar results were obtained by Koper and Piotrowska [1996], who showed that the application of monoculture leads to the decrease of enzymatic activity. A different opinion was expressed by Kucharski and Niewolak [1996, 1997], who found a higher enzymatic activity in monoculture.

Table 1. Activity of dehydrogenases in the soil of apple-tree orchard in the years 2008–2009
Tabela 1. Aktywność dehydrogenaz w glebie sadu jabłoniowego w latach 2008–2009

Locality Stanowisko	$\text{cm}^3 \text{H}_2: 24 \text{h}^{-1} \cdot \text{kg}^{-1}$ d.m. soil $\text{cm}^3 \text{H}_2: 24 \text{h}^{-1} \cdot \text{kg}^{-1}$ ś.m. gleby			Mean value for locality Średnia dla stanowisk
	spring wiosna	summer lato	autumn jesień	
Old row of trees Stary rząd drzew	5.9 a-c	2.5 ab	4.6 a-c	4.4 a
Old herbicide fallow Stary ugór herbicydowy	4.9 a-c	2.0 a	10.5 de	5.8 ab
Old turf Belt Stary pas murawy	10.2 de	6.1 bc	6.8 cd	7.7 bc
4-year break 4-letnia przerwa	5.2 a-c	4.1 a-c	11.8 e	7.0 bc
Virgin soil Nowina	11.0 e	7.1 cd	6.0 a-c	8.0 c
Mean value for terms Średnia dla terminu	7.5 b	4.4 a	7.9 b	

*mean values marked with the same latter do not differ significantly at $\alpha = 0.05$

*średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

Table 2. Activity of proteases in the soil of apple-tree orchard in the years 2008–2009
Tabela 2. Aktywność proteaz w glebie sadu jabłoniowego w latach 2008–2009

Locality Stanowisko	mg tyrozyny · h ⁻¹ · kg ⁻¹ d.m. soil mg tyrozyny · h ⁻¹ · kg ⁻¹ ś.m. gleby			Mean value for locality Średnia dla stanowisk
	spring wiosna	summer lato	autumn jesień	
Old row of trees Stary rząd drzew	1.7 a*	1.4 a	7.8 bc	3.6 a
Old herbicide fallow Stary ugór herbicydowy	1.7 a	0.9 a	11.5 d	4.7 ab
Old turf Belt Stary pas murawy	2.6 a	6.1 b	7.2 bc	5.3 b
4-year break 4-letnia przerwa	3.2 a	2.6 a	9.5 cd	5.1 ab
Virgin soil Nowina	8.9 cd	7.8 bc	18.5 e	11.8 c
Mean value for terms Średnia dla terminu	4.1 a	6.4 b	7.8 c	

*mean values marked with the same letter do not differ significantly at $\alpha = 0.05$

*średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

Enzymatic activity of soil also differs depending on the terms of sampling. Two-years studies have shown a significantly lower activity of dehydrogenases and urease in the summer season, while in proteases and acid phosphatases, it happened in spring (tab. 1–4). This fact partially agrees with the results of the studies carried out by Bielińska and Domżał [1998], who showed the highest enzymatic activity in samples taken in spring and in autumn.

Table 3. Activity of acid phosphatase in the soil of Apple-tree orchard in the years 2008–2009
Tabela 3. Aktywność fosfatazy kwaśnej w glebie sadu jabłoniowego w latach 2008–2009

Locality Stanowisko	mg p-nitrofenolu kg ⁻¹ · h ⁻¹ d.m. soil			Mean value for locality Średnia dla stanowisk
	spring wiosna	summer lato	autumn jesień	
Old row of trees Stary rząd drzew	4.5 a*	9.6 de	8.7 d	7.6 a
Old herbicide fallow Stary ugór herbicydowy	6.9 c	8.7 d	8.9 d	8.2 b
Old turf Belt Stary pas murawy	7.4 c	12.8 g	6.9 c	9.1 c
4-year break 4-letnia przerwa	7.4 c	10.6 f	5.8 b	7.9 ab
Virgin soil Nowina	10.2 ef	12.8 g	9.1 d	10.7 d
Mean value for terms Średnia dla terminu	7.3 a	10.9 c	7.9 b	

*mean values marked with the same letter do not differ significantly at $\alpha = 0.05$

*średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

Table 4. Activity of urease in the soil of apple-tree orchard in the years 2008–2009
Tabela 4. Aktywność ureazy w glebie sadu jabłoniowego w latach 2008–2009

Locality Stanowisko	$\mu\text{mol urea h}^{-1} \cdot \text{g}^{-1} \text{ d.m. soil}$			Mean value for locality Średnia dla stanowisk
	spring wiosna	summer lato	autumn jesień	
Old row of trees Stary rząd drzew	12.9 cd*	10.1 b	14.6 d	12.5 b
Old herbicide fallow Stary ugór herbicydowy	12.6 c	7.4 a	6.3 a	8.6 a
Old turf Belt Stary pas murawy	9.9 b	5.9 a	12.6 c	9.5 a
4-year break 4-letnia przerwa	17.7 e	9.5 b	9.8 b	12.3 b
Virgin soil Nowina	23.1 f	13.2 cd	16.5 e	17.6 c
Mean value for terms Średnia dla terminu	15.2 c	9.2 a	11.9 b	

*mean values marked with the same latter do not differ significantly at $\alpha = 0.05$

*średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

Table 5. Content of total organic carbon in the soil of apple-tree orchard in 2008–2009
Tabela 5. Zawartość węgla organicznego ogółem w glebie sadu jabłoniowego w latach 2008–2009

Locality Stanowisko	$\text{C}_{\text{org.}} \text{ w g} \cdot \text{kg}^{-1} \text{ d.m. soil}$			Mean value for locality Średnia dla stanowisk
	spring wiosna	summer lato	autumn jesień	
Old row of trees Stary rząd drzew	9.4 e*	10.3 g	19.6 l	13.1 e
Old herbicide fallow Stary ugór herbicydowy	10.7 h	6.6 a	11.6 i	9.64 c
Old turf Belt Stary pas murawy	7.6 d	10.1 f	7.2 b	8.3 a
4-year break 4-letnia przerwa	7.6 d	11.9 j	7.4 c	9.0 b
Virgin soil Nowina	13.5 k	11.9 j	11.9 j	12.5 d
Mean value for terms Średnia dla terminu	9.8 a	10.2 b	11.6 c	

*mean values marked with the same latter do not differ significantly at $\alpha = 0.05$

*średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

The factor which may exert an effect on the activity of enzymes is the amount and availability of organic matter in the soil [Bielińska and Węgorzek 2005]. This happens because enzymes play an important role in the transformation of nitrogen and organic carbon [Pawluczuk 1990]. On the basis of studies carried out by Bielińska and Żukowska [2002], it follows that enzymatic activity is proportional to the content of organic carbon in the soil. Our results did not confirm such dependence. On the average, the highest total organic carbon soluble in water was found in the soil from the orchard

Table 6. Content of organic carbon soluble in water in the soil of apple-tree orchard in 2008–2009

Tabela 6. Zawartość węgla organicznego rozpuszczalnego w glebie sadu jabłoniowego w latach 2008–2009

Locality Stanowisko	C _{org. sol.} w H ₂ O w g · kg ⁻¹ d.m. soil			Mean value for locality Średnia dla stanowisk
	spring wiosna	summer lato	autumn jesień	
Old row of trees Stary rząd drzew	0.48 d*	0.55 f	0.97 h	0.67 e
Old herbicide fallow Stary ugór herbicydowy	0.52 e	0.47 cd	0.64 g	0.54 d
Old turf Belt Stary pas murawy	0.55 f	0.55 f	0.46 c	0.52 c
4-year break 4-letnia przerwa	0.42 b	0.54 f	0.33 a	0.43 a
Virgin soil Nowina	0.54 f	0.46 c	0.48 d	0.49 b
Mean value for terms Średnia dla terminu	0.50 a	0.51 b	0.57 c	

*mean values marked with the same latter do not differ significantly at $\alpha = 0.05$ *średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

Table 7. Content of indole-3-acetic acid (IAA) in the soil of apple-tree orchard in 2008–2009

Tabela 7. Zawartość kwasu indolilo-3-octowego (IAA) w glebie sadu jabłoniowego w latach 2008–2009

Locality Stanowisko	µg · kg ⁻¹			Mean value for locality Średnia dla stanowisk
	spring wiosna	summer lato	autumn jesień	
Old row of trees Stary rząd drzew	81.6 b*	71.3 a	106.5 j	86.5 a
Old herbicide fallow Stary ugór herbicydowy	91.1 f	88.5 e	93.8 g	91.1 d
Old turf Belt Stary pas murawy	100.3 i	88.6 e	108.3 k	99.1 e
4-year break 4-letnia przerwa	84.5 d	110.8 l	71.4 a	88.9 b
Virgin soil Nowina	91.1 f	97.7 h	83.2 c	90.7 c
Mean value for terms Średnia dla terminu	89.7 a	91.4 b	92.6 c	

*mean values marked with the same latter do not differ significantly at $\alpha = 0.05$ *średnie oznaczone tymi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$

planted directly in the rows after the grubbed up trees (tab. 5 and 6), while the enzymatic activity was in these places the lowest. The smallest content of organic carbon was generally found in the soil sampled from the locality, where apple-trees were planted in belts of grubbed up orchard, while the lowest content of organic carbon solu-

ble in water was identified in the orchard planted in the locality where a 4-year break in apple-tree growing was applied (tab. 5 and 6). Content of the total organic carbon and the organic carbon soluble in water depended also on the term soil sampling. The lowest contents of both carbon forms in the soil were found in spring, while the highest content were shown in autumn.

An important group of compounds encountered in soil are auxins with indole-3-acetic acid (IAA) in the first place, which are created not only by higher plants, but also by soil microorganisms such as bacteria, actinomycetes, fungi and algae [Pokojska-Burdziej 1982, Szajdak 2004]. The lowest mean content of IAA acid was found in the soil of the orchard established directly in the rows of the grubbed up trees, while the highest content was in the soil, where the new apple-trees were planted in the turf belts of the grubbed up orchard (tab. 7). The applications of 4-year break in apple-tree cultivation did not contribute to any significant increase of IAA acid content in the soil. Analysis of the effect of the sampling term on the content of indole-3 acetic acid showed that its lowest content was found in samples taken in spring, while the highest content was shown in samples taken in autumn.

CONCLUSIONS

1. Activity of dehydrogenases, proteases, acid phosphatase and urease in soil depended on the locality and on the term of sampling. Activity of the studied enzymes was the highest in the orchard planted in the locality after its previous agricultural use. Application of replantation significantly decreased the enzymatic activity of soil.

2. Plantation locality exerted an influence on the soil content of organic substances: total organic carbon and organic carbon soluble in water, and it was the highest in the soil of the orchard planted directly in the rows of grubbed up trees.

3. A higher content of organic substances in the soil of a replanted orchard did not contributed to the increase of enzymatic activity.

4. Content of indole-3-acetic acid in soil depended on the locality where apple-trees were planted. The least amount of IAA acid was contained in the soil of orchard planted directly in the rows of the grubbed up trees.

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**WPLYW WIELOLETNIEJ UPRAWY JABŁONI I STOSOWANIA
REPLANTACJI NA WŁAŚCIWOŚCI GLEBY.
CZĘŚĆ I. WŁAŚCIWOŚCI BIOCHEMICZNE GLEBY**

Streszczenie. Badania przeprowadzono w sadzie doświadczalnym w latach 2008–2009. Jabłonie odmiany Topaz posadzono na pięciu „stanowiskach glebowych”: 1 – bezpośrednio w rzędach wykarczowanego sadu jabłoniowego, 2 – w pasach ugoru herbicydowego wykarczowanego sadu jabłoniowego, 3 – w pasach murawy wykarczowanego sadu jabłoniowego, 4 – na stanowisku z czteroletnią przerwą w uprawie jabłoni, bez zabiegów przygotowawczych i 5 – glebie po uprawach rolniczych – nowina. W trakcie realizacji badań dokonano oceny właściwości biochemicznych gleby na podstawie oceny aktywności enzymów: dehydrogenaz, proteaz, fosfatazy kwaśnej i ureazy, zawartości IAA, zawartości węgla organicznego ogółem i węgla organicznego rozpuszczalnego w wodzie. Badania wykazały istotny wpływ stanowiska glebowego i terminu pobierania prób na właściwości biochemiczne gleby wyrażone aktywnością enzymatyczną. Aktywność trzech badanych enzymów była istotnie niższa na stanowisku 1 w porównaniu z glebą na stanowisku 5. Również w przypadku kwasu indolilo-3-octowego najniższą jego zawartość stwierdzono w glebie z kombinacji 1, a najwyższą w glebie z kombinacji 3. Wcześniejszy sposób użytkowania gleby miał również istotny wpływ na zawartość materii organicznej w glebie. Zawartość węgla organicznego ogółem i węgla organicznego rozpuszczalnego w wodzie była istotnie niższa w glebie z kombinacji 5. Aktywność enzymatyczna i zawartość węgla organicznego była najwyższa w terminie jesiennym, a najniższa w okresie letnim.

Słowa kluczowe: replantacja, aktywność enzymów w glebie, IAA, węgiel organiczny ogółem, węgiel organiczny rozpuszczalny w wodzie

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