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MYCORRHIZAL INOCULATION OF APPLE IN REPLANT SOILS – ENHANCED TREE GROWTH AND MINERAL NUTRIENT STATUS

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Abstract. The field experiment (2009–2012) was conducted to assess the influence of different biofertilizers (AMF liquid/granular inocula, humic and seaweed extracts) on the growth and yielding of 'Topaz'/M.26 apple planted on SARD soils in Poland. During conversion to organic orchard trees' growth, fruit yield, their quality indices as well as nutritional status of leaf and fruit was ascertained. Fruit polyphenol content and their free radical scavenging activity were assessed. Moreover, the mycorrhizal root parameters (mycorrhizal and arbuscules frequency) were also presented. The most vigorous trees were inoculated with liquid inocula MicoPlant M and MicoPlant S. The plants treated with MicoPlant S gave the highest total yield (12.12 kg/tree) and revealed the best productivity (> 1 kg cm^{-2}) as well as the average fruit weight. The liquid suspended inocula were more effective than granular one in terms of mycorrhizal root colonisation. Investigated biofertilizers increased P, K and Cu content of leaf. Organic soil extract (HumiPlant) decreased P and K content of fruit, while seaweed extracts (AlgaminoPlant) increased Ca amount of fruit. These treatments had the lowest K:Ca ratio. Used biofertilizers influenced apples polyphenol content as well as their antioxidant status.

Key words: mycorrhiza, replant disease, macro- and microelements, antioxidants

ABBREVIATIONS

AMF – arbuscular mycorrhizal fungi;
SARD – specific apple replant disease;
GAE – gallic acid equivalent;
DPPH – 2,2-diphenyl-1-picrylhydrazyl;
FRAP – ferric reducing antioxidant power;
SSC – soluble solids content;
f.w. – fresh weight;
d.w. – dry weight

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INTRODUCTION

In sustainable productions of horticultural crops, the arbuscular mycorrhizal (AM) symbiosis is a crucial component for improving the biological equilibrium between microorganisms in the mycorrhizosphere [Tommerup 1992]. AMF are strongly affected by anthropogenic activities [Giovannetti and Gianinazzi-Pearson 1994], and intensive agricultural practices, such as crop rotation, fertilization, pest control and tillage impact AMF, reducing population biodiversity [Helgason et al. 1998, Daniell et al. 2001].

Therefore, in contrast to native ecosystems or organic agriculture, where mycorrhizas are so common, in industrial agriculture the biodiversity level is drastically decreased. Orchard soil-plant system with continuous monoculture, deterioration of soil chemical, physical and biological properties, restricts development and function of mycorrhizal symbioses. Continuous monocultures can both decrease populations of AMF spores and shift the AMF species composition toward species which may not be beneficial to the crop [Douds and Millner 1999, Manici et al. 2003]. Significant shifts in the microbial community of apple rhizosphere were identified that correlate with the increase in replant disease [Mazzola 1999, Zydlik et al. 2006].

Specific apple replant disease is common to all major apple growing regions of the world. It is experienced in most part of world when a new apple planting is made on land just removed from apple production. Also in Poland, which is the 4th leading world producer of apples [FAO 2012], SARD is widely acknowledged as a serious problem [Pacholak et al. 2009].

Therefore, different strategies are employed to overcome this issue. Soil fumigation and pasteurizations are considered the most effective treatment for pre-plant control and other soil-borne diseases [Magarey 1999]. However, soil fumigants destroy the natural equilibrium between pathogens and antagonistic organisms [Uthede and Smith 1999]. This practice is discouraged by Integrated Fruit Production programs. In organic fruit growing there is no alternative to the biocides. The concept of biological control is gaining popularity as more chemical controls. There are very few successful biological treatments for ARD. Natural microbial antagonists with the potential to control ARP include species such as fluorescent pseudomonas, *Trichoderma harzianum*, *Burkholderia cepacia*, *Bacilus subtilis*, *Enterobacter aerogenes* and *Glomus* spp. [Mazzola and Manici 2012]. The addition of various amendments (compost, biofertilizers, cover crops, organic materials) to soil in the planting hole or to the soil surface can lead to significant growth responses in orchards affected by apple replant problem [Mazzola and Gu 2000, Laurent et al. 2008, van Schoor and Stassen 2008].

The benefits of seaweeds as sources of plant growth-promoting substances and nutrients have led to their use as soil conditioner – biofertilizer and plant biostimulator [Sharma et al. 2012a, b]. Seaweeds are a source of plant growth regulators (auxins and auxin-like compounds, cytokinins), betaines, sterols and vitamins precursors (kahydrin) [Khan et al. 2009]. Numerous studies have revealed a wide range of benefit effects on plants, such as improved crop performance and yield, elevated resistant to biotic and abiotic stress. Seaweed extracts promote root growth and development [Zhang et al. 2004, Pacholczak et al. 2013] and improve nutrient uptake by roots [Spinelli et al. 2009]. They also enhance plant defense against pest and diseases. Wu et al. [1998]

demonstrated that application of seaweed extracts to plants can result in decreased levels of nematode attack.

Humic substances (HS) are very important components of soil that affect physical, chemical and biological properties and improve soil fertility [Magdoff and Weil 2004]. The potential bioactivity of products containing HS, most commonly humic acids (HA) has been well documented [Nardia et al. 2002]. HS have indirect and direct beneficial effects on plant growth. In the soil rhizosphere humic substances improve both availability of nutrients and soil porosity, aeration and activities of microorganisms [Kulikova et al. 2005]. On the other hand, HA can regulate mechanisms involved in plant growth stimulation with efficiency comparable to plant auxin [Trevisan et al. 2010]. Many of positives effects of HA on plant physiology including nutrient uptake and root architecture are especially manifest to degraded soil with low organic matter and low microbial biomass [Hartz and Bottoms 2010]. Mitigating activity of HS can be also defined as a phenomena of depressing the negative effects of abiotic stress factors such as unfavorable temperature, pH, nutrient unbalance, salinity, etc. [Kulikova et al. 2005]. These many features of humic substances together with a major demand of environmentally friendly and sustainable agriculture have contributed to expand the significance of humic substances nowadays.

One of proposed approaches is manipulating microbial resources to the orchard soil system to induce a state of general soil suppressiveness to replant disease. It is assumed to reduce overall disease pressure to which young trees are exposed during establishment of successive plantings on the site [Mazzola and Mancini 2012]. Kohler et al. [2009] and Li et al. [2012] indicate that inoculations with AMF reactivate the soil microbial community and consequently improved soil quality and plants' resistance to SARD. AMF form their extended hyphal network can efficiently absorb and translocate water and mineral nutrients [Ryan and Angus 2003], especially from nutrient-poor soils. AMF have also a positive effect on root longevity, increasing the lignification of plant cell walls, which protects roots against infection by soil borne pathogens [Harrier and Watson 2004]. The benefits rendered to the host plants also include the tolerance of abiotic stresses, such as drought, soil compaction salinity or cold [Hildebrandt et al. 2007, Miransari 2010, Wu et al. 2013] and higher photosynthetic efficiency [Auge 2000] what also help to withstand the symptoms on replant disease.

There is no simple alternative for broad spectrum chemical fumigants controlling replant disease because etiology of replant problem in particular location is due a complex of relating factors [Traquair 1984]. The biological methods are likely to be less consistent in their effectiveness than chemical biocides. Currently researchers have to focus on alternatives to chemical fumigation and search biological and cultural practices developing sustainable methods and that could also be used on organic farms [Mazzola and Manici 2012]. Therefore, the aim of the presented study is to assess the effect of mycorrhizal inocula and biostimulants and biofertilizers on the growth, fruiting, mineral status of apple grown in soil with replant problems.

EXPERIMENTAL PROCEDURES

Experimental design. The field experiment was established in the Experimental Station orchard in Garlica Murowana, near Kraków (Poland) in autumn 2009. As the test plant apple trees (*Malus domestica* Borkh.) cv. Topaz grafted on M.26 rootstock were used. 'Topaz' ('Rubin' × 'Vanda') is a scab resistant (V_f) cultivar [Kruczyńska 2002]. Along with its good quality fruits is one of the most promising apple cultivars for organic orchards. One year old maiden budded trees were planted in a randomized block design in four replications of five trees each. Moreover, the additional plots of trees were planted for further mycorrhizal assessment. At the place of nursery/experimental orchard an fruit trees (apples) had been continuously grown for over 20 years. The detailed soil parameters before planting are presented in tables 1 to 3. The following combinations were used:

1. Control trees;
2. MicoPlant E – granular inoculum, 2200 propagules g^{-1} (*Glomus intraradices*, *G. mossae* and *G. agregatum*, *Trichoderma* sp., 30 g per plant, mixed with soil before tree planting);
3. MicoPlant M – suspended liquid inoculum, 3500 propagules g^{-1} (*Glomus intraradices*, *G. mossae* and *G. agregatum*, 4 g L^{-1}, root quick dip before planting);
4. MicoPlant S – liquid inoculum, 1500 AMF propagules g^{-1} (*Glomus intraradices*, *G. mossae*, *G. agregatum*, *G. etunicatum*, *G. deserticola*, *G. monosporus*, *G. brasilianum*, *Gigaspora margarita*, *Rhizopogon sp*., *Scleroderma* sp., *Suillus* sp., *Laccaria* sp., and as well as bacteria strains *Bacillus* sp. and *Azotobacter* sp., 8 g L^{-1}, plants watered with inoculum just after planting);
5. AlgaminoPlant – colloidal suspension of seaweed extracts (18%) from *Sargassum*, *Laminaria*, *Ascophyllum* and *Fuscus* supplemented by potassium salts of amino acids at 10%; 4 mL L^{-1}, foliar spray;
5. HumiPlant – extract made from organic soils containing humic (120 g kg^{-1}) and fulvic (60 g kg^{-1}) acids as potassium salts (30 g K kg^{-1}), and Mg, Ca, Mn, B, S, Mo, Zn and Cu; 100 mL L^{-1}, soil spray.

The mycorrhizal inocula were used only once before planting, while seaweed and humic extracts were used three times each growing season (May–July).

Table 1. Soil texture of the experimental orchard

Percentage of particles (diameter in mm)					
1–0.1	0.1–0.05	0.05–0.02	0.02–0.006	0.06–0.002	<0.006
23%	13%	31%	17%	8%	8%

Plant parameters. The tree trunk diameter was measured every year (end of September) at a height of 30 cm above the soil, the result being calculated per trunk cross-section area (TCSA, cm^2). When the trees began the fruit bearing period, the total yield

as well as the average weight of fruits were recorded. Moreover, the productivity index was calculated (the total yield divided by the TCSA, expressed in kg cm^{-2}).

Table 2. Mean values of pH_{H2O}, soil organic matter, available macroelements (mg dm^{-3}, 0.03 M CH_3COOH extraction)

Soil layer	pH_{H2O}	SOM (%)	Ca (mg dm^{-3})	K (mg dm^{-3})	Mg (mg dm^{-3})	P (mg dm^{-3})	S (mg dm^{-3})
0–20	5.22	1.43	558	123	90.0	21.7	6.20
20–40	6.19	–	489	80.1	66.6	5.40	3.80

Fruit analyses. From each plot 100 apples were separately weighted and mean fruit weigh was estimated. Fruit firmness was measured with an Effegi penetrometer. Soluble solids concentration (SSC) of juices was determined by a digital refractometer (Model PR-100, Atago) at 22°C. Titratable acidity (TA) was ascertained titrating the juice with 0.1 M NaOH to pH 8.1 and expressed as % of malic acid. The total polyphenols content was evaluated by Folin-Ciocalteu method [Singleton 1999]. Additionally, the total antioxidant activity of investigated juices was measured by FRAP assay [Benzie and Strain 1996]. The DPPH assay was done according to the method of Brand-Williams et al. [1995] with some modifications.

Table 3. Soil available microelement and heavy metals concentration (mg kg^{-1}, 1 M HCl extraction)

Soil layer	B (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	As (mg kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})
0–20	0.39	6.32	1345	165	12.8	2.34	0.47	1.02
20–40	0.52	2.87	1042	114	12.1	1.89	0.41	0.91

Determination of mycorrhizal colonisation. One year after planting, trees from additional plots were harvested. Roots were washed from the soil, and cleared in cold 10.0% KOH (24 h). After acidification with 5.0% lactic acid, roots were stained in 0.05% trypan blue according to Koske and Gemma [1989]. For each treatment 90 one-cm root pieces were mounted in polyvinyl alcohol lacto-glycerol on microscope slides. Roots were examined using differential interference contrast microscopy (Axio Imager M2, Carl Zeiss). Mycorrhizal colonization was determined as described by Truvelot et al. [1986]. Mycorrhizal frequency (F%), absolute mycorrhizal intensity (m%) and the abundance of arbuscules present in the root fragments (a%) were assessed in each root segment. The mycorrhizal parameters were calculated using the Mikoryza software ver. 1.1. (Orłowski 2001).

Determination of mineral content. Plant materials for chemical analysis, leaves and fruits were washed thoroughly with distilled water and dried at 70ºC for 48 h. Tissues samples were mineralized in 65% extra pure HNO_3 (Merck) in a CEM MARS-5 Xpress microwave oven [Pasławski and Migaszewski 2006]. Macro- (P, K, Mg, Ca, S), microelements (B, Cu, Fe, Mo, Zn) as well as heavy metals and trace elements (Al, Ba, Cd, Cr, Li, Ni, Pb, Sr, Ti and V) content in leaves and fruits was assessed using ICP-OES technique (Teledyne Prodigy, Leeman Labs). Total N was analyzed by Kjeldahl method.

Statistical analysis. All data were subjected to two way analysis of variance (ANOVA). Significant means ($p < 0.05$) were separated with Duncan's multiple range test using Statistica 9.0 programme (Statsoft Inc.)

RESULTS AND DISCUSSION

Plant parameters. Some authors reported the effect of AMF on vegetative plant properties. Li et al. [2012] demonstrated AMF communities increased cucumber plant height, shoot dry weight, root dry weight, and leaf area. Also Sharma et al. [2012a, b] pointed out the significant correlation between 'Royal Delicious' apple roots mycorrhizal colonisation and trees' diameter/yield.

Used inocula significantly influenced the trees' growth (tab. 4). The most vigorous were trees inoculated with MicoPlant M and MicoPlant S (11.48 and 11.92 cm^2, respectively). The plants treated with MicoPlant S gave the highest total yield (12.12 kg/tree) and revealed the best productivity (> 1 kg cm^{-2}). However, the rest of used AMF inocula did not influence the crop load (with the exception MicoPlant E) nor yield efficiency. Also used seaweed and organic soil extracts had no impact on the parameters. The robust growth and heavier crop load noted for MicoPlant S was positively correlated with the highest fruit weight. The most pronounced effect of MicoPlant S could be caused by

Table 4. Trunk cross-sectional area, the total yield and yield efficiency, mean fruit weight, fruit firmness, soluble solids content and titratable acidity as influenced by different biofertilizers

Treatment	InitialTCSA (cm^2)	FinalTCSA (cm^2)	Totalyield (kg)	Yield efficiency (kg cm^2)	Fruit weight (g)	Fruit hardiness (kG)	SSC (%)	TA (g 100 g^{-1})
Control	1.02 a*	8.89 ab	7.41ab	0.83 ab	114 a	8.61 b	12.8 ab	0.92 a
MicoPlant E	1.01 a	10.58 b	10.32 c	0.97 b	121 abc	8.45 b	12.9 bc	0.91 a
MicoPlant M	1.02 a	11.48 c	9.96 b	0.87 ab	117 ab	8.96 b	13.3 c	0.84 a
MicoPlant S	1.00 a	11.92 c	12.12 d	1.01 c	149 d	7.65 a	12.3 a	0.91 a
AlgaminoPlant	1.01 a	8.56 ab	6.50 a	0.76 a	116 ab	8.55 b	13.2 c	0.90 a
HumiPlant	1.02 a	7.63 a	7.24 ab	0.94 b	127 c	8.31 b	13.4 c	0.88 a

Means within the column, designated with the same letter do not differ at significance at P = 0.95

synergistically interacting microbes (AMF + bacteria strains) used in this bioinoculant. The same synergistic effect reported Singh et al. [2013] during the establishing organic apple orchard. Used seaweed and organic soil extracts had no impact on the tree parameters.

Mycorrhizal colonisation. Industrial farming practices are apparently detrimental for AMF, as recent studies indicate that AMF performance is declining with agricultural intensification [Ryan and Graham 2002, Gosling et al. 2006]. However, little is known about how the duration of organic farming affects AMF. Some authors reported that organic agriculture has been shown to increase AMF root colonization and propagule numbers [Mäder et al. 2000, Galvez et al. 2001, Oehl et al. 2003, 2005], although low input practices used in such management system do not always allow the level of biodiversity to increase, even after a long time [Franke-Snyder et al. 2001]. Hence, understanding the structure and the dynamics of AMF populations as affected by diverse agricultural practices represents an important prerequisite for the success of organic farming.

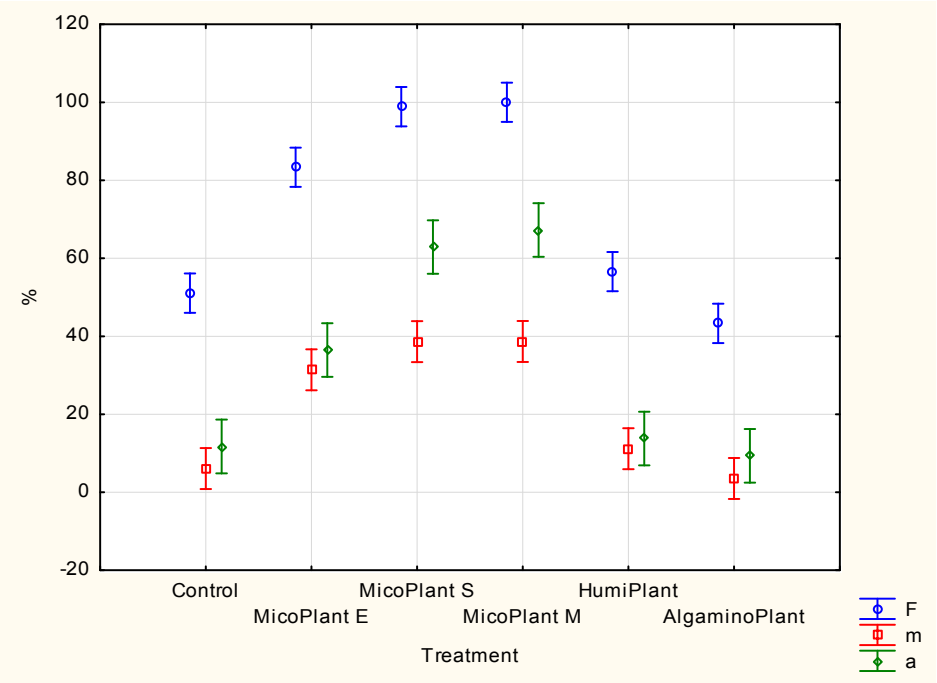

Fig. 1. Mycorrhizal colonization parameters determined in the roots of apple 'Topaz'/M.26. The parameters are: F% – mycorrhizal colonization frequency, m% – intensity of colonization within individual mycorrhizal roots, a% – arbuscule abundance in root fragments where arbuscules were present

In our study, used inocula strongly influenced the mycorrhizal colonization (figs 1 and 2). However, they were not equally efficient. The highest mycorrhizal frequency was obtained for liquid suspended inocula MicoPlant S and MicoPlant M (98 and 100%,

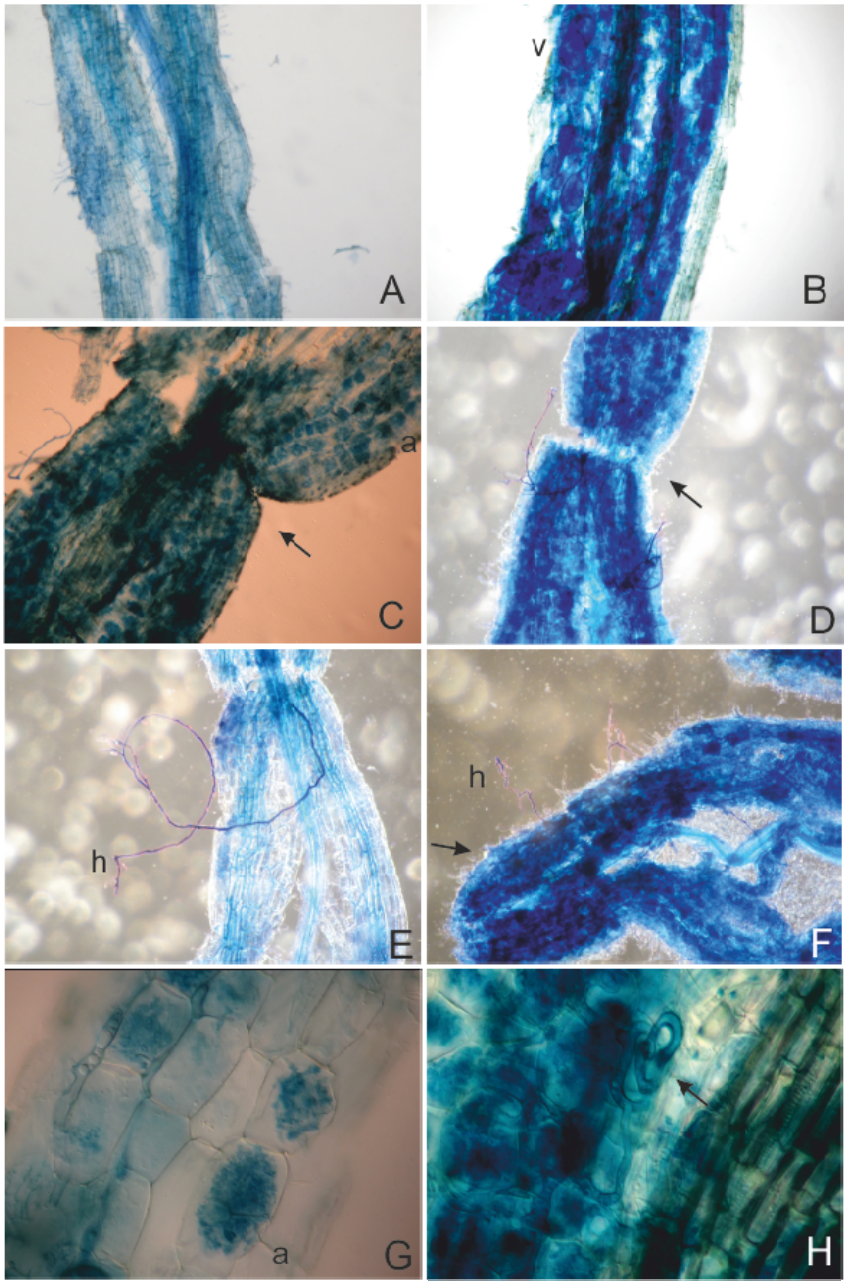

Fig. 2. Apple ('Topaz'/M.26 rootstock) root anatomy. A – non-mycorrhizal root; B – typical pattern of mycorrhization in a root (v – vesicles); C, D – Root narrowing caused by the root's growth cessation. These places were significantly prone to AMF infection; E, F – hyphae (h) penetrating soil, G – arbuscules (a), H – typical formation of Paris type mycorrhiza in apple roots, dense coils of fungal hyphae (arrow) are observed

respectively), while for granular MicoPlant E it was lower (83%). It could be assumed that except the fungus strain used, the formulation which allow better contact inoculum with the root system (quick dip, pouring) is more effective. Control trees reached only F = 51%. The big differences in mycorrhizal frequency is symptomatic for specific apple replant disease (SARD) soils. The extensive use of pesticides naturally reduced the mycorhhizal fungi population. Organic soil and seaweed extracts have no effect on mycorrhizal status. In a pot experiment Bennewitz and Hlušek [2006] with Jonagold/M.9, the AMF root infection was 46% for inoculated, while only 4% for non-treated ones. In different soils Otto [1989] recorded the mycorrhizal frequency for apple seedlings from 39.5 to 98.5%. These results were similar to obtained in our study. As presented in Fig. 2 (C, D, E) in apple roots some narrowing is present, which is a result of root growth cessation. These places are particularly prone to AMF infection.

However, the most prominent mycorrhizal parameter is the abundance of arbuscules present in the root fragments (a%). Due to their large surface area they are thought as putative sites of most nutrient exchange between host and symbiont [Peterson et al. 2004]. In our study the highest arbuscule aboundance was noted for MicoPlant M and MicoPlant S (67.2 and 62.9%, respectively). In the remaining combinations, vesicles, mycelia and arbuscules were present at low abundance. Control roots reached only 11.7% of arbuscules, what might resulted in not fully functional mycorrhiza. These differences might be significant for water/nutrient transport in analysed roots.

Nutrient plant status. The nutrients contents in leaves are summarized in Table 5 and 6. Nitrogen level in apple leaves ranged from 2.56 to 2.82 %. The highest values was detected in 2011 (2.70% N) than in the second year of study (2.59% N). In 2011 the lowest concentration of nitrogen for control trees and MicoPlant M and AlgaminoPlant treatment was recorded. The highest amount of N in leaves of MicroPlant S and MicoPlant E and HumiPlant usage was found. In 2012 olny AlgaminoPlant treatment elevated N content in apple leaves. Several studies have shown that AM fungi contribute to up to 90% of plant P demand [van der Heijden et al. 1998]. Bennewitz and Hlušek [2006] measured higher leaf P concentration for inoculated trees. In our study it was proved only in the first year of the experiment (0.18, 0.18% for AlgaminoPlant and Control, respectively as compared to MicoPlant S inoculum 0.21%). The leaves sampled from control trees revealed the lowest potassium leaf content (1.58%), while the highest was measured for HumiPlant (1.75%) and AlgaminoPlant (1.85%). The reason was probably the chemical formulation of used extracts which contains K, respectively Humiplant – 30 g K kg^{-1} as potassium salts of fulvic and humic acids and AlgaminoPlant potassium salts of amino acids (10%). Numerous studies have revealed a wide range of beneficial effects of AM on nutrients plant concentration. Kohler et al. [2008] found the effect of AMF (*G. intradices* and *G. mosseae*) on better uptake of N, P, Ca, Fe, and Mn in lettuce under water stress. Clark and Zeto [2008] demonstrated the nutrients enhanced most in host plants grown in many soils (e.g., high and low soil pH) are P, N, Zn, and Cu, but K, Ca, and Mg are enhanced when plants are grown in acidic soils. Gąstoł and Domagała-Świątkiewicz [2010] found higher Mg, Ca and Na leaf content in inoculated apple, while Sedláček et al. [2013] higher accumulation of grapevines leaf Ca. However, Singh et al. [2013] reported non-significant correlation between AM fungi root colonization and N, P, Fe, Cu and Zn contents of leaves.

Table 5. Leaf macroelements content (%) as influenced by mycorrhizal inoculation and different biofertilizers application

Treatment			N	P	K	Ca	Mg	S
Year	2011	control	2.64	0.181	1.93	0.78	0.22	0.231
		MicoPlant M	2.67	0.199	2.20	0.80	0.22	0.233
		MicoPlant S	2.82	0.207	2.15	0.90	0.23	0.253
		MicoPlant E	2.71	0.194	2.13	0.87	0.21	0.237
		AlgaminoPlant	2.68	0.176	2.15	0.94	0.21	0.242
		HumiPlant	2.72	0.200	2.15	0.91	0.23	0.253
	2012	control	2.58	0.149	1.23	1.77	0.25	0.190
		MicoPlant M	2.56	0.155	1.48	1.51	0.23	0.193
		MicoPlant S	2.59	0.156	1.48	1.50	0.26	0.195
		MicoPlant E	2.57	0.160	1.53	1.64	0.23	0.204
		AlgaminoPlant	2.73	0.157	1.63	1.36	0.28	0.197
		HumiPlant	2.56	0.144	1.44	1.44	0.21	0.183
Means for	treatment	control	2.60	0.157	1.58	1.50	0.24	0.201
		MicoPlant M	2.59	0.167	1.68	1.32	0.23	0.204
		MicoPlant S	2.65	0.170	1.66	1.34	0.25	0.210
		MicoPlant E	2.60	0.169	1.69	1.43	0.23	0.213
		AlgaminoPlant	2.70	0.165	1.85	1.19	0.26	0.217
		HumiPlant	2.63	0.168	1.75	1.22	0.22	0.216
	year	2011	2.70	0.192	2.12	0.87	0.22	0.241
		2012	2.59	0.154	1.45	1.57	0.24	0.194
	LSD p = 0.05	treatment (A)	0.12	0.010	0.13	0.22	0.03	0.014
		year (B)	0.07	0.007	0.08	0.13	0.02	0.016
		A × B	0.10	0.022	0.21	0.30	0.05	0.024

Within microelement content in leaves of apple grown in orchard with replant problem we noted the decreased amount of copper for control leaves (5.24 mg Cu kg^{-1} d.w.) and elevated for Humiplant (6.0 mg Cu kg^{-1} d.w.) and seaweed extracts (6.30 mg Cu kg^{-1} d.w.) and granular inoculum MicoPlant E (6.06 mg Cu kg^{-1} d.w.) (tab. 6). However, this tendency was strongly year dependent. Treatment with MicoPlant S, MicoPlant E and AlgaminoPlant augmented Fe in apple leaves, especially in 2012. In 2011 the highest content of zinc in leaves of plants treated with HumiPlant (66.1 mg Zn kg^{-1} d.w.) was observed.). Humiplant augmented lead concentration in apple leaves in both years of investigation (1.01 and 2.55 mg Pb kg^{-1} d.w. in 2011 and 2012, respectively) (tab. 7). HumiPlant formulation contents of some nutrients as Fe, S, B, Mo, Zn and Cu. On the other hand, chelators, such as humic substances, could be used for increasing solubility of metal cations, and thus their bioavailability to plants [Chen et al. 2004, Kulikova et al. 2005]. However, the effects of HS on ions uptake appear to be more or less selective and variable in relation to their concentration and to the pH of the soils [Nardia et al. 2002]. There are some inconsistent results reported in the literature concerning the role of mycorrhizae in the absorption and translocation of metals into the plants. Some re-

ports indicate higher concentrations of heavy metals in plants due to AMF [Khan et al. 2000], whereas others have found reduced metals plant concentrations [Hildebrandt et al. 2007, Garg and Aggarwal 2012]. Used biofertilizers had no statistically or consistent effect on the content of the rest of investigated micro (B, Mn) and trace elements (Cd, Cr, Ni, Sr and Ti).

Table 6. Leaf microelement content (mg kg^{-1} D.W.) as influenced by mycorrhizal inoculation and different biofertilizers application

Treatment			B	Cu	Fe	Mn	Zn
Year	2011	control	20.8	6.02	87	329	57.0
		MicoPlant M	22.4	7.02	87	286	56.5
		MicoPlant S	22.2	6.66	86	294	57.4
		MicoPlant E	21.8	6.81	94	288	59.4
		AlgaminoPlant	21.7	6.53	86	267	54.9
		HumiPlant	22.3	7.74	87	332	66.1
	2012	control	23.5	4.95	153	249	38.9
		MicoPlant M	23.7	5.17	149	250	36.8
		MicoPlant S	22.3	5.29	170	256	35.5
		MicoPlant E	25.7	5.78	173	293	38.0
		AlgaminoPlant	22.7	6.14	177	274	35.3
		HumiPlant	23.1	4.70	144	240	35.4
Means for	treatment	control	22.7	5.24	135	271	43.8
		MicoPlant M	23.3	5.67	132	260	42.2
		MicoPlant S	22.3	5.66	147	266	42.1
		MicoPlant E	24.6	6.06	152	292	43.8
		AlgaminoPlant	22.2	6.30	138	271	43.7
		HumiPlant	22.7	6.00	120	280	48.5
	year	2011	21.8	6.79	88	299	58.5
		2012	23.6	5.32	161	261	37.1
	LSD p = 0.05	treatment (A)	Ns	0.61	21	ns	5.5
		year (B)	Ns	0.41	14	ns	3.7
		A × B	ns	ns	33	ns	9.1

Fruit parameters. Used biofertilizers significantly influenced both external and internal quality indices of apples (tab. 4). Soil amendment HumiPlant and MicoPlant S inoculum significantly increased mean fruit weight (127 and 147 g as compared to 114 g for Control). However, the increased fruit weight for MicoPlant S was in the line with the lower fruit firmness and decreased soluble solids content. On the contrary, the beneficial effect on SSC was recorded for MicoPlant M, AlgaminoPlant and HumiPlant (13.3; 13.2 and 13.4° Brix, respectively as compared to 12.8° Brix for Control fruits). In study of Spinelli et al. [2009] seaweed extracts from *Ascophillum nodosum* increased apple average fruit weight and sugar content.

Table 7. Leaf trace elements content (mg kg^{-1} D.W.) as influenced by mycorrhizal inoculation and different biofertilizers application

Treatment			Cd	Cr	Ni	Pb	Sr	Ti
Year	2011	control	0.181	0.354	1.43	0.89	21.6	2.67
		MicoPlant M	0.153	0.316	1.46	0.70	19.5	2.40
		MicoPlant S	0.160	0.335	1.20	0.71	20.8	2.84
		MicoPlant E	0.139	0.308	1.23	0.86	23.2	2.16
		AlgaminoPlant	0.149	0.419	1.43	0.62	19.9	2.00
		HumiPlant	0.188	0.359	1.65	1.01	21.0	2.58
	2012	control	0.116	0.525	3.10	2.00	45.9	2.03
		MicoPlant M	0.159	0.542	2.68	2.27	38.4	1.55
		MicoPlant S	0.147	0.457	3.18	2.11	37.9	1.79
		MicoPlant E	0.147	0.866	4.53	2.15	43.1	2.28
		AlgaminoPlant	0.183	0.489	2.96	2.41	35.8	1.75
		HumiPlant	0.179	0.501	2.99	2.55	38.8	1.31
Means for	treatment	control	0.133	0.478	2.64	1.70	39.2	2.20
		MicoPlant M	0.157	0.480	2.35	1.84	33.2	1.78
		MicoPlant S	0.151	0.423	2.64	1.73	33.3	2.08
		MicoPlant E	0.145	0.714	3.63	1.80	37.7	2.25
		AlgaminoPlant	0.168	0.459	2.30	1.64	29.0	1.85
		HumiPlant	0.183	0.440	2.42	1.89	31.1	1.85
	year	2011	0.161	0.34	1.40	0.18	21.0	2.44
		2012	0.150	0.57	3.29	2.20	40.5	1.83
LSD p = 0.05		treatment (A)	Ns	Ns	1.18	0.46	5.62	0.80
		year (B)	Ns	Ns	0.92	0.23	3.80	0.48
		A × B	ns	ns	2.60	0.62	10.80	1.28

Mycorrhiza affects also the synthesis of secondary matabolites. In Ulrichs et al. [2008] experiment tomato plants inoculated with AMF (*Glomus* sp.) built higher lycopene and b-carotene content in fruits than those without inoculation. In the presented study biofertilizers also influeneced some of fruit secondary metabolites i.e. polyphenols (fig. 3). The lowest phenolics were recorded for MicoPlant S, MicoPlant E and the Control (0.43, 0.44 and 0.46 g GAE L^{-1}, respectively). The higher level was noted for MicoPlant M (0.51) and seaweed extracts (0.52), while the highest for HumiPlant (0.61 g GAE L^{-1}). The similar pattern was recorded for FRAP values: MicoPlant S (4.78 mmol Fe^{2+} L^{-1}), followed by control fruits (5.48) and MicoPlant E (5.60 mmol Fe^{2+} L^{-1} – fig. 4). The highest antioxidant status as measured by this method was obtained for HumiPlant (7.06 mmol Fe^{2+} L^{-1}). DPPH antioxidant assay revealed the lowest free radical scavenging activity for control fruits (17% of inhibition), the others ranged from 20.0 to 38.3% (AlgaminoPlant – fig. 5). The most probably explanation of these differences is an indirect influence of the biofertilizers. It could be linked with the impact on the tree vigour and photosynthetic system efficiency. In some cases the differences were caused by a simple ‘dilution factor’, in the case of bigger fruits.

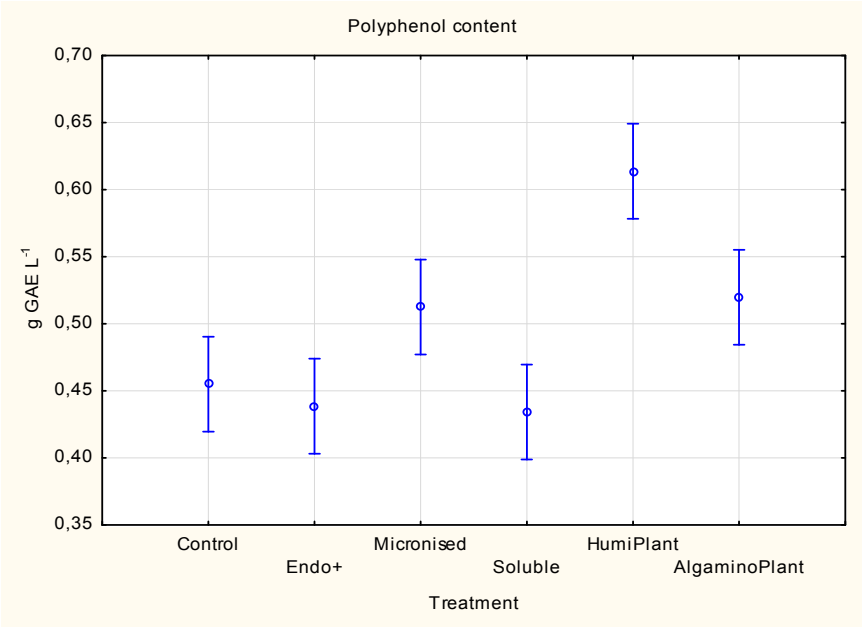

Fig. 3. Mean polyphenol content in apples (g GAE L^{-1}) as influenced by different biofertilizers

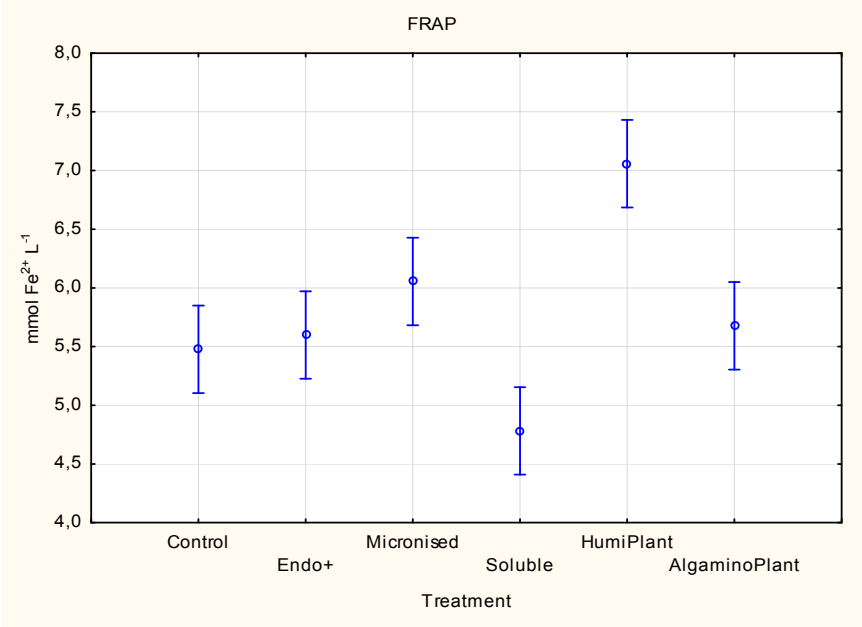

Fig. 4. Ferric reducing antioxidant power (FRAP, mmol Fe^{2+} L^{-1}) of apples as influenced by different biofertilizers

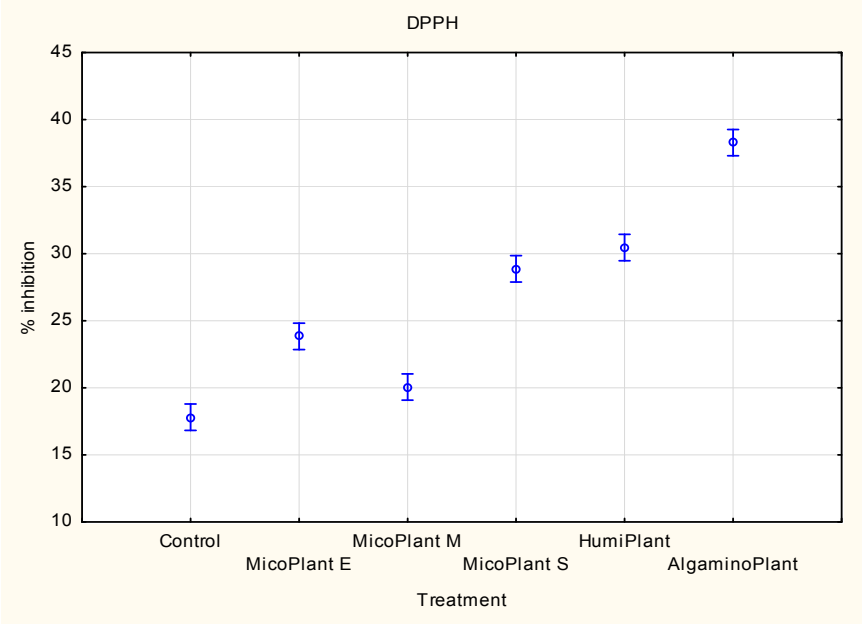

Fig. 5. Antioxidant determination (DPPH assay, % of inhibition) of apples as influenced by different biofertilizers

In Ulrich's et al. [2008] experiment tomato plants inoculated with AMF (*Glomus* sp.) built higher lycopene and ß-carotene content in fruits than those without inoculation. The most probably explanation of these differences is an indirect influence of the biofertilizers. It could be linked with the impact on the tree vigour and photosynthetic system efficiency. The mechanisms of actions of seaweed extracts and plant physiological responses on these treatments are mainly unknown. Zhang et al. [2004] showed the combination of HA + seaweed extract enhanced bentgrass plant root mass (21–68%), and foliar α-tocopherol (110%) and zeatin riboside (38%) contents. This was the first report indicating that application these natural products contain cytokinins resulted in increased endogenous cytokinins level. HS, especially those with a low molecular mass, are taken up by plants and, therefore may also actively modify the plant metabolism, in particularly cell membrane function, nutrient uptake, plant growth and development [Nardia et al. 2002]. Trevisan et al. [2010] demonstrated the auxinic activity of HS is probably the main biological factor responsible for the positive effects exerted by humic substances on plant physiology.

However, the investigated biofertilizers significantly influenced the fruit macronutrient accumulation (tab. 8). Mean phosphorus fruit content calculated for both years of the study varied from 54.1 mg P kg^{-1} f.w. (HumiPlant) to 79.7 mg P kg^{-1} f.w. (MicoPlant M). The same pattern, the lowest values for potassium (809 mg K kg^{-1} f.w.) were noted for HumiPlant, while the highest for mycorrhizal inocula MicoPlant E, MicoPlant S and Control (1035, 1053 and 1080 mg K kg^{-1} f.w., respectively. No differ-

ences in magnesium content were found (the range 42.7 to 51.6 mg Mg kg^{-1} f.w.). In our study, the calcium fruit level was the lowest for the control (44.3 mg Ca kg^{-1} f.w.) and MicoPlant S (45.0) while the highest for AlgaminoPlant (55.9 mg Ca kg^{-1} f.w.). Seaweed extracts are known source of plant growth regulators including auxin and auxin-like compounds [Khan et al. 2009]. In several fruit crop species including apple, it has been documented that an exogenous application of auxin influences some fruit parameters like: size, firmness, and mineral composition [Basak 2006]. Also HS are also generally considered to improve plant growth and enhancing nutrient supply [Kulikowa et al. 2005]. The lowest K:Ca ratio was obtained for AlgaminoPlant (16.9) and HumiPlant (17.45), while for the rest of treatments it was significantly higher (range from 21.9 to 24.8; MicoPlant E and Control, respectively). The similar tendency was proved for Mg:Ca fruit ratio, the lowest for treatments with AlgaminoPlant and HumiPlant. The used inocula did not influence the parameter. The same was observed for sodium content, the lowest for HumiPlant and AlgaPlant (2.07 and 2.14 mg Na kg^{-1} f.w.), the highest level for MicoPlant M (3.10 mg Na kg^{-1} f.w.), while the moderate for the rest treatments.

Table 8. Fruits macroelements content (%), fruit K : Ca and Mg : Ca ratios as influenced by mycorrhizal inoculation and different biofertilizers application

Treatment			P	K	Ca	Mg	S	Na	K/Ca	Mg/Ca
Year	2011	control	87.2	1181	39.65	45.25	35.9	3.43	29.8	1.28
		MicoPlant M	107.3	1302	40.58	50.93	35.8	3.63	32.1	1.35
		MicoPlant S	70.0	861	22.54	46.24	25.1	3.38	38.2	1.52
		MicoPlant E	89.7	1271	42.34	47.30	30.4	2.98	30.2	1.20
		AlgaminoPlant	54.9	774	49.51	51.85	28.3	1.72	15.7	0.85
		HumiPlant	53.7	889	53.29	50.01	35.4	2.37	17.5	0.86
	2012	control	71.4	1020	47.07	48.45	32.5	2.13	21.8	1.01
		MicoPlant M	63.2	904	53.36	52.43	33.4	2.79	17.1	0.95
		MicoPlant S	70.9	1004	54.01	39.26	32.8	2.13	18.8	0.92
		MicoPlant E	63.1	894	54.76	48.37	28.5	2.09	16.8	0.85
		AlgaminoPlant	76.4	1097	61.01	49.47	41.2	2.48	17.8	0.93
		HumiPlant	54.4	745	45.02	42.38	31.8	1.83	17.4	0.96
Means for	treatment	control	77.3	1080	44.29	48.62	33.8	2.62	24.8	1.11
		MicoPlant M	79.7	1053	48.57	51.60	34.3	3.11	22.7	1.09
		MicoPlant S	70.6	963	45.02	45.53	30.6	2.49	24.34	1.10
		MicoPlant E	73.1	1035	50.10	47.73	29.2	2.42	21.9	0.98
		AlgaminoPlant	66.8	953	55.90	50.66	35.5	2.15	16.9	0.90
		HumiPlant	54.1	809	48.70	42.72	33.4	2.07	17.4	0.91
	year	2011	75.1	1033	43.37	46.41	32.2	2.81	25.6	1.13
		2012	66.6	944	52.54	48.70	33.4	2.24	18.3	0.94
	LSD p = 0.05	treatment (A)	9.9	151	8.74	ns	ns	ns	2.96	0.14
		year (B)	6.2	94	5.42	ns	ns	0.49	1.84	0.08
		A × B	14.1	214	12.44	11.62	ns	ns	4.22	0.20

Table 9. Fruits microelements and trace elements content (mg kg^{-1} F.W.) as influenced by mycorrhizal inoculation and different biofertilizers application

Treatment			B	Cu	Fe	Mn	Zn	Mo	Sr	Ba	Ti
Year	2011	control	2.17	0.23	1.43	0.89	0.79	0.217	0.138	0.28	0.031
		MicoPlant M	2.47	0.27	1.42	0.84	0.66	0.019	0.121	0.25	0.022
		MicoPlant S	1.64	0.27	1.26	0.80	0.79	0.013	0.084	0.15	0.022
		MicoPlant E	2.02	0.23	1.31	0.79	0.87	0.026	0.142	0.23	0.016
		AlgaminoPlant	1.09	0.19	1.26	0.93	0.22	0.013	0.149	0.19	0.085
		HumiPlant	1.29	0.71	3.23	1.59	0.42	0.014	0.152	0.29	0.096
	2012	control	1.47	1.10	2.43	1.24	0.25	0.009	0.190	0.34	0.242
		MicoPlant M	1.50	1.11	2.14	1.09	0.24	0.009	0.185	0.31	0.137
		MicoPlant S	1.59	0.65	1.86	1.39	0.36	0.014	0.179	0.29	0.094
		MicoPlant E	1.58	0.54	1.65	0.97	0.18	0.026	0.177	0.26	0.023
		AlgaminoPlant	1.31	0.37	1.90	1.64	0.46	0.012	0.229	0.31	0.171
		HumiPlant	1.25	0.53	2.43	1.47	0.31	0.013	0.150	0.31	0.393
Means for	treatment	control	1.74	0.77	2.05	1.11	0.46	0.087	0.171	0.32	0.164
		MicoPlant M	1.87	0.80	1.87	1.00	0.40	0.288	0.162	0.29	0.094
		MicoPlant S	1.61	0.54	1.69	1.04	0.49	0.014	0.152	0.25	0.073
		MicoPlant E	1.75	0.42	1.52	0.91	0.44	0.025	0.164	0.25	0.021
		AlgaminoPlant	1.21	0.29	1.62	1.32	0.36	0.012	0.194	0.25	0.133
		HumiPlant	1.27	0.61	2.79	1.52	0.36	0.014	0.151	0.30	0.263
	year	2011	1.72	0.33	1.74	1.01	0.59	0.049	0.136	0.23	0.051
		2012	1.45	0.72	2.07	1.26	0.30	0.087	0.185	0.30	0.177
	LSD p = 0.05	treatment (A)	0.14	ns	0.86	0.30	ns	ns	ns	ns	ns
		year (B)	0.09	0.30	ns	0.19	0.10	ns	0.02	0.04	0.10
		A × B	0.20	ns	ns	ns	0.24	ns	ns	ns	ns

As far as the microelements fruit content is concerned, the lowest boron content was noted for AlgaminoPlant and HumiPlant (1.21 and 1.26 mg B kg^{-1} f.w., respectively), the medium level was recorded for MicoPlant S, Control and MicoPlant E, while the highest for MicoPlant M (1.87 mg B kg^{-1} f.w.). Although we found some differences in copper content (the range 0.29 to 0.79 mg Cu kg^{-1} f.w.) they were not statistically significant. No differences were in Ba, Li, Fe, Cr, Ni, zinc, strontium, molybdenium, titanium fruit content was noted. The application of HumiPlant has increased the cobalt fruit content (11.0 µg Co kg^{-1} f.w.) as compared to others (2.37 to 7.99 µg Co kg^{-1} f.w. The used inocula has tendency to lower the fruit manganium content, while AlgaminoPlant and HumiPlant increase Mn level. The use of HumiPlant decreased cadmium fruit level 0.6 (µg Cd kg^{-1} f.w.) as compared to others (the range 2.7 to 5.9 µg Cd kg^{-1} f.w.). The season influenced the content of following elements: Ca, P, B, Cu, Mn, Zn, Na, Sr, Ba, Li, Ti, while for the others no effect was observed.

AMF can filter out toxic heavy metals and consequently keep them away from the plants. Metal are accumulated in the cell wall and in electron-dense granules in the cytoplasm of the fungi. In addition, vesicles might serve as storage compartments for metals [Hildebrandt et al. 2007]. González-Chávezet al. [2004] indicated glomalin –

glycoprotein presumably produced by arbuscular mycorrhizal fungi, which can bind metals in soil. This could explain some of the results obtained in our study.

CONCLUSIONS

AM fungi are especially important for sustainable farming systems because they are efficient when nutrient availability is low and when nutrients are bound to organic matter and soil particles. Due to their significant role in plant nutrient acquisition is considerable concern in using AMF as "bio-fertilizers". Mycorrhizal inoculation enhanced the vegetative growth of apple trees grown during the conversion of orchard planted on SARD affected soils. The most effective in increasing the vigour, yield and mycorrhizal frequency was a multi-strain AMF inoculant. The liquid suspended inocula were more effective than granular ones.

A different theory about the hormone-like substances of humic substances and seaweeds extracts activity has been assumed. However, our conclusions of the effectiveness of using of HS (Humiplant) and seaweeds extracts (AlgaminoPlant) could be speculative rather than theoretical and it would need a more detailed investigation to be considered. We found investigated biofertilizers increased P, K and Cu leaf content. Organic soil extract (HumiPlant) decreased P and K fruit content, while seaweed extracts (AlgaminoPlant) increased Ca fruit amount. These treatments had the lowest K:Ca ratio, indicating good storage properties. Used biofertilizers influeneced fruit polyphenol content as well as their antioxidant status.

The obtained results permit us to definite the final conclusion that biological methods revealed some beneficial effect on the growth, yielding and mineral nutrition of apple trees in the first phase of conversion to organic orchard. However, further investigation needs to be done to assess the long term effect of their use.

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WPŁYW SZCZEPIONEK MIKORYZOWYCH NA WZROST I STATUS MINERALNEGO ODŻYWIENIA DRZEW JABŁONI ROSNĄCYCH W SADZIE Z CHOROBĄ REPLANTACYJNĄ

Streszczenie. W latach 2009–2012 oceniano wpływ różnych bionawozów (granulowane i płynne inokula mikoryzowe, ekstrakty z glonów morskich i gleb organicznych) na wzrost jabłoni 'Topaz'/M.26 rosnących w sadzie z występującą chorobą replantacyjną. Podczas konwersji sadu z produkcji konwencjonalnej na ekologiczną oceniano wigor drzew, plon oraz jego jakość, ze szczególnym uwzględnieniem wartości biologicznej owoców (zawartości polifenoli, potencjał antyoksydacyjny). Określono także wpływ preparatów na parametry opisujące mikoryzę drzew (frekwencja mikoryzowa, obfitość arbuskuli) oraz skład mineralny liści i owoców. Najsilniejszym wzrostem odznaczały się drzewa inokulowane przed posadzeniem szczepionkami mikoryzowymi w postaci płynnej: MicoPlant M oraz MicoPlant S. Jabłonie traktowane preparatem MicoPlant S dały największy plon (12,12 kg/drzewo), były też najbardziej produktywne (>1 kg cm^2), a owoce posiadały największą średnią masę. Ocena kolonizacji mikoryzowej systemu korzeniowego wykazała większą efektywność szczepionek w formie płynnej w porównaniu z granulowanymi. Badane bionawozy zwiększyły zawartość P, K i Cu w blaszkach liściowych. Ekstrakty z gleb organicznych (HumiPlant) zmniejszył koncentrację P i K w jabłkach, podczas gdy ekstrakt z glonów morskich (AlgaminoPlant) zwiększył poziom Ca. W owocach tych kombinacji odnotowano najmniejszą proporcję K:Ca. Zastosowane bionawozy istotnie wpłynęły na zawartość polifenoli w jabłkach, a także ich potencjał antyoksydacyjny.

Słowa kluczowe: mikoryza, choroba replantacyjna, makro-, mikroelementy, antyoksydanty

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