doi: 10.26114/pja.iung.361.2018.34.02

Polish Journal of Agronomy
2018, 34, 11–22

The impact of phytosanitary treatments in the soil with signs of fatigue on the growth of apple seedlings and populations of bacteria and fungi

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Abstract. The object of research was the soil from an orchard after 27 years of apple cultivation, showing signs of fatigue resulting in weaker growth of apple seedlings planted into it, and the soil sampled on the same farm, but not used for growing fruit trees. The physicochemical properties of both soils did not differ significantly. In sick soil, no pathogens of fungal or fungi-like origin were found, and the number of nematodes was below the threshold of economic threat. There is a suspicion that abiotic factors could be responsible for soil fatigue. It has been demonstrated that the cultivation of spring and winter wheat, triticale and white mustard before planting apple 'Antonovka' seedlings, had a positive impact on their growth. The best effect was obtained after the cultivation of white mustard or after the introduction of mustard seed meal into the soil, which was also confirmed by measurements of the intensity of apple seedlings photosynthesis. The cultivation of these phytosanitary plants or the addition of mustard seed meal positively influenced the biological potential of the soil expressed by a significant increase in the population of bacteria from the genera *Pseudomonas* and *Bacillus*. There was also a very large increase in the number of *Trichoderma* spp. fungi after the addition of mustard seed meal into the soil and winter wheat cultivation.

Keywords: replant disease, cereals, white mustard, *Pseudomonas* spp., *Bacillus* spp., *Trichoderma* spp.

INTRODUCTION

In the era of the intensification of fruit production, of which one element is the need to renew orchards, due to, among others, the aging of trees and changes in the preferences of fruit consumers, replant disease becomes an important problem. The disease is the result of the so-called soil fatigue (or soil sickness) and occurs in trees planted in sites after a freshly removed orchard, especially of the same species. It is often the reason for their poor growth, reduced productivity, and even dieback (Granatstein, Mazzola, 2001). The problem of replant disease in orchards in Poland was already signalled more than 30 years ago by Rebandel (1987), and more widely studied both from the point of view of causes and the possibility of limiting its harmfulness by the Department of Pomology at the then Agricultural Academy in Poznań (Pacholak et al., 1989; Pacholak et al., 2004; Rutkowski, Pacholak, 1999; Zydlik, 2004). Szczygieł and Zepp (1993), while analysing soil samples from 121 orchards, concluded that this disease occurred in over 50% of orchards in Poland, especially in ones planted on light soils. Previous studies have shown that replant disease is caused by a set of biotic and abiotic factors occurring in the soil, responsible for processes leading to the creation of conditions unfavourable for the growth and yield of plants. These include the accumulation of pathogens and other harmful microorganisms, nematodes and toxic compounds. From the point of view of soil sickness etiology, it is believed that the greatest importance can be attributed to the soil fungi of the genera: *Cylindrocarpon*, *Rhizoctonia*, *Penicillium*, *Alternaria* and fungus-like organisms such as *Phytophthora* sp., *Pythium* sp., or tumorigenic bacteria and nematodes (Dullahide et al., 1994; Mazzola, 1998; Manici et al., 2003). Also poor soil structure, nutrient imbalance or inappropriate pH are the components of the complex of this disease. Among the abiotic factors, the remains of the roots of trees which are the source of toxic phenolic compounds deserve attention (Politycka, Adamska, 2009; Politycka et al., 2001). The roots of apple trees secrete, for example, phlorizin, a compound showing strong negative allelopathic effects (Traquair, 1984; Hofmann et al., 2009; Nikola et al., 2016).

The complex nature of specific replant disease makes it most difficult to easily determine what harmful factors occur in a given orchard. What is more, these factors can be subject to changes in both quantitative and qualitative

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terms in a relatively short period, even during one season. This leads to certain difficulties in making a decision about the choice of a possible preventive measure or measures to control the disease. The withdrawal of methyl bromide, in accordance with the provisions of the Montreal Protocol of the United Nations (1987), necessitates the search for both environmentally safe chemical compounds and alternative methods (Sobiczewski et al., 2014). Their assortment is extremely poor, and in Poland since 2018 only one agent for soil disinfection in apple orchard based on dazomet (Basamid) has been registered.

One of the earliest recommended ways to reduce the effects of soil fatigue in practice is not to plant the same plant species in it for several years. Often, however, it is difficult to accept because of the need to quickly renew orchards. Katan (2004) emphasizes that the protection of plants against soil-borne diseases should include various methods aimed at their elimination. These contain appropriate crop rotation, fertilization, regulation of water conditions, regulation of weed infestation, and chemical soil disinfection where possible. It was found that the introduction of organic matter into the soil stimulated its biological activity related to the diversity of beneficial microorganisms with antagonistic activity against soil pathogens (Lazarovits, 2001).

Studies have demonstrated that some plant species produce certain compounds which effectively eliminate harmful organisms in the soil. For example, wheat was proven to be particularly useful for this purpose (Mazzola, Gu, 2000). Some of its cultivars, such as 'Eltan', grown in soil showing signs of fatigue, even for only a month, caused such quantitative and qualitative changes in the populations of bacteria and fungi colonizing this soil, that it resulted in almost complete elimination of the negative effects of fatigue. On the one hand, there was a drastic reduction in the number of fungi responsible for the replant disease, and on the other, an increase in the number of bacteria considered useful. This group includes mainly fluorescent *Pseudomonas* spp. among which *P. putida* species deserves to be distinguished. This bacterium produces, among others, chelated compounds (siderophores) which bind iron in soil, constituting an important nutrient for pathogens (Mazzola, Gu, 2000; Gu, Mazzola, 2003). Another way is to introduce into the soil a mixture of rapeseed (*Brassica napus*) and mustard (*B. juncea*) seed meal before planting apple trees. It was proven that the effect of the meal consisted, on the one hand, in supplying a significant amount of nitrogen to the soil, and, on the other hand, in exerting a phytosanitary effect (Mazzola et al., 2007). The meals also stimulated the activity of certain specific microorganisms, mainly bacteria colonizing the roots of the trees, and at the same time limiting pathogenic fungi.

The aim of the presented research was to assess the impact of spring wheat, winter wheat, rye, triticale and white mustard growing in soil showing signs of fatigue or of an addition of mustard seed meal to such soil on the growth and photosynthetic activity of apple seedlings as well as on bacterial and fungal populations inhabiting the sick soil.

MATERIAL AND METHODS

The research was carried out in 2007 and 2008 in the greenhouse of the Research Institute of Horticulture in Skierniewice (RIH). The experimental object was the soil originating from the Przybroda Crops-Orchard Experimental Farm, run by the University of Life Sciences in Poznań, where after 27-year-old apple cultivation problems related to replant disease had been found (Pacholak, Rutkowski, 2001; Zydlik, 2004). The control was the soil from the same farm that had never been used for fruit tree growing (virgin soil). Soil samples from the apple orchard were collected in mid-March in each research year, from a depth of 15–30 cm from 10–12 sites in a row of trees, about 1.5 m from tree trunks (sick soil). At the same time and from the same depth, soil samples determined as virgin soil were collected.

Physicochemical analysis of the soils

The collected soil samples, after thorough mixing, were transported to the laboratory of the RIH. After removing segments of roots and other organic parts, they were subjected to analyses. The mechanical composition of the soil samples was determined with the sieve method, and the content of phosphorus and potassium was determined in air-dried soil samples sifted through a sieve (2 mm) with the Egner-Riehm method, and the magnesium content with the Schachtschabel method. Soil pH was determined with the potentiometric method. It was found that both the soil with signs of fatigue and soil unused for orchard cultivation had similar mechanical composition, with the highest share (approx. 40%) of the fraction with a diameter of 0.25–0.1 mm. These soils were classified as a group of light loamy sands. Analysis showed that the virgin soil was slightly acidic (pH_{H2O} 5.83), and the sick soil – was acidic (pH_{H2O} 4.7). The content of macroelements P, K and Mg in both soils was in the high abundance class.

Cultivation of phytosanitary plants

Seeds of the phytosanitary plants were sown in one, two or three crop cycles into the sick soil placed in flat boxes with dimensions of 600 × 400 × 116 mm. In each cycle the plants were grown for 4 weeks under standard greenhouse conditions (humidity 50–80%, daytime temperature 20–25 °C, night-time – 15–18 °C, intensity of quantum irradiation 500–700 µmol m^{-2} s^{-1} on a sunny day). Phytosanitary plants were not fertilized and they were watered with the amount of water sufficient to maintain the level of 80–100% of field capacity, i.e. in the range of very available water. After each crop cycle, the above ground part of plants was excised at the soil surface and discarded, and the root system was ground and mixed with the soil. The same phytosanitary plant was sown into the substrate prepared in this manner in the next cultivation cycle.

In 2007, the phytosanitary plants were: spring wheat (*Triticum vulgare* Vill cv. Zebra), winter wheat (cv. Finezja), winter triticale (*Triticosecale* Wittm cv. Witon) and white mustard (*Sinapis alba* L.). In addition to the cultivation of these plants, the effect of white mustard seed meal introduced at a ratio of 1% in relation to soil weight was studied. In 2008, after analysing the results from the previous season, only winter wheat (cv. Finezja) and white mustard were applied – grown in one, two or three cycles.

Cultivation of apple seedlings

Eight-week-old apple trees of the cultivar Antonovka were planted into 10-litre containers with soil, in which earlier phytosanitary plants had been grown or white mustard seed meal had been added. In 2007, seedlings were planted on three dates: in April (after one cycle of phytosanitary plant cultivation), in May (after two cycles) and in June (after three cycles). The seedlings cultivated in non-treated sick soil or virgin soil were used as the control. In 2008, apple seedlings were planted in May in the soil where previously selected phytosanitary plants had been grown in one, two or three cultivation cycles. Each combination was represented by 20 seedlings (4 containers × 5 plants) grown for 12–13 weeks under standard greenhouse conditions. After completion of cultivation, the height of the seedlings and the total area of their leaves were measured. The leaf surface was evaluated by image analysis (WinDIAS, Delta-T Devices Ltd, United Kingdom). In addition, after completion of seedling cultivation, measurements of the gas exchange rate (photosynthesis rate) of the leaves were taken from 4 random seedlings of each experimental combination (2 leaves on each plant). A portable LCpro + gas exchange evaluation kit (ADC BioScientific Ltd, Great Britain) was used for the measurements.

Microbiological analyses of the soils

A. Determination of the fungi and fungi-like organisms occurrence

Three 10-gram samples were weighed out from carefully mixed samples of sick and virgin soil, which were placed into 250 ml Erlenmeyer flasks with 90 ml of sterile distilled water. The resulting suspension was mixed for 30 minutes on a rotary shaker at 150 rpm, and serial dilutions of suspensions (10^{-2}, 10^{-3}, 10^{-4}) were prepared, 0.1 ml of which (from each suspension) was plated on Martin's medium in Petri dishes. After 3–7 days of incubation at 24 °C, emerging fungal colonies were counted, and then transplanted onto potato dextrose agar medium (PDA, Difco) for the purpose of their identification on the basis of macro- and microscopic morphological characteristics. The seeding was made in 4 replicates.

B. Determination of the number of fungi and bacteria in the soils after the cultivation of phytosanitary plants or addition of mustard seed meal

At the end of each phytosanitary plant cultivation cycle, three soil samples with a weight of 10 g were taken from each combination, in which the number of bacteria and fungi (cfu – colony forming units) was determined. Mycological analysis was performed as in the studies on phytopathogenic fungi and fungi-like organisms (A), and in the case of bacteria, appropriate dilutions of the soil solution were applied onto King's B and nutrient agar with 5% sucrose (NSA) media (Schaad et al., 2001). The number of individual microorganisms was determined after 3–7 days of incubation at 24 °C, converting it into 1 gram of soil dry matter. In addition, on the basis of the morphological features of the bacteria, fluorescent bacteria have been classified to the genus *Pseudomonas*, and on the basis of the spore test and colony and cell morphology (Schaad et al., 2001) – to the genus *Bacillus.* On the other hand, based on the appearance of the cultures and microscopic observations of the mycelium and spores, the fungi of the genus *Trichoderma*, and in 2008 also fungi of the order Mucorales were separated.

Statistical analysis of the results

The obtained results were subjected to the analysis of variance. The significance of differences between the means of photosynthesis intensity, height of seedlings and leaf surface, was assessed with the Duncan's test (significance level 0.05). The results of the morphological measurements of apple seedlings were shown graphically, taking into account the standard error. The number of bacteria and fungi (cfu) was subjected to a log transformation of $y = \log(x + 1)$. The significance of differences between the means of morphological measurements results assessed using the Newman-Keuls test (significance level 0.05).

RESULTS

Growth of apple seedlings in the soil after the cultivation of phytosanitary plants or introduction of white mustard seed meal

In 2007, three series of experiments were carried out successively (3 cycles of phytosanitary plant cultivation), which were conducted under various greenhouse conditions, depending on external conditions. This caused relatively large differences in the growth of apple seedlings cultivated in spring, when the temperature in the greenhouse did not exceed 25 °C, and the cultivation in subsequent periods, when the temperature exceeded 35 °C, which significantly limited the growth of seedlings (Fig. 1 and 2). Regardless of the cultivation period, seedlings growing in the soil with signs of fatigue had the lowest plant height (Fig. 1) and the smallest foliage area (Fig. 2). Taking into account both parameters, it was shown that the

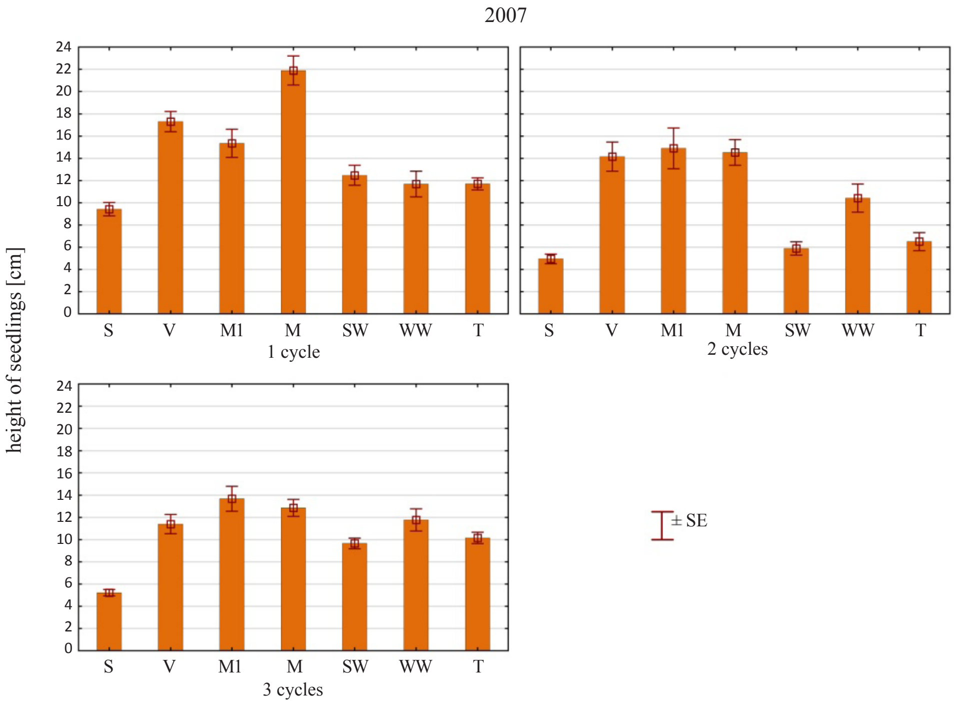

Figure 1. The height of apple seedlings cv. Antonovka growing in the sick soil after the cultivation of phytosanitary plants or amendment with white mustard seed meal (2007): S – sick soil , V – virgin soil, M1 – sick soil with amendment of 1% of white mustard seed meal, M – cultivation of white mustard, SW – cultivation of spring wheat, WW – cultivation of winter wheat, T – cultivation of triticale.

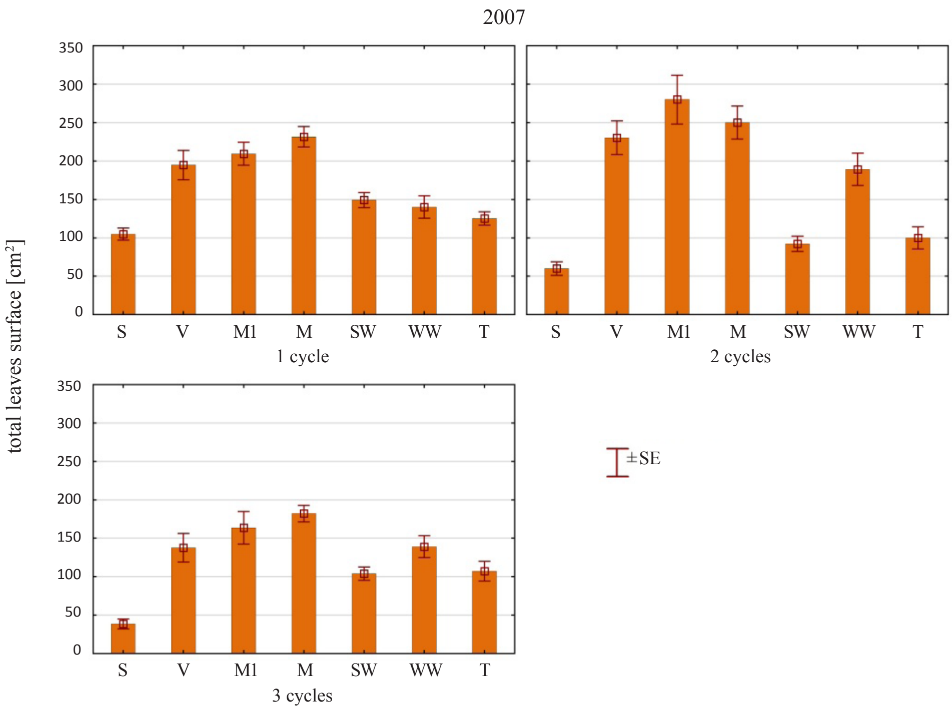

Figure 2. The total surface of the apple seedlings cv. Antonovka leaves growing in the sick soil and after cultivation of phytosanitary plants or amendment with white mustard seed meal (2007); notes see Fig. 1.

2008

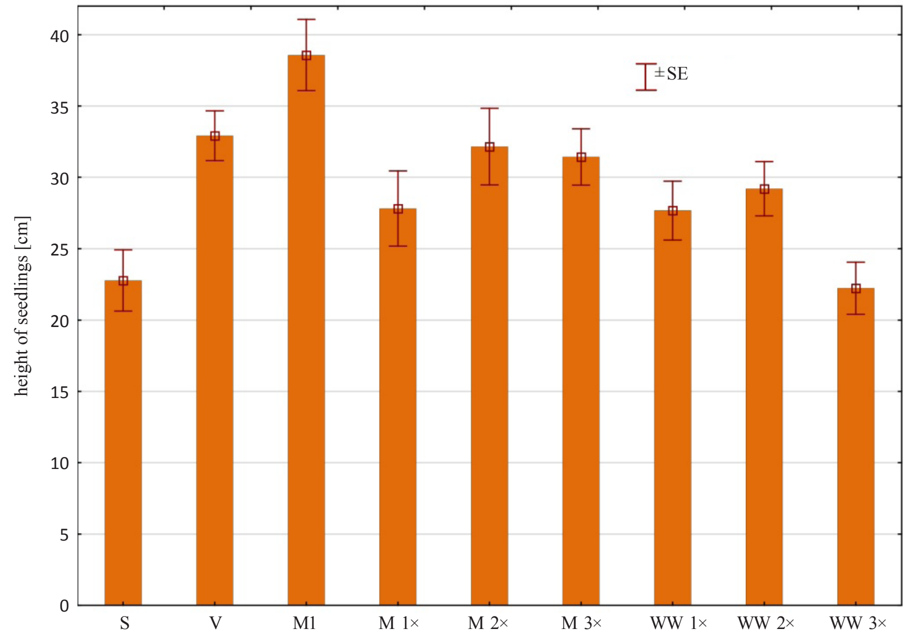

Figure 3. The height of apple seedlings cv. Antonovka growing in the sick soil and after the cultivation of phytosanitary plants or amendment with white mustard seed meal (2008): S – sick soil, V – virgin soil, M1 – sick soil with amendment of 1% of white mustard seed meal, M 1×, M 2×, M 3× – white mustard after 1, 2 or 3 cycles of cultivation; WW 1×, WW 2×, WW 3× – winter wheat after 1, 2 or 3 cycles of cultivation.

2008

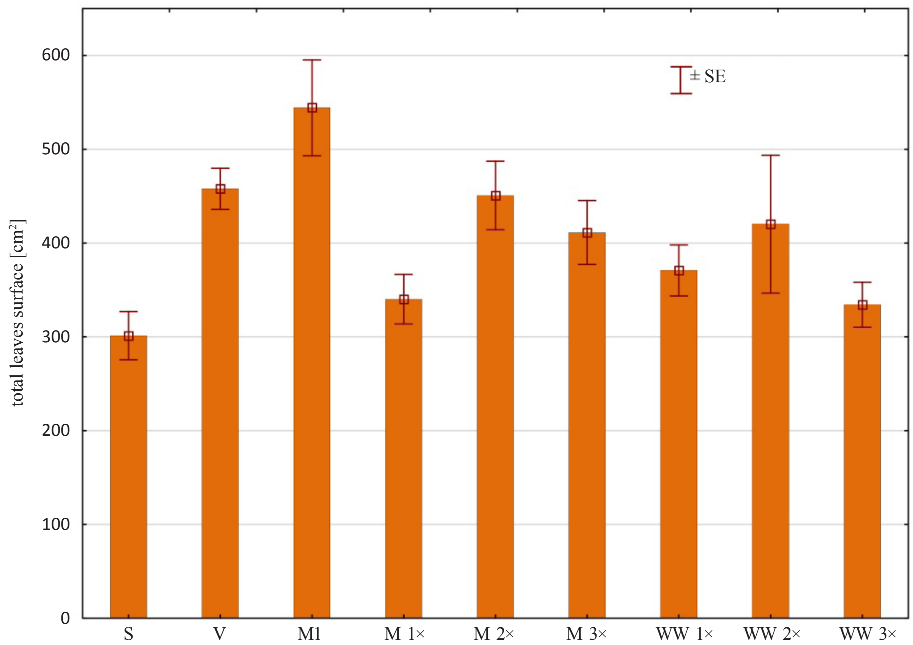

Figure 4. The total surface of the apple seedlings cv. Antonovka leaves growing in the sick soil and after cultivation of phytosanitary plants or amendment with white mustard seed meal (2008); notes see Fig. 3.

seedlings showed significantly stronger growth in this soil after adding mustard seed meal to the soil and after the cultivation of all phytosanitary plants. In this group, the seedlings growing in the soil in which spring wheat and triticale had been cultivated earlier produced the shortest plants with the smallest foliage area. On the other hand, the strongest growth was exhibited by the seedlings grown in the soil previously planted to white mustard or to which the mustard seed meal had been added, and the growth was often more vigorous than in the virgin soil.

The results obtained in 2008 confirmed the positive effect of the addition of mustard seed meal to the sick soil and of the cultivation of white mustard and winter wheat on the strength of seedling growth expressed as plant height and total surface of the foliage (Fig. 3 and 4). Both parameters were the highest in seedlings grown in this soil after the addition of mustard seed meal. Very good results were also obtained after two cycles of cultivating white mustard and winter wheat.

The measurements of leaf gas exchange intensity as an indicator of the physiological state of apple seedlings demonstrated that the majority of treatments used in 2007 caused an increase in the value of this parameter, reaching the highest value in combinations with white mustard (Table 1). The high efficiency of this species as a phytosanitary plant was visible after only one cycle of cultivation or addition of mustard seed meal to the soil. High values of photosynthesis intensity were also demonstrated in 'Antonovka' seedlings growing in combination with the cultivation of winter wheat. Already after one cycle of the cultivation of this plant, the photosynthesis rate of seedlings was higher compared to the control (non-treated sick soil). After three cycles of cultivation, the beneficial effect of using spring wheat was established. Also in 2008, high efficiency of mustard and winter wheat was demonstrated in improving the efficiency of the photosynthetic apparatus in apple seedlings, especially after 3 cycles of the cultivation of these plants (Table 2).

Table 1. The intensity of photosynthesis (μmol CO_2 m^{-2} s^{-1}) of apple seedlings cv. Antonovka growing in sick soil and after cultivation of phytosanitary plants (1, 2, 3 cycle) or amendment with white mustard seed meal (2007).

Treatment	Cycles of cultivation		
	1	2	3
Sick soil	5.69 a	6.80 a	6.66 a
Virgin soil	8.30 b	8.38 ab	8.53 ab
Sick soil + 1% white mustard seed meal	8.71 b	10.11 b	9.23 b
Sick soil, white mustard	8.06 b	8.91 ab	8.27 ab
Sick soil, spring wheat	6.04 a	7.45 ab	9.60 b
Sick soil, winter wheat	7.54 b	9.50 ab	9.25 b
Sick soil, triticale	5.71 a	7.70 ab	8.20 ab

Values in each column followed by the same letter are not significantly different.

Table 2. The intensity of photosynthesis (μmol CO_2 m^{-2} s^{-1}) of apple seedlings cv. Antonovka growing in sick soil and after cultivation of white mustard or winter wheat (1, 2, 3 cycles) or amendment with white mustard seed meal (2008).

Treatments	
Sick soil	7.85 a
Virgin soil	9.28 bc
Sick soil + 1% white mustard seed meal	8.60 abc
Sick soil, white mustard – 1 cycle	8.03 ab
Sick soil, white mustard – 2 cycles	9.40 c
Sick soil, white mustard – 3 cycles	11.12 d
Sick soil, winter wheat – 1 cycle	9.57 c
Sick soil, winter wheat – 2 cycles	9.24 bc
Sick soil, winter wheat – 3 cycles	11.50 d

Values followed by the same letter are not significantly different.

The occurrence of fungi, fungi-like organisms and bacteria in the soils

Mycological analysis of the composition of fungal communities during both years of research showed that both in the soil with signs of fatigue and in the virgin soil there were no fungi and fungi-like organisms from the genera *Rhizoctonia*, *Cylindrocarpon*, *Pythium* and *Phytophthora*, considered by many authors as the causal agents of the replant disease. Most of the fungi present were saprotrophic soil organisms. In the sick soil, 15 taxa of fungi were identified in 2007, with 13 taxa in 2008, while in virgin soil, respectively 19 and 18 taxa (data not included).

The comparison of the number of fungal communities occurring in the soil after many years of cultivating apple trees and in virgin soil in 2007 showed significantly more fungi in the first of mentioned soil only before the beginning of the first phytosanitary plant cultivation cycle (Table 3). Within the remaining periods of the study, no significant differences were found. It should be noted that the samples of both soils were stored until the time of analysis in cold storage at a temperature of approx. 2 ^{o}C. In 2008, the number of fungal populations in the sick soil was also significantly higher than in soil not used for growing of fruit trees, and also higher than in both studied soils in 2007 (Table 4).

In 2007, after one cycle of phytosanitary plants cultivation and the addition of mustard seed meal, there was a significant increase in the number of fungi in all experimental combinations; the highest one after the addition of mustard seed meal and the lowest after the mustard plantation (Table 3). Also after two and three cultivation cycles, there was an increase in the number of fungi compared to the control combination (sick soil); the highest one in combination with the addition of mustard seed meal, then after the cultivation of winter and spring wheat, and the weakest one after the cultivation of mustard and triticale. A very large increase in the number of fungi in the combination with added mustard seed meal resulted mainly from the incre-

Table 3. Number of fungal propagules (cfu × 10^3 in 1 g of soil dry weight) occurring in sick soil and after cultivation of phytosanitary plants (1, 2, 3 cycle) or amendment with white mustard seed meal (2007).

Treatment	1 cycle		2 cycles		3 cycles	
	total number	*Trichoderma* spp.	total number	*Trichoderma* spp.	total number	*Trichoderma* spp.
Sick soil	50.5 b	11.9 b	31.5 a	1.2 bc	35.7 a	32.2 a
Virgin soil	35.4 a	2.9 b	34.6 a	0.01 ab	27.1 a	24.9 a
Sick soil + 1% white mustard seed meal	6 205.0 e	5 002.5 c	2 592.6 d	1 497.7 c	2 935.0 d	1 613.4 d
Sick soil, white mustard	144.3 c	0.02 a	379.5 b	0.00 a	302.7 b	185.5 b
Sick soil, spring wheat	217.9 d	9.3 b	940.1 c	0.00 a	949.7 c	0.00 a
Sick soil, winter wheat	238.6 d	14.8 b	1 272.8 c	3.0 bc	1 224.5 c	1145.3 d
Sick soil, triticale	220.3 d	63 1.b	274.3 b	34.4 c	325.7 b	310.3 c

Values in each column followed by the same letter are not significantly different.

Table 4. Number of fungal propagules (cfu × 10^3 in 1 g of soil dry weight) occurring in sick soil and after cultivation of white mustard or winter wheat (1, 2, 3 cycle) or amendment with white mustard seed meal (2008).

Treatment	Total number	*Trichoderma* spp.	Mucorales
Sick soil	117.5 b	10.8 ab	0.01 a
Virgin soil	77.4 a	9.3 ab	0.01 a
Sick soil + 1% white mustard seed meal	1750.4 e	618.0 d	293.5 e
Sick soil, white mustard – 1 cycle	176.3 b	0.3 a	72.1 c
Sick soil, white mustard – 2 cycles	267.3 c	5.0 ab	19.8 b
Sick soil, white mustard – 3 cycles	294.4 cd	17.4 ab	20.5 b
Sick soil, winter wheat – 1 cycle	287.3 cd	0.5 ab	122.7 d
Sick soil, winter wheat – 2 cycles	1105.8 d	48.8 b	458.7 f
Sick soil, winter wheat – 3 cycles	1268.1 d	136.1 c	826.6 g

Values in each column followed by the same letter are not significantly different.

ase in the number (CFU) of *Trichoderma* spp. fungi, which after one cycle constituted 80.6%, after two cycles 57.8%, and after three cycles 55.0% of all fungi populations.

In 2008, a significant increase in the number of fungi was found in the soil to which mustard meal was added, winter wheat was cultivated in 1, 2 and 3 cycles and mustard in 2 and 3 cycles (Table 4). The highest increase was recorded after the addition of mustard seed meal. In this soil, *Trichoderma* spp. representing 35.3% of all fungi, occurred in abundance. The second largest group was the fungi of the order Mucorales (*Mucor* spp. and *Rhizopus* spp.), being the most abundant in the soil in which winter wheat was grown in 3 and 2 cycles and mustard meal was added.

In 2007, after one cycle of growing phytosanitary plants or adding mustard seed meal to the sick soil, a significant increase in the total number of bacteria was found in all combinations, compared to non-treated sick soil (Table 5). The highest number of bacteria was recorded in the soil into which mustard meal had been introduced and in the soil planted to white mustard, and the lowest one in the soil after triticale cultivation. The numbers of bacteria populations in the sick soil after spring wheat cultivation was similar to that in the virgin soil. Noteworthy are the changes in the populations of bacteria from the genera *Pseudomonas* and *Bacillus* considered to be indicators of the positive biological potential of the soil. Also in this case, the highest increase in their numbers occurred after adding mustard seed meal to the soil, with the number of *Pseudomonas* bacteria also significantly increased after the cultivation of white mustard and spring wheat. The highest number of these bacteria and bacteria of the genus *Bacillus* were found, however, in the soil not used for orchard cultivation. After two cycles of phytosanitary plants cultivation or addition of mustard seed meal to the soil, the highest number of bacteria was found again in the soil with the addition of mustard seed meal and after white mustard cultivation (Table 5). Their numbers were significantly higher also in the soil after the cultivation of spring wheat and in the virgin soil. The bacteria of the genera *Pseudomonas* and *Bacillus* dominated in terms of numbers in comparison to the other experimental combinations. Again, the addition of the meal stimulated their reproduction to the greatest extent. In the case of the bacteria *Pseudomonas*, spring wheat was also conspicuous for the promotion of their growth (10-fold increase). After three cycles, there was a huge increase in the number of bacteria in the soil with the addition of the meal (almost 300-fold) in relation

Table 5. Number of bacteria (cfu × 10^3 in 1 g of soil dry weight) occurring in sick soil and after cultivation of phytosanitary plants (1, 2, 3 cycles) or addition of white mustard seed meal (2007).

Treatment	1 cycle			2 cycles			3 cycles		
	total number	*Pseudomonas* spp.	*Bacillus* spp.	total number	*Pseudomonas* spp.	*Bacillus* spp.	total number	*Pseudomonas* spp.	*Bacillus* spp.
Sick soil	3.6 a	0.4 c	0.06 c	5.3 a	0.1 a	0.1 b	2.5 a	0.02 a	0.09 a
Virgin soil	83.8 d	69.8 g	2.1 f	20.6 b	17.2 e	nt	60.3 b	0.5 b	1.9 e
Sick soil + 1% white mustard seed meal	229.7 f	11.2 f	0.4 e	467.4 e	7.0 d	0.7 c	661.8 e	3.9 c	1.2 d
Sick soil, white mustard	155.1 e	8.8 e	0.05 b	329.5 e	0.6 c	0.08 b	63.1 b	0.3 b	0.5 c
Sick soil, spring wheat	67.4 d	3.4 d	0.04 a	75.9 d	1.0 c	0.09 b	84.7 c	0.3 b	0.25 b
Sick soil, winter wheat	26.0 c	0.1 a	0.08 d	44.5 c	0.7 c	0.08 b	113.4 d	0.3 b	0.24 b
Sick soil, triticale	13.6 b	0.2 b	0.04 b	63.1 cd	0.3 b	0.05 a	88.1 c	0.3 b	0.23 b

Values in each column followed by the same letter are not significantly different.
nt – not tested

Table 6. Number of bacteria (cfu × 10^3 in 1 g of soil dry weight) occurring in sick soil and after cultivation of white mustard or winter wheat (1, 2, 3 cycle) or addition of white mustard seed meal (2008).

Treatment	Total number	*Pseudomonas* spp.	*Bacillus* spp.
Sick soil	23 a	1.4 cd	2.0 e
Virgin soil	37 b	1.6 cd	0.1 c
Sick soil + 1% white mustard seed meal	155 f	0.9 abc	0.4 d
Sick soil, white mustard – 1 cycle	39 bc	1.2 bcd	0.4 d
Sick soil, white mustard – 2 cycles	64 d	1.6 cd	0.4 d
Sick soil, white mustard – 3 cycles	95 e	2.7 d	0.6 d
Sick soil, winter wheat – 1 cycle	53 cd	0.6 ab	0.05 a
Sick soil, winter wheat – 2 cycles	40 bc	0.5 a	0.07 ab
Sick soil, winter wheat – 3 cycles	55 cd	0.7 abc	0.09 bc

Values in each column followed by the same letter are not significantly different.

to the sick soil, followed by the cultivation of winter wheat (almost 50-fold) (Table 5). The meal also caused the largest increase in the number of the bacteria of the genera *Pseudomonas* and *Bacillus*. For comparison, the latter was the most numerous in the soil unused for orchard cultivation.

In 2008, the largest increase in the number of bacteria was recorded in the soils with the addition of mustard meal and after the cultivation of this plant in three successive cycles (Table 6). The bacteria of the genus *Pseudomonas* were also the most numerous after three cycles of white mustard cultivation, with no significant differences in the size of their population in the soil with the addition of meal and after one and two cycles of mustard cultivation. The bacteria of the genus *Bacillus* were found to be the most abundant in the sick soil. However, in the soil of other experimental treatments, the bacteria of this type dominated after the application of mustard, both as meal addition and after the cultivation of this plant, regardless of the number of cycles.

DISCUSSION

The presented research demonstrated that the soil in which apple trees had been grown for 27 years showed signs of fatigue resulting in weaker growth of the apple 'Antonovka' seedlings of planted into it, compared to soil not used for orchard cultivation. The physicochemical properties of both soils did not differ significantly except a difference in pH which, nevertheless, cannot be considered significant from a practical point of view, being both soils in the typical range of acidic soils. No pathogens of fungal and fungal-like organisms origin were found in the soil with signs of fatigue. Nematological analysis, however, showed, a numerically small presence of nematodes *Pratylenchus* spp. and *Tylenchorhynchus dubius.* Their population size did not exceed the threshold of economic damage, which indicates that nematodes did not adversely affect the growth of apple trees (Chałańska, unpublished data). The obtained results allow it to state that abiotic factors could have been responsible for the fatigue of the examined soil, but their determination was not the subject of the research. Among those factors, we could speculate that pH was not decisive. Indeed soil pH did not affect the growth of ‘Antonovka’ seedlings under acidic conditions (Politycka, 2007 and our own observations) and addition of green manure has been proved to differently modify the soil pH depending on factors such as soil physical and chemical characteristics or composition of the manure (Bertranda et al., 2007; Ritchie and Dolling, 1985). Various authors emphasize the importance of phenolic acids secreted into the soil by apple roots and as a result of the decomposition of plant residues (Zhang et al., 2007; Baerson et al., 2008; Politycka, Adamska, 2003; Politycka, Adamska, 2009). Nicola et al. (2016) proved in model studies that an addition of the ground roots of the M26 apple tree rootstock caused damage to the apple seedlings, of cultivar Fuji planted in this soil, which was associated with the autotoxic activity of root-generated phlorizin. The research by Chengmiao et al. (2016) showed that the concentration of phenolic acids in the apple-tree orchard soil was the highest in the autumn in the 30–60 cm layer.

One of the possibilities for reducing the effects of soil fatigue is the cultivation of phytosanitary plants on a given field as a forecrop, and in the case of nursery or greenhouse plantations, adding peat or compost to such soil. Introduction of organic matter into the sick soil (peat, compost from fish and wood waste and its enrichment with ammonium phosphate fertilizer or superphosphate) clearly improved the growth of apple tress (Neilsen, 1994; Wilson et al., 2004). However, it was proven that excessive doses of these components might also be toxic to the apple tree (Kelderer et al., 2016). The cultivation of phytosanitary plants causes changes in the composition of microorganisms inhabiting the soil, and also improves its structure and the supply of nutrients. Our research also showed that the cultivation of spring and winter wheat, triticale and white mustard, before planting apple ‘Antonovka’ seedlings to the soil with signs of fatigue, benefited their growth, including the size of the foliage surface. The best effect was obtained after the cultivation of white mustard or after the introduction of mustard seed meal into the soil, which was also confirmed by the measurements of the intensity of photosynthesis of apple seedlings. The effect of the plants of the *Brassicaceae* family consists in, among others, the production of various bioactive chemical compounds, including isothiocyanates, resulting from the hydrolysis of glucosinolates (Borek, Morra, 2005). For this reason, they are considered to be biofumigation agents with soil disinfectant abilities, but not of universal character. In addition, when used as a forecrop, they are a valuable source of organic matter in the soil. It is estimated that, depending on the date of sowing and location, these plants can provide up to 2 tons of biomass per hectare of field. Due to the rapid growth of both the aerial part and the root system, they efficiently capture and accumulate nitrogen, which remains after the harvest of the main crop deposited down to the depth of 2 meters in the soil profile.

Photosynthesis is the main factor responsible for the process of biomass accumulation by plants. The low efficiency of the photosynthesis in seedlings growing in the sick soil was one of the reasons for the limitation to the growth of these plants. In the available literature, the data on the impact of a specific replant disease on the efficiency of the photosynthetic apparatus of apple trees is scarce. This is also true of the response to measures taken to mitigate the effects of soil fatigue. Wang et al. (2014) determined the effect of the addition of biocarbon on the growth and intensity of gas exchange in the seedlings of the tea crab apple (*Malus hupehensis* Rehd.) cultivated in sick soil. The use of biocarbon significantly increased the rate of photosynthesis and the content of chlorophyll in the leaves of these plants. There was also a higher activity of antioxidant enzymes in plants grown on soil with the addition of biocarbon. Our research has demonstrated a positive impact of certain phytosanitary treatments on the intensity of CO_2 assimilation, which resulted in a more abundant plant growth.

Most literature data related to restoring fertility to sick soil refers to the elimination of biological factors which cause this phenomenon. The results of studies by Mazzola and Gu (2000) indicate that the cultivation of winter wheat of Eltan, Peneva and Rely cultivars, along with the reduction or even extermination of the population of such soil pathogens as *Rhizoctonia solani*, *Pythium* spp. or *Cylindrocarpon* spp., as well as nematodes *Pratylenchus* spp., resulted in a significant increase in the height of the apple trees cv. Gala along with an increase of the mass of their shoots and roots, in comparison with the control. Considerable importance should be attached to the studies on the usefulness of mustard seed meal in improving the phytosanitary status and fertility of soils with signs of fatigue (Fayzalla et al., 2009; Mazzola, Brown, 2010). Increased content of nitrogen in the leaves of apple trees and in the soil after adding rape seed meal (*B. napus*) was observed (Mazzola et al., 2007). It should be emphasised here that the eradication of *R. solani* in the soil after applying rapeseed meal was not related to its direct inhibitory impact on the mycelium of the pathogen, but rather affected the changes in the structure of the populations of microorganisms colonizing the soil, which resulted in the desired effect (Cohen, Mazzola, 2006). The comparison of the effectiveness of rapeseed, brown mustard and white mustard seed meals proved that each of them, when added to the soil, significantly improved apple tree growth (Mazzola et al., 2007).

Important factors determining soil quality include the microorganisms inhabiting this environment, which influence the biological, physical and chemical processes taking place there, and thus play an important role in the growth of crops. From a practical point of view, important microorganisms are the ones that exert a beneficial effect on plants, ones which in some way co-exist with the plant, giving it something in return, e.g. they produce plant hormones or metabolites that break down soil mineral particles, which increases the availability of nutrients for plants. The model studies that involved consisting in the addition of the bacteria *Pseudomonas luteola* strain BN0834 to the soil in which apple trees of cultivar Ligol grew in containers, showed an increase in the content of P, K and Ca in the leaves and an increase in the length of shoots, in comparison with the non-treated control (Kurek et al., 2013). It should be emphasized that in the case of sick soils, being the result of growing the same plants in a succession for a long time, the biodiversity of the bacteria and fungi living there decreases, which may lead to the accumulation of pathogens and other microorganisms adversely affecting the plants. The results of our research also indicate lower diversity of saprotrophic fungi occurring in the soil exploited for many years for fruit-growing, compared to virgin soil. Monocultures also cause certain simplification in the structure of microorganisms leading to the reduction of the population of fluorescent bacteria *Pseudomonas fluorescens*, capable of, among others, the production of the 2.4-diacetylphloroglucinol metabolite with antifungal activity (Mazzola et al., 2002; Weller et al., 2002; Validov et al., 2005).

The cultivation of phytosanitary plants from the *Brassicaceae* family resulted in changes in the populations of microorganisms present in the soil, as demonstrated in the case of agricultural plants (Majchrzak et al., 2010). The soils with a history of white mustard or winter rape cultivation were characterized by a greater potential for inhibiting pathogens due to the greater prevalence of the fungi of *Trichoderma*, *Penicillium* and *Gliocladium* genera. The results of our research also indicate a very high increase in the number of *Trichoderma* spp. after the addition of mustard seed meal and after winter wheat cultivation. The fungi of *Trichoderma* genus are generally recognized as beneficial organisms, that play a huge role in the decomposition of organic matter and in the reduction of many pathogens and diseases caused by them (Smith et al., 1990). Our research has shown that the cultivation of phytosanitary plants (cereals and mustard) has positively influenced the biological potential of the soil expressed by the increase in the population of bacteria of the genera *Pseudomonas* and *Bacillus.* These microorganisms are considered to be the main source of biological plant protection factors against diseases, both soil-borne and those affecting aerial parts of plants. Egamberdieva et al. (2017) underline the role of metabolites produced by microorganisms in stimulation of plant growth and induction of resistance to biotic and abiotic stresses. Some microorganisms that inhabit the rhizosphere and rhizoplane, as well as those that living freely in the soil, may produce various phytohormones. It has been demonstrated, e.g. that the bacteria *Pseudomonas fluorescens*, *Bacillus megaterium*, *Bacillus cereus*, *Bacillus subtilis* produce cytokinins (Salamone et al., 2001), and *Trichoderma asperellum* – indolyl acetic acid, gibberellins and abscisic acid (Zhao, Zhang, 2015). Aslantas et al. (2007) demonstrated that the artificial colonization of the roots of the apple trees of Granny Smith and Stark Spur Golden cultivars with bacterial strains of the genera *Bacillus*, *Burkholderia* and *Pseudomonas*, producing indolyl acetic acid (IAA) and cytokinins, and having the ability to dissolve phosphates, resulted in an increase of fruit yield and increased tree growth parameters (length and diameter of shoots) in comparison to the control. The authors believe that the tested bacteria are potentially useful in fruit production in terms of the possibility of limiting mineral fertilization. Taking into account the results of our research, it should be concluded that the changes in the composition of microorganisms inhabiting soils with signs of fatigue, after planting phytosanitary plants in the soil and applying mustard seed meal, positively influenced the growth of apple seedlings. However, it is difficult to state unambiguously how individual factors and their interaction influenced the effect that was obtained.

CONCLUSIONS

1. The cultivation of cereal crops and white mustard as a forecrop before the establishment of an apple orchard, provides a perspective for the elimination of the effects of soil fatigue caused by many years of growing fruit trees.
2. White mustard seed meal applied into the soil which has been used for many years for growing an apple trees creates the possibility of limiting the effects of soil fatigue.
3. Growing cereals and white mustard or introducing mustard seed meal into the soil increases the biological potential of the soil and improves its fertility.
4. Due to the fact that the studies were conducted in greenhouse conditions, the obtained results indicate only the potential possibilities of implementing the investigated treatments under on-farm conditions, as the latter may be modified by environmental conditions.

REFERENCES

Aslantas R., Cakmakci R., Sahin F., 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. Scientia Horticulturae, 111: 371-377.

Baerson S.R., Dayan F.E., Rimando A.M., Nanayakkara N.P.D., Liu C.J., Schroder J., Mark Fishbein M., Pan Z., Kagan I.A., Pratt L.H., Cordonnier-Pratt M.-M., Duke S. O., 2008. A functional genomics investigation of allelochemical biosynthesis in *Sorghum bicolor* root hairs. Journal of Biological Chemistry, 6: 3231-3247.

Bertranda I., Delfosseb O., Maryb B., 2007. Carbon and nitrogen mineralization in acidic, limed and calcareous agricultural soils: apparent and actual effects. Soil Biology & Biochemistry 39: 276-288.

Borek V., Morra M.J., 2005. Ionic thiocyanate (SCN-) production from 4-hydroxybenzyl glucosinolate contained in *Sinapis alba* seed meal. Journal of Agricultural and Food Chemistry, 53: 8650-8654.

Chengmiao Y., Xiang L., Wang G., Wang Y., Shen X., Chen X., Mao Z., 2016. How to plant apple trees to reduce replant disease in apple orchard: a study on the phenolic acid of the replanted apple orchard. PLOS ONE, doi: 10.1371/journal.pone.0167347

Cohen M.F., Mazzola M., 2006. Effects of *Brassica napus* seed meal amendment on soil population of resident bacteria and *Naegleria americana* and the unsuitability of arachidonic acid as a protozoan specific marker. Journal of Protozoology Research, 16: 16-25.

Dullahide S.R., Stirling G.R., Nikulin A., Stirling A.M., 1994. The role of nematodes, fungi, bacteria and abiotic factors in the etiology of apple replant problems in the Granite Belt of Queensland. Australian Journal of Experimental Agriculture, 34(8): 1177-1182.

Egamberdieva D., Wirth S.J., Alqarawi A.A., Abd Allah E.F., Hashem A., 2017. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Frontiers in Microbiology, doi: 10.3389/fmicb.2017.02104

Fayzalla E.A., El-Barougy E., El-Rayes M.M., 2009. Control of soil-borne pathogenic fungi of soybean by biofumigation with mustard seed meal. Journal of Applied Sciences, 9(12): 2272-2279.

Granatstein D., Mazzola M., 2001. Alternatives to fumigation for control of apple replant disease in Washington state. Integrated Fruit Production, IOBC/wprs Bulletin vol. 24, 5: 265-271.

Gu Y.-H., Mazzola M., 2003. Modification of fluorescent pseudomonad community and control of apple replant disease induced in a wheat cultivar-specific manner. Applied Soil Ecology, 24: 57-72.

Hofmann A., Wittenmayer L., Arnold G., Schieber A., Merbach W., 2009. Root exudation of phloridzin by apple seedlings (Malus x domestica Borkh.) with symptoms of apple replant disease. Journal of Applied Botany and Food Quality, 82: 193-198.

Katan J.. 2004. Role of cultural practices for the management of soilborne pathogens in intesive horticultral systems. Acta Horticulturae, 635: 11-18.

Kelderer M., Topp A., Rainer A., Cristoforetti A., Manici L., 2016. Efficacy of organic composts against apple replant disease. Eco Fruit Conference Proceedings, pp. 97-102.

Kurek E., Ozimek E., Sobiczewski P., Słomka A., Jaroszuk-Ściseł J., 2013. Effect of *Pseudomonas luteola* on mobilization of phosphorus and growth of young apple trees (Ligol) – pot experiment. Scientia Horticulturae, 164: 270-276.

Lazarovits G., 2001. Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from past. Canadian Journal of Plant Pathology, 23: 1-7.

Majchrzak B., Kurowski T.P., Wachowska U., Jaźwińska E., 2010. Changes in soil microbial communities as a result of growing *Brassicaceae* crops. Acta Agrobotanica, 63(1): 161-169.

Manici L.M., Kelderer M., Erschbaumer G., Caputo F., Babini V., Casera C., 2003. Replant problems in South Tyrol: role of fungal pathogens and microbial population in conventional and organic apple orchards. Plant and Soil, 256(2): 315-324.

Mazzola M., 1998. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. Phytopathology, 88: 930-938.

Mazzola M., Gu Y.-H., 2000. Impact of wheat cultivation on microbial communities from replant soils and apple growth in greenhouse trials. Phytopathology, 90: 114-119.

Mazzola M., Granatstein D.M., Elfving D.C., Mullinix K., Gu Yu-Huan, 2002. Cultural management of microbial community structure to enhance growth of apple in replant soils. Phytopathology, 92: 1363-1366.

Mazzola M., Brown J., Izzo A.D., Cohen M.F., 2007. Mechanism of action and efficacy of seed meal-induced suppression of pathogens inciting apple replant disease differ in a Brassicaceae species and time-dependent manner. Phytopathology, 97: 454-460.

Mazzola M., Brown J., 2010. Efficacy of Brassicaceous seed meal formulations for the control of apple replant disease in conventional and organic production systems. Plant Disease, 94: 835-842. doi:10.1094/PDIS-94-7-0835

Neilsen G.H., 1994. Planting hole amendments modify growth and fruiting of apples on replant sites. HortScience, 29(2): 82-84. doi: 10.21273/HORTSCI.29.2.82

Nicola L., Vrhovsek U., Soini E., Insam H., Pertot I., 2016. Phlorizin released by apple root debris is related to apple replant disease. Phytopathologia Mediterranea, 55(3): 432-442.

Pacholak E., Cwynar M., Suterski Ł., 1989. Wzrost jabłoni w pierwszym roku po replantacji w warunkach zróżnicowanego nawożenia i nawadniania. Ogólnopolska Konferencja Sadownicza, Skierniewice, Prace Instytutu Sadownictwa i Kwiaciarstwa, C, 1-4: 34-35.

Pacholak E., Rutkowski K., 2001. Effect of soil sickness prevention methods on the numer of nematodes and on the growth and yielding of 'Elstar' cultivar apple trees on the different moisture of soil. Zeszyty Naukowe ISK, 9: 7-14. [in Polish + abstract in English]

Pacholak E., Zydlik Z., Rutkowski K., Przybyła Cz., 2004. Effect of fertilization and irrigation on the growth and yielding of apple tree 'Sampion' cultivar in a replanted orchard in the years 1994-2002. Folia Universitatis Agriculturae Stetinensis, Agricultura, 96: 143-148.

Politycka B., 2007. Substrate reaction and phytotoxicity of root residues of apple trees. Nauka Przyroda Technologie, 1(1): 1-6. [in Polish + abstract in English]

Politycka B., Adamska D., Pacholak E., 2001. Changes in content of phenolic compounds in orchard soil after replantation of apple trees in relation to fertilization and irrigation. Folia Horticulturae, 13/1: 55-63.

Politycka B., Adamska D., 2003. Release of phenolic compounds from apple residues decomposing in soil and the influence of temperature on their degradation. Polish Journal of Environmental Studies, 12(1): 95-98.

Politycka B., Adamska D., 2009. Phenolic compounds occurring in the residues of apple roots and their phytotoxicity. Zeszyty Problemowe Postępów Nauk Rolniczych, 539: 593-599. [in Polish + abstract in English]

Rebandel Z., 1987. Problem zmęczenia gleby w sadownictwie. In: Sadownictwo w Wielkopolsce. PWRiL, Warszawa.

Ritchie G.S.P., Dolling P.J., 1985. The role of organic matter in soil acidification. Australian Journal of Soil Research, 23(4): 569-576.

Rutkowski K., Pacholak E., 1999. Wpływ sposobów zapobiegania zmęczeniu gleby na wzrost jabłoni odmiany Elstar.

Zeszyty Naukowe Akademii Rolniczej im. H. Kołłątaja w Krakowie, 351: 135-140.

Salamone D.G., Hynes R.K., Nelson L.M., 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Canadian Journal of Microbiology, 47: 404-411. doi: 10.1139/w01-029

Schaad N. W., Jones J. B., Chun W., 2001. Laboratory guide for identification of plant pathogenic bacteria. 373 pp.

Smith V.L., Wilcox W.F., Harman G. E., 1990. Potential for biological control of Phytophthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. Phytopathology, 80: 880-885.

Sobiczewski P., Meszka B., Bryk H., Malusa E., 2014. Changes in soil microbial populations after fumigation and alternative methods to control soil-borne diseases. Plant Science, Sofia, Bulgaria, LI(6): 79-81.

Szczygieł A., Zepp A.L., 1993. Preliminary study on the occurrence of replant disease in apple orchards in Poland. Acta Horticulturae, 324: 81-84.

Traquair J.A., 1984. Etiology and control of orchard replant problems: a review. Canadian Journal of Plant Pathology, 6: 54-62.

Validov S., Mavrodi O., De La Fuente L., Boronin A., Weller D., Thomashow L., Mavrodi D., 2005. Antagonistic activity among 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. FEMS Microbiology Letters, 242: 249-256.

Wang Y., Pan F., Wang G., Zhang G., Wang Y., Chen X., MaoZ., 2014. Effects of biochar on photosynthesis and antioxidative system of *Malus hupehensis* Rehd. seedlings under replant conditions. Scientia Horticulturae, 175: 9-15.

Weller D.M., Raaijmakers J.M., Gardener B.B.M., Thomashow L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology, 40: 309-348.

Wilson S., Andrews P., Nair T.S., 2004. Non fumigant management of apple replant disease. Scientia Horticulturae, 102: 221-231.

Zhao L., Zhang Y., 2015. Effects of phosphate solubilization and phytohormone production of *Trichoderma asperellum* Q1 on promoting cucumber growth under salt stress. Journal of Integrative Agriculture, 14: 1588-1597. doi: 10.1016/S2095-3119(14)60966-7

Zhang J.H., Mao Z.Q., Wang L.Q., Shu H.R., 2007. Bioassay and identification of root exudates of three fruit tree species. Journal of Integrative Plant Biology, 49(3): 257-261.

Zydlik Z., 2004. Effect of locality on the microbiological condition of soil from replanted apple orchard. Folia Universitatis Agriculturae Stetinensis, Agricultura, 96: 219-224.

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received – 27 September 2018
revised – 24 November 2018
accepted – 29 November 2018

Ministerstwo Nauki i Szkolnictwa Wyższego

„Przetłumaczenie na język angielski wybranych prac zgłoszonych do Polish Journal of Agronomy" – zadanie finansowane w ramach umowy Nr 692/P-DUN/2018 ze środków Ministra Nauki i Szkolnictwa Wyższego przeznaczonych na działalność upowszechniającą naukę