

# INFLUENCE OF SOIL APPLICATION OF BIOLOGICAL AND MINERAL FERTILIZERS ON THE GROWTH, YIELD, AND FRUIT BIOCHEMICAL COMPONENTS OF 'CHARAVNITSA' APPLE, AND ON SOME AGROCHEMICAL SOIL CHARACTERISTICS

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**Abstract**. The six-year data of study of influence of soil application of biological and mineral fertilizers and their complex on the parameters of growth and cropping of apple variety Charavnitsa, budded on the semi-dwarfing 57-545 rootstock, were analyzed. It was found that application of biological fertilizers, the biological complex and the biological-mineral complex had a positive influence on parameters of growth of apple trees, the area of leaf plate and leaf area per tree as well as on mean fruit weight and fruit chemical composition. Application of mineral fertilizers had a negative effect on tree growth and yield. Application of various kinds of fertilizers did not affect the content or composition of pigments of leaves. Mineral fertilizers increased soil acidity and caused a slight increase of potassium, phosphorus and humus content. Application of biological products significantly lowered soil acidity to the optimum level for apple trees and considerably increased the contents of soil potassium, phosphorus and humus.

Key words: apple, growth, yield, fertilizers, biochemical composition

# INTRODUCTION

Adequate supply of basic nutrients is a major factor determining productivity of fruit crops. However, intensive application of chemical fertilizers may cause a number of the undesirable phenomena: deterioration of soil properties, environment pollution and/or decrease of quality of agricultural products.

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Recently in Belarus a great attention has been paid to the problems of adaptive intensification in horticulture. It is related to the ecological situation in the Republic. Introduction of new achievements of biotechnology, including the use of various biological preparations is necessary.

Nitrogen nutrition is a basic problem in plant production and soil fertility. High energy spending for production of nitrogen mineral fertilizers and the ecological risks of their use forced a search of alternative sources of nitrogen for plants. A use of atmospheric nitrogen by soil microorganisms is a unique ecologically safe and energy saving source of nitrogen for plants. In contrast to mineral fertilizers, it does not require energy expenses and it is completely absorbed by plants without polluting environment [Nesterenko 1994, Nesterenko et al. 1994, Bulavin et al. 1997]. Therefore an intensive research on biological fixation of atmospheric nitrogen are carried out in many countries.

Modern conditions demand introduction of new achievements of biotechnology including use of biological preparations of different purpose. Biological nitrogen fixation should be an important component of adaptive intensification in horticulture. According to Umarov [1986] associative nitrogen fixation reaches 75% of the total nitrogen acting in biological nitrogen fixation as a whole. Introduction of active forms of associative diazotrofic microorganisms may considerably increase the natural potential of associative nitrogen fixation.

Associative diazotrofs are active producers of phytohormones. They improve nitrogen nutritional status of plants, and show a significant growth stimulating effect. This results in acceleration of growth and development and improvement of crop quality, by increasing the total and protein nitrogen content. It also, in some cases, causes an increase of phosphorus and potassium content. Bacterization of soils promotes increase of yield and improves fruit quality.

Preparations of nitrogen-fixing bacteria (root diazotrofs) of various kinds have been developed in the Institute of Soil Science and Agrochemistry. One of them is a biological product – Azobacterin. Experiments carried out in the laboratory of microbiology, biochemistry and soils detoxification of this have shown, that Azobacterin (*Azospirillum brasiliense*) has not only a specific action, but may also have a wide spectrum of application for soils detoxification.

Use of preparations of associative bacteria is ecologically safe and energy-saving means of increase of productivity in agriculture. Increase of efficiency of use of biological nitrogen allows reducing the use for mineral nitrogen fertilizers by 30 kg per hectare on the average. Besides, biological fertilizers cannot be overdosed and this is their important quality.

Positive results of application associative diazotrofs in fruit production were obtained in the China, Thailand and India [Khalafallah et al. 1984, Maurya and Sanoria 1986, Natula and Guptata 1987, Cacciari et al. 1989]. The researchers of the Institute of Microbiology of the Academy of Sciences of the province Hebei (city of Baoding) have found that apple trees treated with associative nitrogen-fixation bacteria passed all phenophases faster, their yield increased, compared with the control, fruits were larger and had a higher content of starch and sugars.

The second actual problem in agriculture and fruit growing is increasing potassium deficiency. One of the possible solutions of this problem is the use of silicate bacteria,

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the soil microorganisms that are capable to transfer hardly soluble forms of potassium minerals in forms available to plants.

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Potassium makes 2.35% of the earth's crust. However, 95% soil potassium is in minerals. Silicate bacteria are capable to withdraw free ions from crystal lattices of minerals; they can also liberate the ions of some microelements, such as molybdenum. As only 20-30% of mineral fertilizers can be used by plants and 70-80% remain in unavailable mineral forms, replacement of mineral fertilizers by biological is may improve this situation. According to the scientists of the Baoding Institute of Microbiology Chinese soils can be supplied by natural stocks potassium for two centuries due to application of preparation BPF, a potassium biological fertilizer. Hence application of mineral K is not necessary. Baoding Institute of Microbiology released two forms of biological potassium fertilizers (BPF): (1) liquid solution, containing two million bacteria per 1 ml, (2) packages of 500 g of peat-moss substrate, contains 2 million bacteria. It has been calculated that an input of 1-3 Yuans (13-40 cents) per 1 hectare, in form of BPF, may increase profitability of production 20–30 times. It was also found that application of preparation BPF in the orchard resulted in strengthening mechanical tissues of apple trees. Trees got more resistant to viral and bacterial diseases and to the sucking pests. The increase of the sizes of fruits, more intensive fruits coloring, reduction of terms of maturing, increase of the maintenance of dry substances and sugars, longer periods of storage of fruits was marked.

Influence of biological fertilizers on fruit crops in Belarus has not been investigated. In this connection in the Institute for Fruit Growing, National Academy of Sciences of Belarus, at Samokhvalovitchi, Minsk region the experiments on application of biological fertilizers in apple orchard were set up in spring 1998.

#### MATERIAL AND METHODS

'Charavnitsa' apple trees on semi-dwarfing rootstock 57-545 were planted on greybrown podzolic loamy soil, in spring 1998. Available  $P_2O_5$  content was 276 mg·kg<sup>-1</sup>, K<sub>2</sub>O content – 153 mg·kg<sup>-1</sup>, pH (KCl) – 4.12, humus content – 1.15%. Spacing was  $4 \times 2$  m, planting density – 1250 trees per hectare. The experiment was set up in randomized design, in 3 replications, with 5 trees per plot. Soil in the orchard was maintained under clean cultivation in alleyways and herbicides along tree rows. Trees were trained in a hedgerow system.

The root system of trees designed for application of biological fertilizers was treated by solutions of bacterial preparations before planting. Repeated treatment with solutions of bacterial preparations, into the rhizosphere, around the tree trunks, was carried out in spring 1998 and 2003.

In course of the experiment trunk cross section area, its annual increments, leaf area of per tree, yield and mean fruit were recorded. The content of pigments in leaves was determined by spectrophotometer method. For estimation of biochemical composition of fruits soluble solids content, sugar content, titratable acidity was also determined by the standard methods [Khalafallah et al. 1984, Maurya and Sanoria 1986]. The content of pectin substances was determined by colorimetric carbolic method, total phenolic

compounds – by colorimetric method with use the of the Folen-Denis reagent, ascorbic acid – based on reaction with  $\alpha$ - $\alpha'$  dipiridil at the presence of iron chloride and phosphoric acid.

#### **Biological preparations and mineral fertilizers**

Azobacterin was applied, as preparation of nitrogen-fixation associative diazotrofic Azospirillum bacteria, the product of the Institute for Soil Science and Agrochemistry, Belarus.

BPF, as preparation of silicate bacteria, the product of the Institute of microbiology Baoding, China was also used. Mineral fertilizers – ammonia saltpeter, double superphosphate, and potassium chloride were applied at the rates of  $N_{90}P_{60}K_{90}$ .

#### **Experimental treatments**

- 1. The control without application of fertilizers.
- 2. Mineral fertilizers N<sub>90</sub> P<sub>60</sub> K<sub>90</sub>.
- 3. BPF Biological K.
- 4. Azobacterin Biological N.
- 5. Mineral fertilizers N<sub>90</sub> P<sub>60</sub> K<sub>90</sub> + Biological N and K.
- 6. Complex Azobacterin with BPF.

### **RESULTS AND DISCUSSION**

Tree vigor depended on fertilizer treatments (tab. 1). The trunk cross-sectional area was the smallest in trees treated with mineral fertilizers treatment 2), significantly higher (except for the data of 2002) than-with complex application of biological and mineral fertilizers (treatment 5). All treatments of application of biological fertilizers

Table 1. Influence of application of various kinds of fertilizers on trunk cross-sectional area (TCSA) and mean shoot length of 'Charavnitsa' apple trees in the years 1998–2003

Tabela 1. Wpływ zastosowania różnego rodzaju nawozów na pole przekroju poprzecznego pnia (PPPP) i średnią długość pędy jabłoni 'Charavnitsa' w latach 1998–2003

Fertilizer treatment Kombinacja nawożenia	1998	1999	2000	2001	2002	2003		
Trunk cross-sectional area – Pole przekroju poprzecznego pnia, cm								
1. Control	1.4	3.20	8.00	13.25	21.35	30.50		
2. Mineral NPK	1.4	3.00	7.40	12.96	19.86	29.88		
3. Bio K	1.7	3.50	8.80	14.17	22.11	33.06		
4. Bio N	1.6	3.65	8.95	15.63	23.68	33.78		
5. Mineral NPK + Bio NK	1.7	4.17	9.00	16.60	26.69	38.20		
6. Bio NK	1.5	3.57	8.60	14.73	23.07	33.39		
LSD <sub>0.05</sub> NIR <sub>0,05</sub>	0.20	0.50	0.96	1.56	7.73	2.57		
	Mean shoot ler	ıgth – Średr	iia długość pę	du, cm				
1. Control	18.0	31.1	41.4	41.1	44.7	35.0		
2. Mineral NPK	19.1	35.9	41.5	36.1	46.2	39.2		
3. Bio K	23.3	34.0	45.9	45.9	49.7	50.3		
4. Bio N	20.8	34.0	41.4	39.2	45.0	42.2		
5. Mineral NPK + Bio NK	22.1	29.4	39.9	34.5	44.5	44.7		
6. Bio NK	21.1	37.6	46.8	46.8	50.2	48.1		

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surpassed in this parameter the control. A similar relationship was noted in the dimensions of tree crowns (tab. 2). However, between particular fertilizer treatments no significant differences in crown dimensions – between particular fertilizer treatments were found. No significant effect of treatments on mean shoot length was recorded (tab. 1)

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Fertilizer treatment Kombinacja nawożenia	Trunk cross- sectional area Pole przekroju poprzecznego pnia cm <sup>2</sup>	Increment of the trunk cross- sectional area Przyrost pola przekroju poprzecznego pnia, cm <sup>2</sup>	Height of the crown Wysokość korony m	Width of the crown (crosswise a row) Szerokość korony (w poprzek rzędu), m	Width of the crown (along a row) Szerokość korony (wzdłuż rzędu) m
1. Control	30.50	9.15	3.13	1.97	1.91
2. Mineral NPK	29.88	10.02	3.05	1.83	1.87
3. Bio K	33.06	10.95	3.05	1.86	1.82
4. Bio N	33.78	10.10	3.03	1.89	1.79
5. Mineral NPK + Bio NK	38.20	11.51	3.14	1.90	1.84
6. Bio NK	33.39	10.32	3.15	1.89	1.91
LSD <sub>0.05</sub> NIR <sub>0,05</sub>	2.57				

Table 2. Effect of various kinds of fertilizers on parameters of tree growth in 2003Tabela 2. Wpływ różnych rodzajów nawożenia na parametry wzrostu drzew w 2003 r.

Significant differences in the trunk cross-sectional area and its increment were observed between the treatments 4, 5 and 6 (tab. 1). The highest values were noted in the treatment 5, i.e. with combined mineral and bio-fertilization. The longest shoots were recorded in the treatment 3 (biological K), 1,4 times longer than in the control (tab. 1).

Influence of fertilizer treatments was found in such parameters as mean leaf area, the tree leaf area per tree, number of shoots and their mean length (tab. 3). So, in the treatment 6 (biological NK) the area of leaves on spurs was by 40% and on shoots by 18% larger, in comparison with the control. When calculated per tree, the highest leaf area was noted in the treatment 2 (mineral NPK), reaching more than 22 m<sup>2</sup> per tree, by 96% more than in the control.

Table 3. Leaf area and its distribution, depending on the mode of fertilization, 2003 Tabela 3. Powierzchnia liści i jej rozmieszczenie, w zależności od sposobu nawożenia, 2003 r.

Fertilizer treatment Kombinacja nawożenia	The area of 1 leaf Powierzchnia 1 liścia cm <sup>2</sup>		Total le Ogólna powi	eaf area ierzchnia liści	Number of shoots per tree Liczba	Mean shoot length Średnia
	on spurs na krótko- pędach	on shoots na długo pędach	$m^2 \cdot tree^{-1}$ $m^2 \cdot drzewo^{-1}$	$10^2 \cdot m^2 \cdot ha^{-1}$	pędów na drzewo	diugose pędu cm
1. Control	8.87	19.28	11.28	14.09	233	35.0
2. Mineral NPK	11.42	19.69	22.13	27.67	381	39.2
3. Bio K	9.80	20.57	13.97	17.46	333	50.3
4. Bio N	11.85	21.92	14.78	18.47	428	42.2
5. Mineral NPK + Bio NK	11.43	20.45	13.55	16.93	495	44.7
6. Biological NK	12.41	22.72	15.83	19.79	648	48.1
LSD <sub>0.05</sub> NIR <sub>0,05</sub>	2.35	1.78	6.25	7.81	199.7	14.0

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Trees in all treatments started to bear fruit in 2000, i.e. in the  $3^{rd}$  year after planting. The biennial yield (2000–2001) in the treatment of application of a biopreparations a significant increase of yield was noted, in comparison with the control. In 2002 trees practically did not fruit. In 2003, the  $6^{th}$  year after planting, the orchard for the first time reached the commercial level of bearing.

Table 4. Influence of different kinds of fertilizers on intensity of flowering, number of fruits, mean fruit weight and yield, 2003

Tabela 4. Wpływ różnych rodzajów nawozów na intensywność kwitnienia, liczbę i ciężar owoców oraz plon, 2003 r.

Fertilizer treatment	Flowering index	Number of fruits per tree	Mean fruit weight	Yield – Plon	
Kombinacja nawożenia	Wskaźnik kwitnienia	Liczba owoców·drzewo <sup>-1</sup>	Średnia masa owocu, g	kg∙tree <sup>-1</sup> kg∙drzewo <sup>-1</sup>	t∙ha⁻¹
1. Control	2.7	44	168	7.4	9.25
<ol><li>Mineral NPK</li></ol>	2.6	35	155	5.5	6.87
3. Bio K	2.7	46	167	7.7	9.63
4. Bio N	2.1	57	159	9.1	11.38
<ol><li>Mineral NPK + Bio NK</li></ol>	2.1	59	170	10.0	12.50
6. Bio NK	2.9	53	171	9.0	11.25

Table 5.	The content of pigments	s, phenolic compound	ds and dry matter	in leaves, 2003
Tabela 5.	Zawartość barwników,	związków fenolowyc	h i suchej masy w	liściach, 2003 r.

Fertilizer treatment Kombinacja nawożenia	Chlor con Zawa chlo mg· a	ophyll itent artość rofilu dm <sup>-2</sup> b	Ratio of chlorophylls Stosunek chlorofilu a/b	Total carotenoid content Ogólna zawar- tość karotenoidów. mg·dm <sup>-2</sup>	Total phenols Ogólna zawartość związków fenolowych mg·100 g <sup>-1</sup>	Dry matter content Zawartość suchej masy %
		On spurs	s – Na krótkope	ędach		
1. Control	3.73	1.68	2.23	2.06	986	41.5
2. Mineral NPK	3.64	1.60	2.27	1.96	964	42.3
3. Bio K	3.28	1.39	2.38	1.84	968	39.4
4. Bio N	3.37	1.51	2.27	1.98	947	40.6
5. Mineral NPK + Bio NK	3.27	1.37	2.39	1.81	964	41.9
6. Bio NK	3.52	1.48	2.37	1.95	968	40.3
		On shoot	ts – Na długop	ędach		
1. Control	3.96	1.68	2.36	1.81	916	41.9
2. Mineral NPK	3.84	1.53	2.50	2.09	948	42.5
3. Bio K	3.71	1.56	2.38	1.95	948	36.8
4. Bio N	3.55	1.44	2.48	1.82	914	41.8
5. Mineral NPK + Bio NK	3.52	1.60	2.21	1.84	921	42.1
6. Bio NK	3.85	1.62	2.37	1.97	930	40.7

In 2003 flowering of trees of variety Charavnitsa was weak (tab. 4). The lowest flowering index was noted in treatments 4 (bio N) and 5 ( $N_{90}P_{60}K_{90}$  + Bio NK) – 2.1. This, however, was not reflected in productivity, which was high, reaching 11.38 and 12.50 t·ha<sup>-1</sup> respectively. At the same time in the control treatment flowering index was 2.7, while yield only 9.25 t·ha<sup>-1</sup>. The lowest yield (6.87 t·ha<sup>-1</sup>) and the lowest mean fruit weight 155 g was in the treatment 2 –mineral  $N_{90}P_{60}K_{90}$ .

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Fertilizer treatments practically have not affected composition of pigments or the content of phenolic compounds in the leaves in 2003 (tab. 5). The content of pigments was slightly higher in the treatment 3 - biological K. The dry matter content in the leaves was by 5% lower in this treatment. Shoot leaves contained, in general, less pigments than spur leaves.

Fertilizer treatments affected biochemical composition of fruits (tab. 6). Total sugar content was higher in all fertilizer treatments, than in the control. The highest values of total sugars was noted in the treatment 6 - 10.83%, in the treatment 3 - 10.07% and in the treatment 4 - 10,3%, compared with only 9.35% in the control. Soluble solids content in fruits was also the lowest in the control, while it was the highest in the treatment 6, with Azobacterin and BPF. The content of pectin substances also was the lowest in the control (0.77\%), while the highest in the treatment 6, biological NK - 0.94%. The lowest acidity was in the treatment 5 – mineral NPK+bio NK.

Table 6. Effect of different kinds of fertilizers on biochemical composition of fruits, %, 2003 Tabela 6. Wpływ różnego rodzaju nawozów na skład biochemiczny owoców, %, 2003 r.

Fertilizer treatment Kombinacja nawożenia	Dry matter Sucha masa	Soluble solids Ekstrakt	Acidity Kwasowość	Total sugars Cukry ogółem	Total pectins Ogólna zawartość pektyn	Ascorbic acid Kwas askorbinowy
1. Control	15.2	13.3	0.61	9.35	0.77	2.84
2. Mineral NPK	16.6	13.3	0.69	9.96	0.84	2.97
3. Bio K	16.1	13.7	0.71	10.07	0.90	3.24
4. Bio N	16.5	13.8	0.65	10.30	0.93	2.91
5. Mineral NPK + Bio NK	15.9	13.4	0.58	9.91	0.82	2.90
6. Biological NK	16.7	13.9	0.64	10.83	0.94	2.67

Table 7. Influence of various kinds fertilizers on physico-chemical soil properties, 2003 Tabela 7. Wpływ różnego rodzaju nawozów na właściwości fizykochemiczne gleby, 2003 r.

Fertilizer treatment Kombinacja nawożenia	pH KCl	P <sub>2</sub> O <sub>5</sub> mg·kg <sup>-1</sup>	K <sub>2</sub> 0 mg·kg <sup>-1</sup>	Humus Próchnica, %
1. The control	5.10	164.5	271.5	1.14
2. Mineral NPK	4.55	172.5	283.0	1.18
3. Bio K	5.73	253.5	382.0	1.26
4. Bio N	6.53	273.0	369.5	1.41
5. Mineral NPK + Bio NK	4.67	308.0	314.0	1.31
6. Biological NK	6.00	218.0	352.0	1.26
LSD <sub>0.05</sub> NIR <sub>0,05</sub>	0.09	8.0	2.6	0.043

Treating soil with mineral fertilizers resulted in increased soil acidity, as shown by pH values, which in treatments 2 an 5 reached values 4.55 and 4.67 respectively, compared with 5.1 in the control (tab. 7). Some increase in K, P and humus content was noted in all fertilizer treatments. The content of soil potassium, phosphorus and humus has considerably increased. In the treatment with BPF the content of available potassium reached 140.7%, of phosphorus 154% and of humus 110.5% of the corresponding values in the control. In the treatment with application of Azobacterin the respective

figures were 136% for potassium, 165% for phosphorus and 123.7% for humus. In the treatment 6 (complex biological fertilization) the increase of K and P content was lower than with application of the same products separately, reaching, in relation to control – 129% for K and 132% for P. Application of biological preparations resulted in a considerable and significant increase of soil pH, raising it to the level close to optimum for apple trees.

# CONCLUSIONS

1. Application of biological preparations BPF and Azobacterin in the apple orchard caused an increase of trunk cross-sectional area, mean shoot length mean leaf area and total leaf area.

2. Treatments with preparations BPF and Azobacterin applied to the rhizosphere of apple trees resulted in an increase of number of fruits per tree, increase of mean fruit weight and an increase of yield.

3. Treatment with mineral fertilizers  $N_{90} P_{60} K_{90}$  decreased mean fruit weight by 8% and yield by 36%, in comparison to the control, and on 29–40% in comparison with the treatments with application of biological preparations.

4. Applications of Azobacterin and BPF affected biochemical composition of fruits, resulting in an increase of sugar content by 15–21.6% as well as an increase of soluble solids, pectin and ascorbic acid content.

5. Treatment with different kinds of fertilizers practically did not have any effect on the composition of pigments and on the content of dry matter or phenolic compounds in the leaves.

6. According to the data obtained in 2003, the most effective were the treatments of application of a bio-mineral complex and a complex of bacterial preparations.

7. Treatment with biological fertilizers significantly lowered soil acidity, to the optimum for apple trees and significantly increased the contents of available soil K, P and humus.

8. Application of mineral fertilizers significantly increased soil acidity and slightly increased the contents of potassium, phosphorus and humus in the soil.

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## WPŁYW NAWOZÓW BIOLOGICZNYCH I MINERALNYCH ZASTOSOWA-NYCH DO GLEBY NA WZROST, PLONOWANIE, SKŁAD BIOCHEMICZNY OWOCÓW JABŁONI 'CHARAVNITSA' ORAZ NA NIEKTÓRE WŁAŚCIWO-ŚCI AGROCHEMICZNE GLEBY

Streszczenie. W ciągu sześciu lat prowadzono badania nad wpływem nawożenia mineralnego i nawożenia preparatami biologicznymi na wzrost i owocowanie jabłoni odmiany Čaravnica na półkarłowej podkładce 57-545. Stwierdzono, że w wyniku zastosowania nawozów biologicznych – oddzielnie i kompleksowo oraz w kompleksie z nawozami mineralnymi – uległ wzmożeniu wzrost drzew, zwiększyła się średnia powierzchnia liści i ogólna powierzchnia liści na drzewach, a także zwiększył się średni ciężar owoców i poprawił ich skład chemiczny. Same nawozy mineralne nie miały wpływu na wzrost drzew i ich plonowanie. Zastosowanie różnego rodzaju nawozów nie miało wpływu na zawartość i skład barwników w liściach. Nawozy mineralne zwiększyły kwasowość gleby oraz spowodowały nieznaczny wzrost zawartości potasu, fosforu i próchnicy w glebie. Preparaty biologiczne obniżały w istotny sposób kwasowość gleby – do poziomu optymalnego dla jabłoni, a także zwiększały znacznie zawartość potasu, fosforu i próchnicy w glebie.

Słowa kluczowe: jabłoń, wzrost, plon, nawozy, skład biochemiczny

Accepted for print - Zaakceptowano do druku: 9.12.2004

Hortorum Cultus 4(1) 2005