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The Effects of Bioinoculants Based on Mycorrhizal and *Trichoderma* spp. Fungi in an Apple Tree Nursery under Replantation Conditions

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Abstract: Both mycorrhizal and *Trichoderma* spp. fungi are known for antagonistic effects against certain biological pathogens causing apple replant disease (ARD). The aim of this study was to assess the effectiveness of the bioinoculants based on endomycorrhizal and *Trichoderma* spp. fungi on the biological properties of soil as well as the parameters of the apple tree growths in a fruit tree nursery under replantation conditions. A two-year experiment was conducted on Jonagold apple trees grafted on to M.9 rootstock in western Poland. The trees were planted in the replant soil—from areas used for the production of apple trees, and in the crop rotation soil, that had not been used for nursery purposes before. A mycorrhizal inoculum and preparations containing *Trichoderma* spp. fungi were applied to the replant soil. Biological properties of the soil and the growth of the aerial and underground parts of the apple trees were assessed. The enzymatic (dehydrogenases and protease) and respiratory activity of the replant soil was significantly lower than that of the crop rotation soil. The apple trees grew worse when exposed to the ARD conditions. The effectiveness of applied bioinoculants in mitigating the effects of replantation in the nursery were shown. Both the treatment mycorrhization and the application of bioinoculants containing *Trichoderma* spp. increased the respiratory and enzymatic activity of the replant soil. The growth of the root system and the aerial parts of the trees (including leaves) was much better after the combined use of both types of fungi than in the replant soil that had not received the fungal treatment.

Keywords: replant soil; fruit tree nursery; mycorrhizal inoculum; *Trichoderma* spp.; vegetative growth; dehydrogenase and protease activity; respiratory activity

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1. Introduction

Intensive fruit farming production is characterized by high efficiency, which is achieved through a high planting density for trees and the rapid onset of the fruiting period. In order to ensure this effect, it is necessary to use high-quality planting material, i.e., fruit trees on dwarf rootstocks with well-developed crowns that are free from viruses. Due to the market requirements concerning the selection of species and cultivars of fruit plants and due to consumers' increasing requirements concerning the quality of the fruit, growers need to change plantings very often. Apart from that, the life of orchards is reduced by poorly growing rootstocks [1]. In order to meet fruit growers' requirements nurseries also have to change their offerings frequently. If there is not enough new land, that was not used for nursery production before, new nurseries may be established in the places of old ones. This increases the risk of apple replant disease (ARD), also known as the 'replant problem', 'soil sickness', or 'soil fatigue'. There have been numerous studies describing the effects of ARD, mainly in apple orchards, but much fewer publications assessing the consequences of ARD in fruit tree nurseries. Regardless of the type of cultivation, ARD may reduce the productivity of replant soil, which results in worse vegetative growth of plants [2–5], and lower yield and fruit quality [4,6,7].

The problem of ARD is increasing along with the growing intensity of fruit production. Therefore, it is important to find an effective way to mitigate the consequences of ARD. Improvement of the productivity of replant soil is a time-consuming procedure and it is not always sufficiently effective because sometimes it is difficult to precisely identify its causes. ARD may be caused by both abiotic and biotic factors. The former includes high or low soil pH, excessive soil salinity, and inappropriate soil structure. However, according to Spath et al. [8], the influence of abiotic factors on the occurrence of ARD is relatively small. The effectiveness of chemical soil disinfection (fumigation) in the elimination of the consequences of ARD suggests that biotic factors are more important. ARD may be caused by nematodes [4,8], phenolic compounds formed as a result of the decomposition of root debris [9,10], actinobacteria, and bacteria, mainly of the *Bacillius* and *Pseudomonas* genera [11]. ARD may also be caused by fungi of the *Cylindrocarpon*, *Rhizoctonia*, *Alternaria*, *Oomycetes*, *Phytophtora*, and *Pythium* genera [12–14].

The productivity of replant soil can be improved by means of mineral or organic fertilization. However, in the Polish context there are not enough organic fertilizers in modern agriculture, whereas mineral fertilization involves environmental limitations. Excessive mineral fertilization may increase soil acidity, cause nutritional imbalance, and deteriorate the yield quality [15]. Microorganisms are of key importance for long-term soil fertility. Therefore, preparations containing various groups of beneficial microorganisms (bacteria, fungi) which exhibit antagonism against plant pathogens, are being used with increasing frequency in agricultural practice. For example, preparations containing fungi of the *Trichoderma* genus and mycorrhizal fungi are used for biological crop protection. The common phenomenon of mycorrhiza is an example of symbiosis between plants and non-pathogenic fungi. Mycorrhizal fungi facilitate the uptake of water and nutrients by the root system [16]. In return, they receive assimilates from plants. *Trichoderma* spp. fungi can be found wherever organic matter is decomposed. The effectiveness of root colonization with these fungi increases when plants are exposed to stress [17]. Most studies with *Trichoderma* spp. are conducted on agricultural species and vegetables. There are fewer studies on fruit plants. Regardless of the research objects, studies have proved that preparations containing *Trichoderma* fungi effectively increase the resistance of crops to stress factors, such as drought [17,18] and salinity [19]. The fact that these preparations contain the fungi that are antagonistic to the plant pathogens and are considered to cause ARD seems to be their most desirable feature in mitigation of the consequences of this disease. Both mycorrhizal fungi [20] and *Trichoderma* spp. [21,22] exhibit antagonism against plant pathogens. The latter are parasites of phytopathogenic fungi such as *Pythium*, *Fusarium*, and *Rhizoctonia*, as well as nematodes [23]. The following *Trichoderma* strains are the most suitable for fighting plant pathogens: *T. harzianum*, *T. asperellum*, *T. viride*, *T. gamsii*, and *T. polysporum*.

The aim of the study was to assess the influence of the bioinoculants containing endomycorrhizal and *Trichoderma* spp. fungi on the biological properties of soil and the parameters of the growth of apple trees in an apple tree nursery under replantation conditions.

2. Materials and Methods

2.1. Experimental Design

Between 2015 and 2016, an experiment was conducted in a fruit tree nursery located in the village of Puszczykowo-Zaborze, Greater Poland Voivodeship, Poland (52°25′45.553″ N, 17°11′32.755″ E). The experiment was conducted on apple trees of the Jonagold cultivar, grafted on rootstock M.9 (winter grafting), and planted in plastic containers with a capacity of 8 L. The trees were planted in the same type of soil but with differing previous use, so-called crop rotation soil, which was not used for nursery purposes before, and replant soil, in which apple trees had been produced for two seasons. On the crop rotation soil, a 10-year break in the production of fruit trees was used. During this period, agricultural crops were grown, namely spring wheat, rye, and corn. In the last two years before

the planned nursery crop, mustard was grown for green manure. The replant soil was not treated to improve its productivity. The soils had the following content of selected macronutrients (mg 100 g^{-1} dm): the replant soil: phosphorus (P) 15.0, potassium (K) 14.2, magnesium (Mg) 6.0; the crop rotation soil: P 19.0, K 24.0, Mg 9.8. The soil pH was 5.4 and 7.2, respectively. Soil organic matter content in replanted soil was 0.86% and in crop rotation soil it was 1.27%. Salinity in both cases was 0.2 g dm^3 NaCl, and the content of floatable fraction was 20% (sand with high loam). Both types of soil were sampled before the start of the experiment in April 2015.

The following treatments were used in the experiment: (1) (control) trees planted in crop rotation soil (CRS); (2) trees planted in replant soil (RS); (3) trees planted in replant soil with a mycorrhizal inoculant (MI); (4) trees planted in replant soil with *Trichoderma* spp. (T); and (5) trees planted in replant soil with the mycorrhizal inoculant and *Trichoderma* spp. (MI + T). There were ten replicates in each treatment. One plant was one replicate (5 treatments × 10 trees = 50 trees in the experiment).

The plants were treated with a Mykoflor mycorrhizal inoculant (Mykoflor, Poland) containing propagules of arbuscular mycorrhizal fungi (1.000 propagules per 1 g): *Rhizophagus irregularis* (formerly *Glomus intraradices*), *Funneliformis mosseae* (formerly *Glomus mosseae*) and *Claroideoglomus etunicatum*. These species are often used for the mycorrhization of crops, including apple trees, in both field and pot experiments [24,25]. Hydrogel was used as a support. The inoculum did not contain other groups of beneficial microorganisms. The mycorrhizal preparation was applied only once, with a syringe, at an amount of 1 g per plant, when the trees were being planted.

The apple trees were inoculated with *Trichoderma* spp. fungi contained in Trianum G and Trianum P preparations. Both of them contained *Trichoderma harzianum Rifai*, strain P22, as the active substance, namely 1.5 × 10^{-8} CFU g^{-1} (Trianum G) and 10^{-9} CFU g^{-1} (Trianum P). Trianum G was applied once, during planting, by mixing 375 g of the preparation granules with 1 m^3 of replant soil. During vegetation, the trees were irrigated with a Trianum P suspension three times, each time at a dose of 20 g per 100 L of water. The first treatment was applied at the end of April, the others after four weeks.

In each growing season the trees were fertilized with multicomponent Basocote Plus 6M fertilizer (NPK 16 + 8 + 12, microelements) at a dose of 3 g dm^3. Additionally, during the growing season, the trees received foliar treatment with 1% liquid Florovit. The crop protection treatments were applied in accordance with the recommendations for nursery production. The weather conditions during the experiment were analyzed on the basis of measurements of the weather station located in the immediate vicinity of the experiment site. The climatogram in Figure 1 shows the average temperatures and total monthly rainfalls at a ratio of 1 °C: 4.5 mm of rainfall.

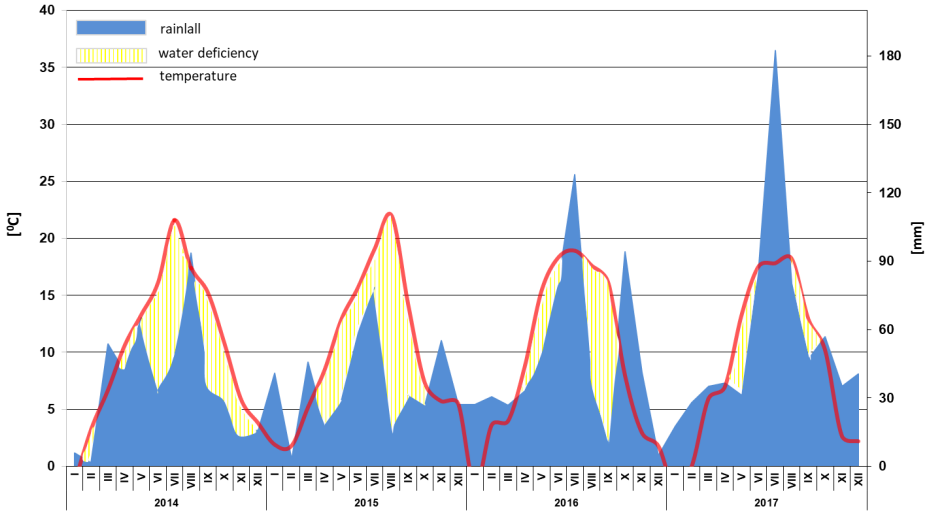

Figure 1. The climatogram in the years 2015–2016.

2.2. Measurements of the Vegetative Growth of the Trees

The assessment of the growth of the trees was based on measurements of the diameter of their trunks (mm); their height (cm); the number and total length of their side shoots; the weight of their root system (g); the weight, length, and width of their leaves (g); and their surface area (cm^2). The content of chloroplast pigments in the leaves (chlorophyll *a* and *b*) and carotenoids was also analyzed. The growth force of the trees was measured in the autumn in the second year of the experiment. The diameters of the apple tree trunks were measured 10 cm above the budding site, in accordance with the guidelines for assessment of the quality of nursery material. The height of the trees was measured from the root neck to the top of the main shoot. When measuring the number of side shoots and their total increments, all shoots longer than 1 cm were taken into account. The root system of the trees was weighed (with an accuracy of 1 g) after the roots had been rinsed with water under constant pressure to remove soil. In late July, 50 leaves were randomly collected from the trees in each combination for measurements. The leaves were weighed (with an accuracy of 0.1 g) in quadruplicate. Next, all the leaves were scanned to measure their surface area with DigiShape software. The content of assimilation pigments, namely chlorophyll *a* and *b* and carotenoids, was measured in the fresh leaf mass by extraction with dimethyl sulfoxide [26].

2.3. Analysis of Biological Properties of Soil

The biological properties of the soil were assessed in the first and second years of the experiment. The assessment included the analysis of the activity of two soil enzymes proteases and dehydrogenases, the soil respiratory activity, and the number of spores of endomycorrhizal fungi. The biological properties of the soil were analyzed at three terms: in the spring, in mid-May; in the summer, after intensive growth of the trees; and in the autumn, after the leaves had discolored. Samples were collected from the rhizosphere of each replicate (container). Next, they were mixed into one 0.5-kg sample, which was representative for each treatment. The protease activity was measured with the spectrophotometric method described by Ladd and Butler [27], with a 1% sodium caseinate solution as a substrate. The measurements were made with a spectrophotometer, at a wavelength of 578 nm, after a one-hour incubation of the samples at 50 °C. The protease activity was expressed as mg of tyrosine h^{-1} kg^{-1} dm of soil. The dehydrogenase activity was measured with the colorimetric method described by Thalmann [28], with a 1% triphenyl tetrazolium chloride solution (TTC). The measurements were made with a spectrophotometer, at a wavelength of 485 nm (TTC test), after a 24-hour incubation of the samples at 30 °C. The dehydrogenase activity was expressed as cm^3 H_2 24 h^{-1} kg^{-1} dm of soil. The soil respiratory activity (mg CO_2 kg^{-1} 48 h^{-1}) was measured with the absorption method described by Gołębiowska and Pędziwilk [29], on the basis of the amount of CO_2 released. The enzymatic and respiratory activity of the replant soil was performed in four repetitions for each treatment. Results are presented as the average of two years of study.

The number of endomycorrhizal fungal spores (in 100 g of air-dry mass) was measured once in the laboratory of the Department of Agricultural Microbiology, Institute of Soil Cultivation and Fertilization. In the second year of the experiment, in the spring (late May), 250 g of soil was collected from each treatment. The measurements were made according to the methodology developed by Allen et al. [30] The fungal spores isolated on filters with mesh diameters of 150, 50, and 75 µm were counted. They were totaled as the number of spores in the soil sample. There were four replicates of cultures in each combination.

The results were processed statistically with analysis of variance and Duncan's test at a significance level of $\alpha = 0.05$.

3. Results and Discussion

3.1. The Influence of the Site on the Biological Properties of Soil

Apple replant disease decreases soil biodiversity and the activity of soil microorganisms, including fungi [5,31]. The experiment showed that there were fewer mycorrhizal fungi in the replanted soil (755 spores) than in the crop rotation soil (1000 spores) (Table 1).

Table 1. The number of spores of endomycorrhizal fungi in the replant soil (CRS = crop rotation soil, RS = replanted soil, MI = micorrhyzal inoculum).

Treatment	>150 µm	>75 µm	>50 µm	Total in 100 g Air-dm
CRS	35	455	510	1000
RS	30	340	385	755
MI	50	870	890	1810

Fluctuations in the count and activity of soil microorganisms directly affect the enzymatic activity, which is considered to be one of the basic parameters of the quality of soil and changes occurring in this environment under the influence of anthropogenic factors [32,33]. Soil enzymes may originate from various sources. However, most of them come from microorganisms, mainly bacteria, as well as plant roots and root debits. Oxidoreductases (dehydrogenases) and hydrolases (phosphatases, proteases, urease) are the most important enzymes in the soil environment. The experiment showed that both dehydrogenases and proteases exhibited significantly lower activity in the replant soil (RS) than in the crop rotation soil (CRS). There were particularly big differences in the activity of dehydrogenases, enzymes considered to be the main indicator of soil microbial activity [34]. Between 2015 and 2016, the average dehydrogenase activity in the treatment with CRS amounted to 1.84 cm^3 H_2 24 h^{-1} kg^{-1} dm, whereas in the RS it was 0.52 cm^3 H_2 24 h^{-1} kg^{-1} dm (Table 2).

Table 2. Dehydrogenases activity in the soil (cm^3 H_2 24 h^{-1} kg^{-1} dm) in the year 2015–2016 (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma*; MI + T = mycorrhizal inoculum + *Trichoderma*).

Treatment	Spring	Summer	Autumn	Average for Treatment
CRS	1.49 g (0.15)	1.18 de (0.03)	2.86 h (0.49)	1.84 b
RS	0.24 a (0.02)	0.85 c (0.04)	0.48 b (0.14)	0.52 a
MI	0.80 c (0.03)	0.33 ab (0.03)	1.24 ef (0.06)	0.79 a
T	0.87 c (0.04)	0.96 cd (0.02)	1.55 g (0.06)	1.13 ab
MI + T	0.95 cd (0.04)	0.41 ab (0.05)	1.43 fg (0.07)	0.93 ab
Average for term	0.87 b	0.74 a	1.51 c	

Means marked with the same letters do not differ significantly at $\alpha = 0.05$. Values in brackets represent standard deviation.

The earlier soil use also affected the protease activity, which was almost two times lower in the replant soil (RS) than in the crop rotation soil (2.42 and 4.58 mg of tyrosine 24 h^{-1} kg^{-1} dm, respectively) (Table 3).

Table 3. Protease activity in the soil (mg of tyrosine h^{-1} kg^{-1} dm) in the year 2015–2016 (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma*; MI + T = mycorrhizal inoculum + *Trichoderma*).

Treatment	Spring	Summer	Autumn	Average for Treatment
CRS	6.38 de (0.97)	3.26 ab (0.69)	4.09 bc (0.57)	4.58 b
RS	2.01 a (0.43)	1.90 a (0.75)	3.35 ab (0.47)	2.42 a
MI	7.03 e (1.40)	3.77 b (0.74)	6.19 de (1.79)	5.66 b
T	4.64 b–d (1.10)	5.69 c–e (0.67)	6.72 e (0.53)	5.68 b
MI + T	7.48 e (1.12)	13.52 f (1.12)	5.74 c–e (1.52)	8.91 c
Average for term	5.51 a	5.63 a	5.22 a	

Means marked with the same letters do not differ significantly at $\alpha = 0.05$. Values in brackets represent standard deviation.

The authors of this study also observed a decrease in the enzymatic activity of replant soil in their earlier study [31], which may have resulted from soil acidity. The activity of soil enzymes increases along with the soil pH [35,36]. In an acidic environment, the count and activity of soil bacteria decrease and so does the activity of dehydrogenases [37]. The replant soil used in the experiment was more acidic (pH 5.4) than the crop rotation soil (pH 7.2). The activity of soil enzymes may also be influenced by the abundance of soil nutrients. The amount of nutrients in the soil may be positively correlated with its enzymatic activity [38]. In our experiment the content of available phosphorus, potassium, and magnesium in the replant soil was lower than in the crop rotation soil (see Section 2). Another parameter that determines the biological properties of soil is its respiratory activity, measured with the amount of carbon dioxide released. This parameter was significantly affected by the earlier soil use. Between 2015 and 2016, the average respiratory activity in the CRS treatment was three times higher than in the RS (40.31 mg CO^2 kg^{-1} 48 h^{-1} vs. 14.48 mg CO^2 kg^{-1} 48 h^{-1} (Table 4).

Table 4. Soil respiratory activity (mg CO^2 kg^{-1} 48 h^{-1}) in the years 2015–2016 (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma*; MI + T = mycorrhizal inoculum + *Trivhoderma*).

Treatment	Spring	Summer	Autumn	Average for Treatment
CRS	57.50 h (7.40)	30.81 d–f (5.24)	32.63 ef (5.14)	40.31 c
RS	16.58 ab (1.72)	9.24 a (2.04)	11.63 a (1.80)	12.48 a
MI	53.79 h (5.89)	27.27 c–e (3.62)	20.54 bc (3.37)	33.87 b
T	52.10 h (6.24)	32.30 d–f (4.23)	24.05 c–d (2.94)	36.15 bc
MI + T	42.03 g (7.51)	38.32 fg (5.62)	26.32 c–e (3.24)	35.56 b
Average for term	44.4 c	27.59 b	23.03 a	

Means marked with the same letters do not differ significantly at $\alpha = 0.05$ Values in brackets represent standard deviation.

3.2. The Influence of Mycorrhizal Fungi and Trichoderma spp. *on the Biological Properties of Replant Soil*

The inoculant (MI) used in the experiment increased the number of mycorrhizal fungal spores in the replanted soil more than two times—from 755 to over 1800 (Table 1). The larger number of spores indicates a greater population of mycorrhizal fungi. *Funneliformis mosseae*, *Claroideoglomus etunicatum*, and, to a much lesser extent, fungi from the *Gigasporaceae* family dominated in the group of spores > 75 and 150 µm. Sumorok [39] also observed that the Mykoflor inoculant increased the colonization of plant roots with mycorrhizal fungi. The

authors found that the fungal population on the roots of the apple trees treated with the inoculant was over 50% greater than on plants which had not received the treatment. In the experiment conducted by Mikiciuk et al. [40], the population was more than two times larger.

The mycorrhizal fungi and *Trichoderma* spp. increased the protease activity in the replant soil more than two-fold. During the two-year period of the experiment the mean activity of this enzyme increased by 233% in the MI treatment and by 234% in the T treatment, as compared with the RS treatment (Table 3). The highest efficiency was achieved when both types of fungi were applied together (MI + T). The protease activity in this combination was more than three times higher than in the replant soil (RS) (8.91 vs. 2.42 mg tyrosine h^{-1} kg^{-1} dm) and almost two times higher than in the control treatment (CRS) (4.58 mg tyrosine h^{-1} kg^{-1} dm) (Table 3). There were no significant differences in the dehydrogenase activity in various treatments (Table 2). Sheng [41] observed increased dehydrogenase activity in the soil after mycorrhization. However, these authors did not conduct their experiment on fruit trees. The mycorrhizal fungi and *Trichoderma* spp. significantly increased the respiratory activity of the replant soil, which was almost three times higher in the MI (33.87 mg CO_2 kg^{-1} 48 h^{-1}) and T (36.15 mg CO_2 kg^{-1} 48 h^{-1}) treatments than in the RS treatment (12.45 mg CO_2 kg^{-1} 48 h^{-1}) (Table 4).

3.3. Soil Enzymatic Activity in Different Vegetation Periods

Apart from the content of soil colloids, the method of soil cultivation, and the intensity of mineral fertilization, the soil enzymatic and respiratory activities may also be influenced by weather conditions, including humidity and temperature. The experiment showed differences in the soil enzymatic activity, which depended both on the year of the study and the growing season. In 2015, both dehydrogenases and proteases exhibited significantly lower activity than a year later (Table 5).

Table 5. Dehydrogenase (cm^3 H_2 24 h^{-1} kg^{-1} dm) and protease (mg of tyrosine h^{-1} kg^{-1} dm) activity in the years 2015 and 2016.

Enzyme	2015	2016
Dehydrogenases	0.88 a	1.21 b
Proteases	4.77 a	6.13 b

Each parameter was individually analyzed statistically. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

This may have been influenced by variation in the air temperature and the amount of rainfall. In 2016, there was higher rainfall than in 2015 (Figure 1). In 2015, the temperature was 3.4 °C higher than the long-term average. A lower availability of water decreases the activity of soil microorganisms and, therefore, the enzymatic activity [42,43]. The soil enzymatic activity may increase in spring due to the optimal temperature and sufficient moisture of the substrate. In autumn, it may increase as a result of the supply of fresh organic matter in the form of crop residues stimulating the proliferation of soil microorganisms. Tables 2 and 3 show the results of measurements of the activity of these two soil enzymes in different growing seasons. The activity of dehydrogenases was the most diverse. During the two-year research, the average dehydrogenase activity in the soil was the highest in the autumn (1.51 cm^3 H_2 24 h^{-1} kg^{-1} dm), and the lowest in the summer (0.74 cm^3 H_2 24 h^{-1} kg^{-1} dm; Table 2). Yuan and Yue [44] also observed the highest dehydrogenase activity in the autumn, whereas Wilińska et al. [32] observed the lowest dehydrogenase activity in the summer. The activity of dehydrogenases tends to vary considerably in a season-dependent manner, because it is closely related to the activity of soil microorganisms. Lower dehydrogenase activity in the summer suggests low activity of soil microorganisms. Both in 2015 and 2016, at the end of the summer (August–September), there was low or very low rainfall (9.4 mm in September 2016) and high temperatures, which resulted in dry periods (Figure 1).

The dynamics of the protease activity in different growing seasons was evidenced by Sardanas et al. [45], who found that even a slight decrease in the soil moisture reduced the activity of these enzymes by several dozen per cent. Although there were large differences in humidity and temperature during our experiment, the analysis did not show any differences in the soil protease activity in different growing seasons (Table 3).

3.4. The Influence of the Site on the Growth of Apple Trees

Lower productivity of replant soil weakens the growth of plants affected by ARD. The apple trees growing in the replant soil (RS) were significantly shorter than those in the CRS treatment (147.0 vs. 177.8 cm, respectively) (Table 6).

Table 6. The apple tree growth parameters (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma*; MI + T = mycorrhizal inoculum + *Trichoderma*).

Treatment	Height of Trees (cm)	Number of Side Shoots	Total Length of Side Shoots (cm)
CRS	177.8 b (9.15)	10 ab (2.58)	86.16 bc (8.41)
RS	147.0 a (13.54)	7.0 a (1.34)	40.23 a (10.24)
MI	176.7 b (11.02)	10 ab (1.84)	48.33 ab (3.38)
T	175.8 b (9.85)	10 ab (1.53)	87.09 bc (8.24)
MI + T	190.5 c (11.74)	12 b (1.76)	128.09 c (9.85)

Each parameter was individually analyzed statistically. Means marked with the same letters do not differ significantly at $\alpha = 0.05$. Values in brackets represent standard deviation.

The sum of the increments of side shoots on the trees growing in the replant soil (40.23 cm) was more than two times smaller than that of the trees growing in the crop rotation soil (86.16 cm; Table 6). Additionally, the annual increment in the tree trunk diameter in the RS treatment (4.16 cm) was much smaller than in the CRS treatment (4.55 cm; Figure 2).

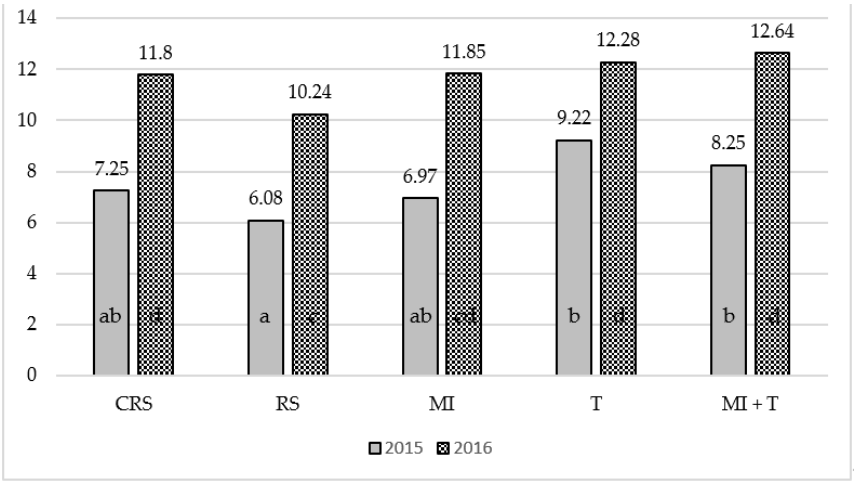

Figure 2. The apple tree trunk diameter (mm) in the years 2015 and 2016 (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma*; MI + T = mycorrhizal inoculum + *Trichoderma*). Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

The biometric parameters of the tree leaves (length and width), area, and especially, the weight in the RS treatment were significantly lower than in the CRS. For example, the weight of the leaves of the trees growing in the replant soil (7.56 g) was almost two times lower than that in the control treatment (14.62 g; Table 7). Weiß and Winkelmann [46] also observed weaker growth of apple rootstocks affected by ARD.

Table 7. The apple leaf growth parameters (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma*; MI + T = mycorrhizal inoculum + *Trichoderma*).

Treatment	Weight of Leaves (g)	Width of Leaves (cm)	Length of Leaves (cm)	Surface Area of Leaves (cm^2)
CRS	14.62 b (1.75)	2.32 c (0.02)	3.13 b (0.03)	42.47 b (1.84)
RS	7.56 a (1.10)	1.72 a (0.87)	2.56 a (0.21)	25.88 a (2.85)
MI	14.33 b (1.90)	2.02 b (0.03)	3.02 b (0.03)	46.11 c (1.53)
T	16.06 c (1.40)	2.16 bc (0.02)	3.49 c (0.02)	45.83 c (1.62)
MI + T	17.52 d (1.20)	1.87 ab (0.02)	2.98 b (0.03)	48.94 d (1.44)

Means marked with the same letters do not differ significantly at $\alpha = 0.05$. Values in brackets represent standard deviation.

A well-developed root system is necessary for plants to have a good supply of water and nutrients, especially when they are exposed to biotic and abiotic stress. Our experiment showed that the root system of the apple trees growing in the replant soil (RS) developed more poorly than on the CRS. This was evidenced by the average weight of the roots of the trees in the RS treatment (134 g), which was significantly lower than in the CRS treatment (240 g; Figure 3).

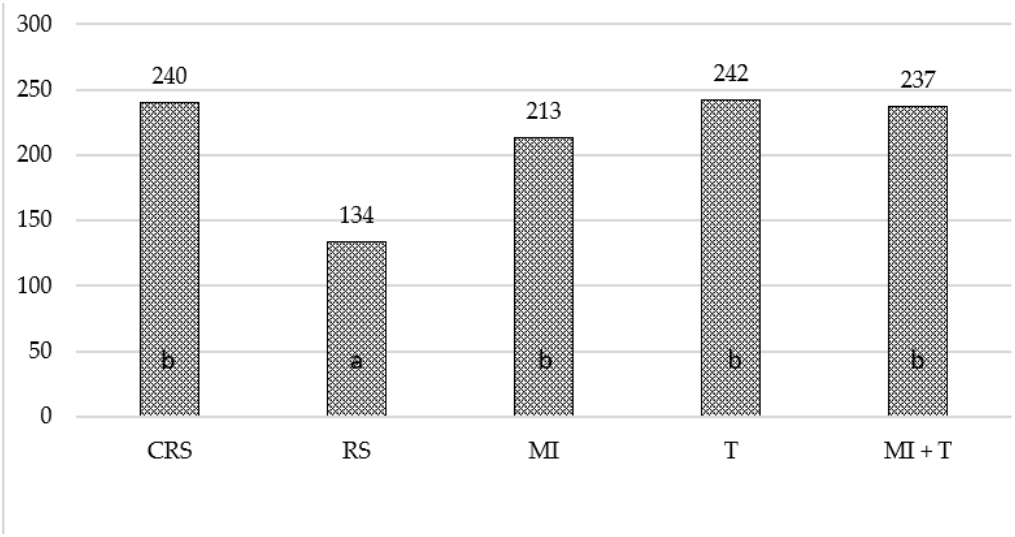

Figure 3. Weight of the roots of the apple trees (g) (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma;* MI + T = mycorrhizal inoculum + *Trichoderma*). Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

3.5. The Influence of Soil Preparations on the Growth Power of Apple Trees

After the mycorrhization and the application of *Trichoderma* spp., the growth of the aerial part of the apple trees growing in the replant soil improved. The apple trees in the MI + T treatment (190.5 cm) were over 20% taller than those in the RS (147.0 cm) and a few percent taller than those in the CRS treatment (177.8 cm) (Table 6). Likewise, the trees in this treatment had the most side shoots and the highest sum of their increments. The mycorrhizal and *Trichoderma* spp. fungi applied separately also improved the vegetative growth of the trees in the replant soil. After inoculation with *Trichoderma* spp. the sum of increments in the apple tree shoots was more than two times greater than in the RS treatment (87.09 cm vs. 40.23 cm) (Table 6). After the mycorrhization treatment (MI) the diameter of the apple tree trunks increased by 4.89 mm over one year. It increased by 4.39 mm (Figure 2) in the MI + T treatment. The leaf surface area of the trees in the MI + T

treatment was almost two times larger than in the RS (48.94 vs. 25.88 cm^2), whereas the leaf mass was more than two times heavier (17.52 vs. 7.56 g; Table 7).

The mycorrhizal fungi and *Trichoderma* spp. improved the growth parameters not only in the aerial part of the trees but also in their root system. The root weight in the treatments treated with the fungi was significantly greater than in those which had not received the treatment (from 159% in the MI to 180% in the T; Figure 3). Figure 4 shows a visual assessment of the apple tree root system in different treatments. The root volume of the trees growing in the replant soil (RS) after the application of mycorrhizal fungi and *Trichoderma* spp. was similar to that of the trees in the CRS treatment.

Figure 4. Root system of apple trees (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma*; MI + T = mycorrhizal inoculum + *Trichoderma*).

Our experiment confirmed the findings of earlier studies which showed that mycorrhization had positive effect on the growth of roots of some species of fruit trees, i.e., apple trees [47], pear trees [48], sour cherry trees [49], and plum trees [50]. The results of studies conducted by other researchers showed that mycorrhizal fungi improved the vegetative growth of apple trees [51], sour cherry trees [52], cherry rootstocks [53], and strawberries [54]. The effectiveness of mycorrhizal fungi is even higher when plants are exposed to stress [55], both biotic (ARD) [56] and abiotic (low supply of soil phosphorus) [57]. Mycorrhizal fungi improve plants' vegetative growth because they form a dense network in soil, which facilitates the uptake of water and nutrients. *Trichoderma* fungi have a similar property, which especially facilitates the uptake of nitrogen [58].

The process of photosynthesis determines the accumulation of biomass by plants. Its proper course largely depends on the content of chlorophyll in the leaves. Available scientific publications provide relatively little information on the influence of ARD on the photosynthesis in apple leaves. Our experiment showed that previous use of the soil had no influence on the content of chlorophyll $a + b$ in the leaves of the apple trees (Appendix A).

Carotenoids increase plant resistance to stress factors [59] because they protect the photosynthetic apparatus from photodestruction. The statistical analysis conducted in our study did not show any significant differences in the content of chlorophyll and carotenoids in the leaves of the apple trees after treatment with mycorrhizal and *Trichoderma* spp. fungi. Our study did not confirm the results of the experiments conducted by other researchers, who observed that mycorrhization treatment increased the content of chlorophyll and carotenoids in the leaves of strawberries [40,60], cucumbers [56], and courgettes [61].

4. Conclusions

Our experiment confirmed the findings of earlier studies which showed that soil productivity declines under ARD conditions. Replant soil was characterized by a lower count of mycorrhizal fungi, lower activity of proteases and dehydrogenases, and lower

respiratory activity. For this reason, the apple trees had poorer growth parameters—they were much shorter, with smaller increments in the trunk diameter, and lower mass of the root system than the trees in combination with crop rotation soil. They also had significantly lower leaf weight and area. The bioinoculants used in the investigation, based on both the mycorrhizal fungi and *Trichoderma* spp. significantly increased the protease activity and the respiratory activity of the replant soil. The highest effectiveness in this regard was observed when both types of fungi were applied together. There were no significant differences in the dehydrogenase activity in the soil or the chlorophyll content in the leaves of the trees. The bioinoculants based on mycorrhizal fungi and *Trichoderma* spp. improved the growth parameters of the aerial and underground parts of the apple trees. Additionally, in this case the combination with both types of the fungi was the most optimal—the leaf surface area tripled, and the leaf weight doubled. The combined application of the mycorrhizal fungi and *Trichoderma* spp. resulted in the greatest height of the apple trees, and the greatest number of side shoots and the sum of their increments. To sum up, the use of bioinoculants containing both mycorrhizal and *Trichoderma* spp. fungi allowed for significant mitigation of the effects of replanting in the apple tree nursery. Protease activity and respiratory activity of replanted soil increased and significantly improved biometric parameters of apple trees. Particularly beneficial in this regard was the combined use of both types of fungi.

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Appendix A

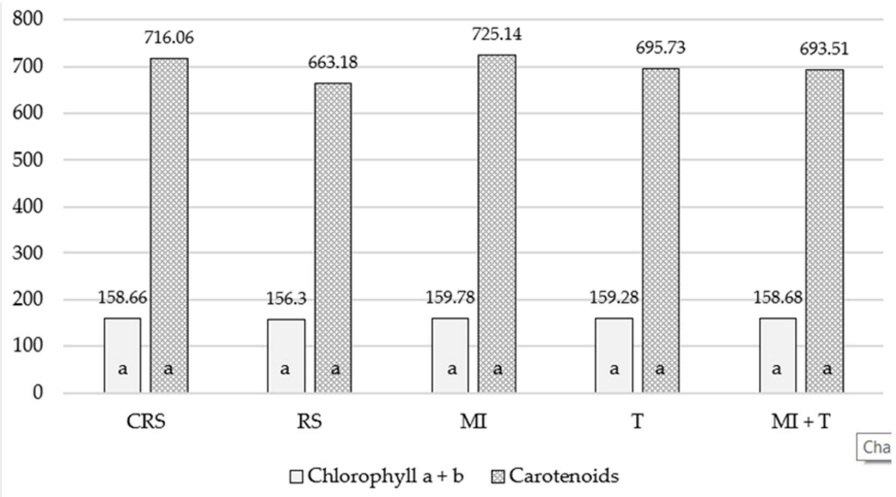

Figure A1. The leaf chlorophyll content (mg kg^{-1}) in the apple trees (CRS = crop rotation soil; RS = replanted soil; MI = mycorrhizal inoculum; T = *Trichoderma;* MI + T = mycorrhizal inoculum + *Trihoderma*). Each parameter was individually analyzed statistically. Means marked with the same letters do not differ significantly at $\alpha = 0.05$.

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