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Effect of *Trichoderma* spp. Fungi and *Phytium oligandrum* on Maiden Apple Tree Growth and Photosynthesis in the Nursery

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Abstract: The conducted experiment evaluated the effects of three fungi—*Trichoderma atroviride* (Ta), *Trichoderma harzianum* (Th) and *Phytium oligandrum* (Po)—on the growth of maidens of two apple cultivars, 'Szampion' and 'Topaz', budded on two rootstocks, M.9 and M.26, in the nursery. The evaluation was based on the number of maidens obtained and their height, trunk diameter and number and length of lateral shoots, as well as the fresh weight of the leaves and the whole maiden. For the weaker-growing maidens of the 'Topaz' cultivar, the activity of the photosynthetic apparatus was additionally measured depending on the rootstock and fungal treatments. The number of maidens obtained improved significantly when Th (8.3–9.0%) and Po (8.4–12.8%) were applied, depending on the rootstock and cultivar used. With the best treatment with the Po fungus, on average, for the two cultivars, maiden apple trees budded on the M.9 dwarf rootstock were characterized by a significantly better height of 6% and trunk diameter of 13% compared to the control. In contrast, trees grown on the M.26 rootstock did not have significantly increased growth after the same treatments, with the exception of the Po fungus, which improved the stem diameter by an average of 10%. The use of fungi stimulated an increase in the number and length of the lateral shoots of maidens of the more easily branching 'Szampion' apple tree cultivar. Based on the fluorescence parameters obtained, it can be assumed that the 'Topaz' cultivar on the M.26 rootstock is less susceptible to stress conditions, especially those related to high temperatures and drought. All fungi used had a positive effect on the activity of the photosynthetic apparatus. Significantly worse values of the fluorescence parameters were obtained for the control combination compared to the fungal treatments.

Keywords: apple; nursery production; rootstocks; cultivars; vigor; branching; chlorophyll content index; fluorescence parameters

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1. Introduction

Poland is the third- or fourth-largest apple producer in the world, depending on the year, with annual production of about 4 million tonnes of fruit [1]. The most common rootstocks used in the nursery production of maiden apple trees are M.9 and M.26. Apple cultivars that have long been important in orchard production include 'Szampion' and 'Topaz'. A factor influencing the efficiency of a modern apple orchard is the quality of the maidens. The appropriate combination of cultivar and rootstock makes it possible to increase the density of trees per unit area. Today, modern technology in the EU is based on the production of maiden apple trees that have a formed crown already in the nursery. When planted in the orchard, apples with a crown formed in the nursery are characterized by early entry into the fruiting period and a shorter investment period [2–4]. The trunk diameter and number of lateral shoots obtained in the nursery are positively related to the later productivity of the orchard [5]. On the other hand, maiden apple trees produced especially on the M.9 dwarf rootstock show weak growth in the nursery. In addition, the environmental conditions in the nursery, including high humidity and nutrient availability, affect the occurrence and spread of soil-borne pathogens. Plant protection against diseases in nurseries very often relies on the standard application of fungicides, which represent a potential source of residue in the soil. A sensible solution seems to be the use of various types of biostimulants as bioprotection agents.

To date, the use of the *Pythium oligandrum* (Po) fungus as a biostimulant relates to annuals such as peas [6], tomato [7], sugar beet [8] and potato [9]. There is little research on the effect of this fungus on the growth of perennials, an example of which is grapevine [10]. The commercial preparation Polyversum WP (Bioagris, Warsaw, Poland) contains the *Pythium oligandrum* fungus and has biologically active properties that block the spread of pathogenic fungi [11]. It also shows the ability to protect the root systems of plants against fungi causing plant diseases [12]. Veselý [13] was the first to observe the mycoparasitism of non-pathogenic Po dwelling in the soil. Benhamou et al. [14] found that Po has the ability to colonize the root systems of many crop species, thus attacking pathogenic fungi in the soil; this improves plant growth and protection against fungal diseases. It does so, in part, by activating the plant's immune system. Compared to other bioprotection agents, this fungus has better effectiveness as it penetrates deeper into the plant tissue, but it must survive in the host tissues for this to happen [15]. Control resulting from the presence of the Po fungus in the plant is a complex process—this is due to the effect on the pathogenic fungus through mycoparasitism and antibiosis, while stimulating plant defense processes [14,16].

Diverse soil-dwelling microorganisms populate the root systems of crops, forming a beneficial microflora, which has a positive effect on the plant and raises its yields; they also include *Trichoderma* spp. [17,18]. It serves as an environmentally friendly biological control agent [19,20], enabling a reduction in the use of synthetic fungicides [21] to protect plants from soil-dwelling pathogenic fungi [22]. Fungal species of the Trichoderma genus have the ability to produce enzymes and antibiotics with anti-fungal properties [23]. They act as a source of competition to soil-borne pathogens in terms of space and nutrients by inhabiting the rhizosphere [24]. These fungi improve plants' resistance to drought stress by increasing the volume of the root system and improving the nutrient and water acquisition [25,26]. Trichoderma is administered both to the leaves and to the soil, but the latter form of application is better in preventing soil-borne pathogens [27]. According to research [27], applying this fungus to the roots in nursery production has a significant effect on root colonization, which increases plants' growth and their resistance to biotic and abiotic stresses. The use of *Trichoderma* spp. fungi in the nursery to control pathogenic fungal species has already been studied in the past [28–30]. Their use resulted in the shortening of the plant production cycle in the nursery, which had economic consequences, and thus increased its production capacity [31].

The aim of the experiment was to assess the impact of inoculating rootstocks planted in a nursery with three fungi and the possible improvement in the growth of maiden apple trees and their life processes.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The study was carried out in 2022–2023 at Poznań University of Life Sciences (52°24′24″ N and 16°55′47″ E). The experiment was set up twice in a randomized complete block design, with four replications, each with 20 maiden apple trees per plot. The experiment consisted of two parts, in each of which one of the two rootstocks under consideration (M.9 and M.26) was planted into the ground nursery in mid-March 2021 and 2022, at 90 cm $\times$ 30 cm spacing. Before planting, virus-free rootstocks of uniform diameter (7–8 mm) were treated with the mycelium of two fungi, Ta and Th, obtained by culturing in a laboratory. The adhesion of the mycelium to the roots of the rootstocks was enhanced by the use of a hydrogel, with which the liquid mycelium was thickened (10^8 spores·1 mL of water). The third treatment was to soak the roots of the rootstocks for 24 h in water with the addition of 1 g $\times$ 1 L^{-1} of Polyversum WP containing the *P. oligandrum* fungus

(10^6 oospores in 1 g of agent), as recommended by the manufacturer (Bioagris, Poland). Control rootstocks were immersed in the hydrogel alone. In the first year of cultivation, the budding of rootstocks was carried out in early August using the T-shaped method, with two apple cultivars, 'Szampion' and 'Topaz'. In the second year of cultivation, in the beginning of March, the rootstocks were pruned directly above the established leaf bud of the apple tree cultivar and the maidens were supported with bamboo stakes. The protection of maidens against diseases and pests was carried out according to the recommendations for an apple orchard. Biometric measurements of the maidens were taken at the end of October 2022 and 2023 on all maidens obtained. Measurements were taken of the tree height (cm) using a vertical measuring staff; the trunk diameter at 10 cm above the budding site (mm), using an electronic caliper; and the length of the lateral shoots (cm), measuring this parameter with a meter staff, while their number was also determined. All leaves from maidens were picked and their fresh weight (kg) was determined; a portable electronic scale was used for this purpose. The maidens were shaken out of the ground after digging and their fresh weight (kg) was determined using the same electronic scale. The number of maidens obtained relative to the budded rootstocks was also calculated.

2.2. Soil and Climatic Conditions for the Growth of Maiden Apple Trees

The rainfall levels during the maiden growth years were 2022–550 mm and 2023–596 mm. The average daily temperature for each month during maiden growth was as follows: April—8.4, May—13.8, June—19.0, July—20.1, August—19.4, September—18.3 and October—10.9 °C. The temperature and rainfall patterns favored intensive plant growth. The experiment was located on podzolic-type soil, class IVb. A soil analysis carried out in July 2022 showed the following respective content: N—28, P—106, K—179, Mg—243, Ca—613 ($mg \cdot L\ g^{-1}$ of soil). These were appropriate values for the normal development of maidens. Weeds in the nursery were controlled mechanically. Nitrogen fertilization was applied in the form of ammonium nitrate at 300 $kg \cdot ha^{-1}$ in three doses.

2.3. Analysis of the Chlorophyll Content Index and Fluorescence Parameters

In July 2023, the chlorophyll content index and fluorescence parameters were measured only on maidens of the weaker-growing 'Topaz' cultivar, where no large differences in growth were observed. The chlorophyll content index (CCI) was measured with the use of the OSI CCM-200 Plus leaf chlorophyll meter (ADC BioScientific Ltd., Hoddesdon, UK). Chlorophyll fluorescence was measured with the use of the OS1-FL modulated fluorometer (Opti-Sciences, Hudson, NH, USA), half an hour after the termination of the period of exposure to light. Three maidens from each treatment with healthy, fully developed leaves from the middle portion of the main shoot were randomly selected for measurement.

2.4. Statistical Analysis

The results of the study were analyzed using STATISTICA 13.1 (Statsoft Polska, Kraków, Poland). The experimental results obtained for the biometric measurements were subjected to a two-way analysis of variance (fungal treatment and apple cultivar), for each rootstock under consideration separately, using Duncan's test, with a probability level of $\alpha = 0.05$. The percentage results of the maidens obtained were subjected to Bliss transformation. The results presented in the Tables 1–7 are averages from two years of research, because the results between the two years did not differ significantly. Meanwhile, the values of the fluorescence parameters for the maidens of the 'Topaz' cultivar were developed using a one-way analysis of variance separately for the two rootstocks and the fungal treatments. These were the results from one year of measurements and are presented in Tables 8 and 9.

Table 1. The influence of the fungus treatments and varieties on the heights of maiden apple trees grown on the M.9 and M.26 rootstocks.

M.9 (cm)		
Treatment	'Topaz'	'Szampion'
Ta	98.1 ± 3.7 a *	113.4 ± 2.3 c
Th	102.9 ± 3.7 ab	107.5 ± 4.0 bc
Po	102.1 ± 2.9 ab	110.5 ± 3.9 c
Control	98.4 ± 1.6 a	101.4 ± 4.0 b
M.26 (cm)		
Treatment	'Topaz'	'Szampion'
Ta	87.4 ± 4.2 ab *	109.9 ± 2.3 d
Th	81.4 ± 4.1 a	109.4 ± 4.0 d
Po	93.6 ± 4.1 bc	111.6 ± 3.9 d
Control	98.5 ± 4.0 c	100.5 ± 4.0 c

* Different letters indicate significant differences at the 5% level, according to Duncan's test, for each rootstock separately. The results did not differ significantly between the years, so they are given as averages from two years in this table and in the remaining ones.

Table 2. The influence of the fungus treatments and varieties on the stem diameters of maiden apple trees grown on the M.9 and M.26 rootstocks.

M.9 (mm)		
Treatment	'Topaz'	'Szampion'
Ta	12.2 ± 0.91 ab *	15.4 ± 0.41 d
Th	12.5 ± 0.54 a–c	15.7 ± 0.41 d
Po	13.8 ± 1.08 c	16.1 ± 0.41 d
Control	11.3 ± 0.41 a	13.2 ± 0.40 bc
M.26 (mm)		
Treatment	'Topaz'	'Szampion'
Ta	9.7 ± 0.49 a	14.8 ± 0.41 d
Th	11.3 ± 0.37 b	14.8 ± 0.41 d
Po	13.3 ± 0.41 c	14.9 ± 0.41 d
Control	11.4 ± 0.41 b	14.0 ± 0.40 cd

* Different letters indicate significant differences at the 5% level, according to Duncan's test, for each rootstock separately.

Table 3. The influence of the fungus treatments and varieties on the number of side shoots of maiden apple trees grown on the M.9 and M.26 rootstocks.

M.9		
Treatment	'Topaz'	'Szampion'
Ta	0.47 ± 0.09 a *	2.40 ± 0.16 c
Th	0.53 ± 0.12 a	3.50 ± 0.16 d
Po	0.50 ± 0.08 a	3.47 ± 0.21 d
Control	0.40 ± 0.08 a	2.07 ± 0.09 b
M.26		
Treatment	'Topaz'	'Szampion'
Ta	0.60 ± 0.08 ab *	2.50 ± 0.25 de
Th	0.87 ± 0.12 b	2.20 ± 0.16 d
Po	0.50 ± 0.08 a	2.60 ± 0.16 e
Control	0.50 ± 0.08 a	1.80 ± 0.16 c

* Different letters indicate significant differences at the 5% level, according to Duncan's test, for each rootstock separately.

Table 4. The influence of the fungus treatments and varieties on the sum of the lengths of maiden apple trees' side shoots, grown on the M.9 and M.26 rootstocks.

M.9 (cm)		
Treatment	'Topaz'	'Szampion'
Ta	7.0 ± 2.16 a *	54.0 ± 2.16 c
Th	20.7 ± 4.19 ab	113.0 ± 19.80 d
Po	17.3 ± 5.56 ab	141.3 ± 9.10 d
Control	10.7 ± 0.94 a	48.3 ± 4.19 bc
M.26 (cm)		
Treatment	'Topaz'	'Szampion'
Ta	19.3 ± 4.03 a *	52.0 ± 8.83 b
Th	17.7 ± 5.56 a	55.0 ± 14.14 b
Po	24.0 ± 6.48 a	82.3 ± 13.22 c
Control	18.3 ± 6.24 a	50.0 ± 20.07 b

* Different letters indicate significant differences at the 5% level, according to Duncan's test, for each rootstock separately.

Table 5. The influence of the fungus treatments and varieties on the fresh weight of the leaves (kg) of maiden apple trees grown on the M.9 and M.26 rootstocks.

M.9 (kg)		
Treatment	'Topaz'	'Szampion'
Ta	0.16 ± 0.01 a–c *	0.19 ± 0.02 c
Th	0.16 ± 0.00 a–c	0.17 ± 0.01 bc
Po	0.15 ± 0.01 ab	0.16 ± 0.01 a–c
Control	0.15 ± 0.02 ab	0.14 ± 0.00 a
M.26 (kg)		
Treatment	'Topaz'	'Szampion'
Ta	0.12 ± 0.01 a *	0.15 ± 0.01 bc
Th	0.13 ± 0.01 ab	0.13 ± 0.01 ab
Po	0.15 ± 0.00 bc	0.16 ± 0.01 c
Control	0.14 ± 0.00 ab	0.15 ± 0.01 bc

* Different letters indicate significant differences at the 5% level, according to Duncan's test, for each rootstock separately.

Table 6. The influence of the fungus treatments and varieties on the fresh weight (kg) of maiden apple trees grown on the M.9 and M.26 rootstocks.

M.9 (kg)		
Treatment	'Topaz'	'Szampion'
Ta	0.40 ± 0.03 ab *	0.43 ± 0.02 b
Th	0.35 ± 0.02 a	0.39 ± 0.01 ab
Po	0.38 ± 0.03 a	0.40 ± 0.00 ab
Control	0.38 ± 0.03 a	0.38 ± 0.01 a
M.26 (kg)		
Treatment	'Topaz'	'Szampion'
Ta	0.40 ± 0.02 a *	0.40 ± 0.01 a
Th	0.41 ± 0.02 a	0.38 ± 0.02 a
Po	0.42 ± 0.03 a	0.38 ± 0.01 a
Control	0.38 ± 0.01 a	0.39 ± 0.02 a

* Different letters indicate significant differences at the 5% level, according to Duncan's test, for each rootstock separately.

Table 7. The influence of the fungus treatments and varieties on the percentage of obtained maiden apple trees grown on the M.9 and M.26 rootstocks.

M.9 (%)		
Treatment	'Topaz'	'Szampion'
Ta	54.0 ± 3.27 a *	60.3 ± 2.45 b
Th	61.7 ± 1.65 bc	69.7 ± 2.45 e
Po	65.0 ± 2.45 cd	66.7 ± 2.45 de
Control	52.7 ± 1.70 a	62.0 ± 2.44 bc
M.26 (%)		
Treatment	'Topaz'	'Szampion'
Ta	80.4 ± 2.38 a *	85.3 ± 1.23 b
Th	85.9 ± 2.15 b	90.4 ± 1.53 c
Po	90.4 ± 1.42 c	93.1 ± 1.22 c
Control	77.4 ± 1.59 a	80.5 ± 1.92 a

* Different letters indicate significant differences at the 5% level, according to Duncan's test, for each rootstock separately.

Table 8. The influence of the rootstock and fungus inoculation on the chlorophyll fluorescence parameters and CCI.

Treatment	F_0	F_M	F_V/F_M	F_V/F_0	NPQ	PI_{ABS}	CCI
rootstock							
M.26	5039 ± 231.3 a *	16753 ± 688.9 a	0.813 ± 0.00 a	4.36 ± 0.09 a	0.68 ± 0.06 a	3.41 ± 0.07 a	51.6 ± 1.90 a
M.9	3933 ± 472.2 b	13936 ± 567.0 b	0.805 ± 0.00 b	4.21 ± 0.06 a	0.67 ± 0.04 a	3.22 ± 0.11 a	53.9 ± 0.57 a
fungus							
Control	4183 ±128.4 c	8821 ± 477.9 c	0.716 ± 0.00 c	2.57 ± 0.04 c	0.80 ± 0.02 a	2.56 ± 0.23 b	43.5 ± 1.39 b
Po	5353 ± 51.7 a	14770 ± 365.9 a	0.810 ± 0.01 a	4.26 ± 0.07 a	0.71 ± 0.01 b	3.17 ± 0.16 a	45.8 ± 1.17 ab
Ta	5120 ± 79.6 b	12770 ± 539.7 b	0.790 ± 0.01 b	4.01 ± 0.04 b	0.71 ± 0.02 b	3.11 ± 0.12 a	45.5 ± 1.34 ab
Th	5320 ± 79.6 ab	14054 ± 107.8 a	0.800 ± 0.01 ab	4.12 ± 0.04 b	0.81 ± 0.01 a	3.56 ± 0.23 a	46.9 ± 1.24 ab

* Different letters for the same parameter separately for the rootstock and fungus indicate significant differences at the 5% level, according to Duncan's test. Data are from 2023.

Table 9. The influence of the rootstock and fungus inoculation on the chlorophyll fluorescence parameters.

Treatment	ABS/RC	TR_0/RC	ET_0/RC	DI_0/RC	qP	Rfd
rootstock						
M.26	2.04 ± 0.15 a *	1.66 ± 0.10 a	1.09 ± 0.03 a	0.38 ± 0.05 a	0.64 ± 0.05 a	1.47 ± 0.06 a
M.9	1.94 ± 0.10 a	1.39 ± 0.07 b	0.72 ± 0.10 b	0.28 ± 0.01 a	0.57 ± 0.10 a	1.27 ± 0.03 a
fungus						
Control	2.04 ± 0.06 b	1.6 ± 0.03 c	0.78 ± 0.02 c	0.45 ± 0.02 a	0.77 ± 0.07 b	1.28 ± 0.12 c
Po	1.97 ± 0.12 b	1.66± 0.02 b	0.92 ± 0.04 a	0.44 ± 0.01 a	0.7 ± 0.07 b	1.72 ± 0.03 a
Ta	2.07± 0.03 b	1.62 ± 0.02 bc	0.86 ± 0.00 b	0.44 ± 0.01 a	0.72 ± 0.05 b	1.68 ± 0.09 a
Th	2.31 ± 0.03 a	1.74 ± 0.03 a	0.91 ± 0.02 ab	0.45 ± 0.02 a	0.91 ± 0.05 a	1.48 ± 0.06 b

* Different letters for the same parameter separately for the rootstock and fungus indicate significant differences at the 5% level, according to Duncan's test. Data are from 2023.

3. Results

3.1. Analysis of Plant Growth

The application of all three fungal treatments during the planting of the M.9 and M.26 rootstocks improved the height of the maiden apple trees of the 'Szampion' cultivar compared to the control (Table 1). For the 'Topaz' cultivar and M.9 rootstock, no significant differences in the results were found (Table 1). For the second rootstock, M.26, and the

'Topaz' cultivar, the best height was obtained for maidens from the control and the smallest form of Th treatment (Table 1).

The trunk diameter of maidens for the M.9 and M.26 rootstocks and the 'Topaz' cultivar was better than for the control only for the Po fungus (Table 2). For the 'Szampion' cultivar and M.9 rootstock, all three fungal treatments significantly improved the trunk diameter of the maidens compared to the control. For this cultivar and the M.26 rootstock, no differences in trunk diameter were found as a result of the treatments (Table 2).

The number of lateral shoots obtained for the M.9 rootstock for the 'Topaz' cultivar did not change significantly as a result of the treatments. For the 'Szampion' cultivar, all three fungi improved the number of shoots compared to the control (Table 3). For the second rootstock, M.26, and the 'Topaz' cultivar, a better shoot number compared to the other treatments was obtained only for the Th treatment. For the 'Szampion' cultivar, the best number of lateral shoots was found for the Polyversum preparation. This was followed by decreasing numbers for the other two fungi and the lowest for the control (Table 3).

The sum of the lateral shoot lengths of the maiden apple trees on the M.9 rootstock did not differ for the 'Topaz' cultivar. For the 'Szampion' cultivar, the maidens were characterized by a greater shoot length than in the control after treatment with the Th and Po fungi (Table 4). For the second rootstock, M.26, and the 'Topaz' cultivar, the parameter under consideration did not differ in value between the treatments applied. For the 'Szampion' cultivar, only the Polyversum preparation significantly improved this growth parameter compared to the control (Table 4).

The fresh leaf weights of the maidens for the M.9 rootstock and the 'Topaz' cultivar did not differ. The 'Szampion' cultivar showed a better weight for the Ta and Th fungi compared to the control (Table 5). For the two apple cultivars on the M.26 rootstock under consideration, no differences in fresh leaf weight were found after the fungal treatment compared to the control. The average leaf weight after the Polyversum treatment differed significantly from that of the other two fungi (Table 5).

The fresh weight of the 'Topaz' cultivar maiden on the M.9 rootstock was not significantly different. For the 'Szampion' cultivar, only the Ta fungi gave a better result than the control (Table 6). For the second rootstock, M.26, no significant differences in plant weight were found between treatments.

The percentage of maiden apple trees for the two cultivars obtained on the M.9 rootstock for the Th and Po fungi was higher than in the control (Table 7). On the other hand, for the M.26 rootstock, the same best values were found after the application of the Po fungus. This was followed by decreasing values for Th and Ta and the lowest for the control.

3.2. Analysis of the Chlorophyll Content Index and Fluorescence Parameters

The effect of the rootstocks on the values of the fluorescence parameters in the 'Topaz' cultivar maidens varied. For some parameters, no significant differences were found. A significant increase in trapped energy flux per active reaction center (TR/RC) and electron transport flux per active reaction center (ET/RC) was observed for the M.26 rootstock (Tables 8 and 9). Moreover, maiden apple trees on this rootstock were characterized by higher maximum photochemical efficiency of PSII (Fv/Fm) and higher minimal and maximal fluorescence (Fo and Fm).

The application of fungi significantly improved the photosynthetic parameters of the maiden apple trees. The lowest values of most parameters were obtained for the control combination. However, it is worth noting that the control plants had the lowest value of minimal fluorescence (Fo), but also a low value of maximal fluorescence (Fm) and Rfd (fluorescence decrease ratio)—the vitality index of PSII. The highest maximum photochemical efficiency of PSII (Fv/Fm) was found for plants treated with the Po and Th fungi. These were the fungi that had the strongest influence on the fluorescence parameters of the maiden apple trees. For the Th fungus, the increase in the following parameters was more pronounced: absorption per active reaction center (ABS/RC, 12–15% higher value compared to the other combinations), (trapped energy flux per active reaction center (Tr/RC, approximately 10% higher value compared to the control) and coefficient of photochemical quenching (qP 16–20% higher value compared to the other combinations) (Table 9). There were no significant differences in the chlorophyll content index (CCI) between the treatments, although the lowest values were obtained for the control.

4. Discussion

4.1. Maiden Apple Tree Growth after Applying the Fungi

In the experiment considered, the apple tree cultivars produced on the M.9 rootstock achieved a low percentage of maidens, ranging from 57.2 to 65.8% depending on the treatment. At the same time, for two of the three fungi considered, the percentage was significantly better than for the control. In contrast, on the M.26 rootstock, the percentage of maidens was high (79.0–91.8%), significantly better than the control for all fungi applied. The efficiency of maidens in an earlier experiment by Świerczyński and Stachowiak [32] for two cultivars, 'Delikates' and 'Elstar', was, respectively, 88.0% and 76.0% for the M.9 rootstock and 70.4% and 75.3% for the M.26 rootstock. Thus, an inverse relationship was obtained between the number of maidens and the rootstock used. However, different apple cultivars were studied, which may have influenced the results. In the experiment conducted, the positive effect on the yields of maiden apple trees was undoubtedly due to the fungi applied, which, with a small exception (the Ta fungus and M.9 rootstock), improved it significantly. Similarly, the higher survival rate of grapevine cuttings treated with *Trichoderm* spp. during rooting in the nursery was confirmed by other researchers [29,33]. As they claim, this was associated with better plant conditions resulting from optimal nutrition and the lower presence of biotic and abiotic stresses. A similar relationship may have occurred in the experiment under consideration.

The treatment of the rootstocks with the fungi significantly improved the height of the maidens of the 'Szampion' cultivar budded on the M.9 rootstock by an average of 13% and on the M.26 rootstock by 9% compared to the control. On the other hand, a positive effect of these fungi on the maiden height was not found for the 'Topaz' cultivar. Similar results were obtained by Andrzejak et al. [34], who studied the effect of the *Trichoderma* spp. fungi and fertilization on the flowering of Begonia x tuberhybrida. Their application had no significant effect on plant growth, and sometimes even worse results than in the control were obtained. Zydlik et al. [35] also found a similar height in maiden apple trees of the 'Jonagold' cultivar on the M.9 rootstock after the application of the Th fungus in replanted soil compared to the control. A significantly better height in maiden apple trees of the 'Topaz' cultivar on the M.26 rootstock was obtained by Grzyb et al. [36], which was 127 cm. However, the different experimental results may have been due to the varying soil and climate conditions, as well as the fact that the studies were conducted on different plant species or cultivars. In addition, they may have also arisen from the use of different inoculum doses and different forms of application. However, it should be stressed that the use of fungi does not always benefit plant growth at an early stage of development. This is because the growing mycelium can deprive the host plant of some of its assimilates until it reaches the target size. At a later stage of growth, the host may benefit more from the saprophytic fungus, which further protects it from soil-borne pathogenic fungi.

The stem diameter of the maidens, for the M.9 dwarf rootstock, improved after the use of the three fungi under consideration. However, for the M.26 semi-dwarf rootstock, only the use of the Po fungus increased the stem diameter compared to the control. This may have been due to the optimum growth conditions in the nursery, which, for the stronger-growing rootstock, were sufficient for its proper growth, but, in the case of the dwarf rootstock, were not. Here, the plant growth was aided by the presence of the fungus. Similar to the height, for the trunk diameter of the stronger-growing maidens of the 'Szampion' cultivar, a growth improvement also occurred in the company of the fungus, while, for the weaker-growing 'Topaz', it did not. It is possible that the assimilates obtained by photosynthesis formed in the aboveground part of the strong-growing cultivar flowed into the root system of the rootstock and, in greater quantities, also served the development of the saprophytic fungus, without limiting the growth of the maidens. For the weaker-growing 'Topaz' cultivar, the smaller quantity of assimilates was largely consumed for the development of the fungus and limited the growth of the maiden. In the experiment of Zydlik et al. [35], no improvement in the diameter of maiden apple trees was found after the application of Th. Therefore, it can be concluded that the trunk diameter depends in particular on the growth strength of the cultivar under consideration and varies according to the soil and climate conditions. In an experiment conducted by Kapłan and Baryła [37], maidens of the 'Szampion' cultivar on the M.9 and M.26 rootstocks not treated with fungi had a stem diameter of 12.3 cm and 13.0 cm, respectively, and these results were similar to those found in the considered experiment for the control. A very similar stem diameter of maidens was also recorded by Grzyb et al. [36] for the 'Topaz' cultivar, which was 12.8 mm. It is therefore possible to identify the repeatability of the growth dynamics in the proportion of maiden apple trees of these two cultivars in the nursery.

In the experiment conducted, the number of lateral shoots in the control sample for the 'Szampion' cultivar obtained on the M.9 and M.26 rootstocks was 2.1 and 1.8, respectively, and these numbers were significantly lower than those obtained by Kapłan and Baryłe [37]—3.6 and 4.1, respectively. It can be assumed that in these authors' experiments, the higher number of lateral shoots reduced the height of the maidens, the value of which was lower than in the experiment under consideration. In the experiment conducted, the application of the fungus brought an improvement in the number of lateral shoots of the maidens. In contrast, a lack of difference in the number of lateral shoots of maiden apple trees when inoculated with Th was shown by other authors [35]. This could have been influenced in particular by the comparison of different cultivars in the two experiments, and also by the fact that the above-mentioned authors studied the growth of maidens in soil replanted with the addition of Th, compared to the non-replanted soil control.

In the experiment described above, particularly maidens of the 'Szampion' cultivar, after treatment with the Po fungus, reached a shoot length of 141.3 cm for the M.9 rootstock and 82.3 cm for the M.26 rootstock. In other research [37], an inverse dependence of the parameter under consideration on the rootstock was obtained for the same cultivar, which was 86.4 cm for the M.9 rootstock and 102.7 cm for the M.26 rootstock. On the other hand, Zydlik et al. [35] obtained no variation in this growth parameter after the application of Th. The potential reasons for these differences have already been explained above. A similar length of the lateral shoots of the 'Topaz' cultivar on the M.26 rootstock was obtained by Grzyb et al. [36], which was 98 cm.

Most often, no improvement in the fresh weight of maidens of the two apple tree cultivars under study was obtained after the rootstock treatments used. However, as time passes after its application, Trichoderma becomes more active. This effect is due to the intensive colonization of maidens' roots by the fungus itself. As demonstrated by other researchers [38–40], the *Trichoderma* spp. fungi have an effect especially on the development of the plant root system, mainly hairy roots. In turn, better growth of the hairy roots leads to stronger plant growth. This may be due to the higher uptake of nutrients from the soil, as well as greater stress tolerance and a lower presence of soil-borne diseases, which is in line with previous observations [28–30,38,41,42]. On the other hand, it was found that the Po contained in the Polyversum preparation was able to stimulate the growth of the root system of red clover, improving its yield. In the case of the experiment carried out, the Polyversum preparation may have had a similar effect on the root systems of the maidens, enabling them to take up significantly more of the nutrients needed for their growth. However, this was not proven on the basis of the fresh weight of the leaves and maidens.

4.2. Chlorophyll Content Index and Fluorescence Parameters after Applying the Fungi

In the study, the F_V/F_M values for all fungal treatments were in the range of 0.79–0.81. Only maidens from the control combination had a significantly lower value for this parameter (0.71), which demonstrates the efficiency of the PSII primary photochemistry. A decrease in its value below 0.75 indicates damage to PSII [43] and a decrease in reaction centers (RCs) as a result of stress [44]. In contrast, a value of the F_V/F_M parameter of around 0.80 or above indicates high potential PSII activity [45]. Thus, the results obtained allow the conclusion that the fungi applied significantly protected the plants under possible stress conditions. This is also evidenced by the significantly large decrease for the control in the values of the fluorescence decrease ratio (Rfd) and performance index on absorption basis (PI_{ABS}), and the consequent decrease in photosynthetic apparatus activity. Previous studies have shown that differences in PI_{ABS} values can be attributed to genetic differences, physiological traits and environmental conditions [46,47]. Similar behavior of this parameter in potato leaves subjected to drought was described by Boguszewska-Mańkowska et al. [48]. On the other hand, all combinations treated with fungi were characterized by a higher F_O value compared to the control, as were maidens of the 'Topaz' cultivar on the M.26 rootstock compared to the M.9 rootstock. The F_O values are the minimum fluorescence levels, assuming that all antenna pigment complexes associated with the photosystem are open (dark-adapted) [49]. An increase in F_O indicates any difficulties and degradation in photosystem II (D1 protein and another part of the PS) or the disruption of energy transfer to the reaction center [50]. This suggests that the maiden apple trees were partially subjected to photosynthetic stress under the applied treatments and that F_O is a more sensitive parameter and not correlated with F_V/F_M. One of the protective mechanisms of the photosynthetic apparatus, especially PSII, against stress-induced damage is the slowing down of electron transport from the reaction centers to plastoquinones [51,52]. In the present study, a significant reduction in the electron transport rate (ET_O/RC) was found for the control combination compared to the fungi-treated combinations and for the 'Topaz' cultivar on the M.9 rootstock. At the same time, no significant increase in energy dissipation at the cost of heat (DI_O/RC) was observed for any of the treatments. A decrease in F_M under the influence of high temperatures in the study was found for the combination not treated with fungi and for the M.9 rootstock. This occurred as a consequence of the thermal inactivation of the oxygen-releasing complex [53]. In turn, low F_V/F_O values under high-temperature conditions indicate the reduced efficiency of the water photolysis reaction in the PSII system and are related to F_V/F_M, meaning that any factor affecting F_V/F_M v/Fm also affects F_V/F_O [54]. The results lead to the conclusion that fungi-treated apple trees and maidens of the 'Topaz' cultivar on the M.26 rootstock show greater tolerance to high-temperature stress.

5. Conclusions

Based on the results obtained, it can be concluded that the application of selected fungal species during rootstock planting most often improves the growth of maiden apple trees. The Po and Th fungi proved to be better, as they intensified the maidens' growth more than Ta. This was particularly evident in the case of most of the maiden growth parameters studied for the stronger-growing 'Szampion' cultivar. For the weaker-growing 'Topaz' cultivar, the application of the fungi did not always have a positive effect at the initial growth stage of the maidens in the nursery. This is important, as it is precisely for the weaker-growing maidens that such an increase in growth is particularly desirable in the nursery. The effect of the fungi was also dependent on the rootstock, being noticeably better for the M.9 rootstock. Unsurprisingly, the branching results of the maidens were influenced in particular by the cultivar budded. Indeed, the 'Szampion' cultivar with its genetic background formed more lateral shoots, but this was also partly stimulated by the presence of the fungi. As can be seen, the research results are variable and dependent on many factors, some of which are beyond one's control, such as the pattern of climate conditions. However, it is worth continuing experiments with the use of beneficial fungi in the production of plants other than maiden apple trees in the nursery, as the results achieved so far are promising.

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