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ORIGINAL RESEARCH PAPER

Fungi complicit in oak powdery mildew infection in the Oława Forest District

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Abstract

Oaks are important forest-forming species in Poland. It is an important species from an economic perspective in Polish forestry. Oak is characterized by high tolerance in terms of soil requirements, which include soil formations from sandy to clayey with a reaction from acidic to indifferent, sometimes even alkaline in pedunculate oak. It is sensitive to late frosts. In recent years, climate change has been observed in Europe and also in Poland. Abiotic stresses caused by unfavorable weather conditions have worsened the health of oak trees, on which other pathogens, primarily fungi, have become established. The research was carried out at two sites in the Oława Forest District in Bystrzyca in 2022. They consisted in collecting 10 representative shoots from each research area, which were then used to isolate fungal species in the laboratory. The results of the experiment confirm that co-occurring species can be observed with the presence of powdery mildew. Oak shoots are colonized by both pathogenic and saprotrophic fungal species. The high occurrence of pathogenic fungi species along with powdery mildew on oak shoots can be associated with the deterioration of the phytosarcoma condition of oaks, however, in order to be able to state this unequivocally, research on this phenomenon should be focused on in the future.

Keywords

pathogen; Polish forestry; *Fusarium* spp.; *Trichoderma* spp.; *Alternaria* spp.

1. Introduction

Oak (*Quercus*) is a tree species belonging to the beech family - *Fagaceae*. More than 900 species can be classified in this family, most of which occur in the northern hemisphere of our planet (Sadowski et al., 2020). The two most widespread and economically important species in Polish forestry are the pedunculate oak (*Quercus robur* L.) and the sessile oak (*Quercus petraea* Liebl.) (Buraczyk et al., 2020). Oak has a high tolerance to soil moisture. Plantations of oaks should be established on clay-limestone or clay-sandy soils, due to their relatively high soil requirements (Andrzejczyk & Sewerniak, 2016; Przybył, 1995). The species is characterized by frost tolerance but is sensitive to late frosts (Będkowski, 2018).

In recent years, oak dieback has been an increasing problem in forests, mainly of new 1–3-year-old regenerations, as well as of young specimens up to 25 years old (Wit et al., 2015). Oak dieback is a large and still not fully diagnosed problem both in Poland and worldwide (Oszako et al., 2009, 2013). On the one hand, climate change and the ensuing unfavorable abiotic factors such as long periods of drought with high tem-

peratures or sulfurous frosts are responsible for this, as well as falling groundwater levels, making it harder for trees to draw water from increasingly deep aquifers (Andrzejczyk, 2009; Corcobado et al., 2014; Oszako et al., 2013). On the other hand, biotic stressors, primarily pathogens such as fungi, are responsible for weakening and ultimately dying (Wit et al., 2015).

A disease that significantly affects the condition of trees, their assimilation apparatus, and non-wooden shoots is oak powdery mildew, caused mainly by *Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. (Roszak et al., 2019). The second species of powdery mildew occurring in Poland is *E. hypophylla* (Sucharzewska, 2009). It arrived in Europe after being imported from North America. This fungus infects young oak specimens up to 3 meters in height, as well as two types of shoots: suckers and holly, and to a lesser extent, adult trees. It mainly affects European oaks; American specimens are more resistant (Tkaczyk, 2022). The mycelium initially covers the leaf blade, followed by leaf darkening and crinkling, so that eventually, the leaves quickly die (Behnke-Borowczyk & Baranowska-Wasilewska, 2017). Other species

of *Erysiphe* spp. Found on oak leaves in the world are: *E. abbreviata* (Peck) U. Braun and S. Takam., *E. calocladophora* (G.F. Atk.) U. Braun and S. Takam., *E. extensa* (Cooke and Peck) U. Braun and S. Takam., *E. gracilis* R.Y. Zheng and GQ Chen, *E. hypophylla* (Nevod.) U. Braun and Cunningt., *E. quercicola* S. Takam. And U. Braun and *E. polygona* D.C. (Kasprzyk et al., 2022).

As powdery mildew infects oak trees at a young age, planting material in cultivated nurseries, which mostly carry out extensive production, is particularly vulnerable. Hence, the study was also carried out on artificial oak restoration, which resembles a field nursery in terms of growing conditions (Buraczyk et al., 2020).

Rain is a factor that, on the one hand, favors the development of powdery mildew and, on the other, can also inhibit infection on plants. It all depends on the amount and length of rainfall and the ambient temperature. Moderate rainfall of around 80 mm, together with a moderate temperature of around 20 °C, is conducive to the development of the disease. Deviations from these parameters have a chilling effect on disease development (Mychayliv et al., 2011). Studies by Sivapalan (1993) also showed the deleterious effect of heavy rainfall on powdery mildew fungi by flushing out spores and damaging the mycelium on the leaf surface. It was also found that the ability of the fungus to establish a parasitic relationship with the host strongly decreased with residence in water.

As a biotroph, the main purpose of *E. alphitoides* is not to kill the host. However, it weakens oaks through infection, making them more susceptible to many other killer fungal species, such as *Armillaria* spp., the fungus that causes Manila root rot (Szewczyk et al., 2015). *Colpoma quercinum* (Pers.) Wallr. is another pathogen responsible for the death of weakened oak shoots. This fungus is called a parasite of weakness. It lives on weakened individuals as well as dead individuals killed by other factors. It can be observed on shoots in the form of transverse, spindle-shaped, dark cups. Infection occurs through ascospores, it penetrates through wounds (Mańka et al., 2005). Infection of oak shoots by powdery mildew also favors secondary infection by blight fungi, e.g., *Fusarium oxysporum* Schltdl. (Topalidou & Shaw, 2016; Wrzosek & Sierota, 2012).

The main aim of the study was to determine if there are fungi in the environment that contribute to oak dieback. If these fungi are present, whether they occur on natural or artificial restorations. Also, to determine whether antagonistic fungi are present and whether there is a difference in their abundance between natural and artificial restoration. Determining whether other pathogenic fungi are also present that can cause young oak trees to die and whether there is an apparent difference between artificial and natural regeneration was another objective of the research undertaken.

2. Material and methods

The study material consisted of one-year and several-year-old seedlings of the pedunculate oak *Quercus robur*. The research was carried out at two sites in the Oława Forest District in Bystrzyca (50°57'31.754" N; 17°23'48.246" E). The first site was a natural regeneration of pedunculate oak, growing between large representatives of the species. The second is a fenced plantation of 2–3-year-old pedunculate oak managed

in accordance with the guidelines of the State Forests. The survey work was carried out in 2022. Four experimental plots of 50 m² (10 m long, 5 m wide) each were marked out in the forest for natural regeneration, as well as four plots with the same parameters for forest crops. The role of control was the number of fields. Each successive plot was also replicate.

In September 2022, 10 representative shoots with symptoms of oak powdery mildew were selected from each test plot. The shoots were harvested to isolate the co-participating fungi.

In the sterile transplant room, the oak shoots were surface disinfected for 1 minute using a 0.5% sodium hypochlorite solution. Each shoot was then cut into 10 pieces, which were placed in Petri dishes with previously prepared potato-glucose medium (PDA). Potato Dextrose LAB-AGAR medium from Biomaxima was used in the experiment. After a period of 1.5 weeks, fungal colonies began to appear in the Petri dishes. The next step was to transplant the pure colonies into previously prepared slants with PDA. Identification of the fungi was done under the microscope, based on their morphological characteristics and using monographs (Ellis, 1971; Nelson et al., 1983; Zycha et al., 1969).

The formula was used to calculate the Margalef Index:

$$Mg = (S - 1) / \ln(N),$$

where: S - number of species, N - number of total individuals.

To calculate the Shannon Index (SHDI) the formula was used:

$$H = - \sum (p_i * \ln p_i),$$

where p_i is the ratio of the number of individuals of a species to the total number of individuals in all species.

The formula used to calculate the Shannon Evenness Index (SEI) was:

$$E = (H_{\max} - H_{\text{obs}}) / H_{\max},$$

where:

H_{\max} is the maximum possible Shannon Index for a given number of groups,

H_{obs} is the observed Shannon Index.

The Berger–Parker Index (BPI) is calculated from the formula:

$$d = 1 - ((S_1/N_1)^2 + (S_2/N_2)^2 + \dots + (S_n/N_n)^2),$$

where:

S_1, S_2, \dots, S_n are the numbers of individuals in each group,

N_1, N_2, \dots, N_n are the total number of individuals in all groups.

3. Results

The results confirm that, along with the presence of powdery mildew, co-occurring species were also isolated. Oak shoots are colonized by both pathogenic and saprotrophic fungal species. A major threat to oak trees in both natural and artificial restoration is the fungi of the genus *Fusarium*, which was one of the most frequently isolated. In artificial restoration, another abundant type is *Alternaria* (Król et al., 2015), and the same relationship is confirmed by the study of Wit et al. (2015). In natural regeneration, the next most abundant type

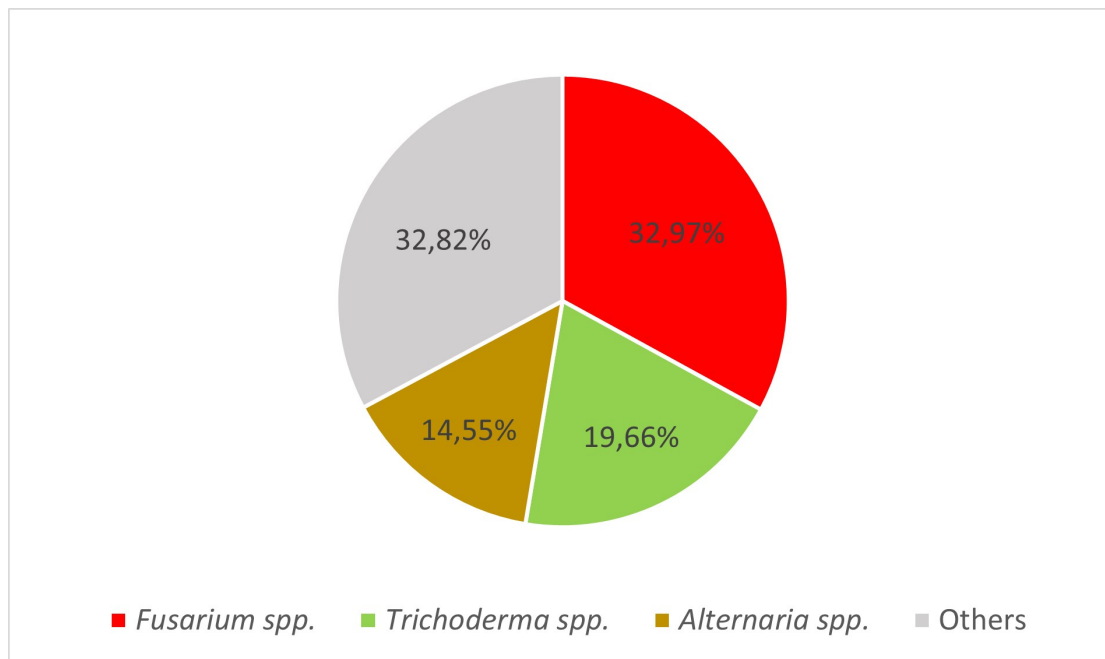


Figure 1 Number of fungal colonies isolated from oak shoots total [%].

was *Trichoderma*. This relationship was also shown in a study by Topalidou and Shaw (2016).

Mycological analysis revealed that the total number of colonies (NC) isolated from oak shoots was 646. In natural regeneration, 308 colonies were obtained, including 8 non-spore colonies (Table S1). In artificial restoration, the number of colonies was almost 10% higher at 338, including 5 non-spore-forming colonies (Table S1). The highest number of fungal colonies - 90 - was isolated in the artificial restoration in the 4th plot. The highest number, 109, were colonies of *Fusarium sporotrichioides*, which constituted 16.87% of all colonies. A total of 33 fungal species were observed, including light and dark non-spore-forming colonies (Table S1).

Fusarium spp. accounted for the largest percentage, 32.97%, of the fungi isolated from oak shoots. The next two groups accounting for the largest percentage were *Trichoderma* spp. - 19.66% and *Alternaria* spp. - 14.55% (Figure 1). In natural regeneration, it can be observed that almost 38% of all colonies were fungi of the genera *Fusarium* spp. and *Trichoderma* spp. The number of colonies of other species was 60, which is 9.29%. *Alternaria* spp. were few in number, only 6 - 0.93%. In the artificial restoration, 90 colonies of *Fusarium* spp. and 88 colonies of *Alternaria* spp. were observed. Other fungi account for 152 colonies, 23.53% of all fungi. *Trichoderma* spp. represent only 8 colonies - 1.24% (Figure 2).

The total number of *Fusarium* spp. colonies was 213, with 123 colonies in the natural regeneration giving 19.04% and 90 colonies in the artificial regeneration giving 13.93% of the total colonies. The most abundant species in both restorations was *Fusarium sporotrichioides*. A total of 109 colonies were observed, representing 16.86% of the total. The next most abundant species was *Fusarium oxysporum*, accounting for 6.97% of the total. The remaining species did not exceed 12 colonies (Figure 3).

Fungi of the species *Alternaria* spp. were abundant in the artificial restoration. A total of 94 colonies were identified. *Alternaria alternata* was the most abundant species. It accounted for 71% of all *Alternaria* spp. Only 6 colonies of the fungus of this genus were captured in natural regeneration (Figure 4).

Trichoderma spp. were abundant in natural regeneration. A total of 119 colonies were observed in it, representing 18.42% of all identified colonies. The most abundant *Trichoderma* spp. were *Trichoderma harzianum* - 9.91% and *Trichoderma piluliferum* - 7.59%. In artificial restoration, only 8 colonies of *T. piluliferum* were observed, representing 1.24% of all colonies (Figure 5).

In natural regeneration, the fungus species *Epicoccum nigrum* was abundant, with 32 colonies, and *Mucor mucedo*, with 10 colonies. The remaining species, occurred in small numbers, not exceeding five colonies. The dominant fungus species in the artificial restoration was *E. nigrum*, with 54 colonies accounting for 8.36% of all colonies. *Phoma glomerata* was also abundant, with 37 colonies giving 5.73% of the total. Other species exceeding 10 colonies were *Stemphylium botryosum*, with 20 colonies, and *Ulocladium consortiale*, with 12 colonies (Figure 6).

For both natural and artificial restoration, selected indices of species diversity were compared: Margalef (MRI), Shannon (SHDI), Shannon evenness (SEI), and Berger-Parker (BPI) (Table 1). The results for the MRI and SHDI indices are similar. On the other hand, the SEI and BPI of the next two indices are slightly different. The SEI is higher for artificial regeneration, and the BPI is higher for natural regeneration. The closer the SEI is to 0, the more even the distribution, and the closer it is to 1, the more the population is concentrated in a few groups. In this case, the SEI index for natural regeneration is around 0.267, indicating that the distribution of fungi is relatively even. For artificial restoration, the SEI

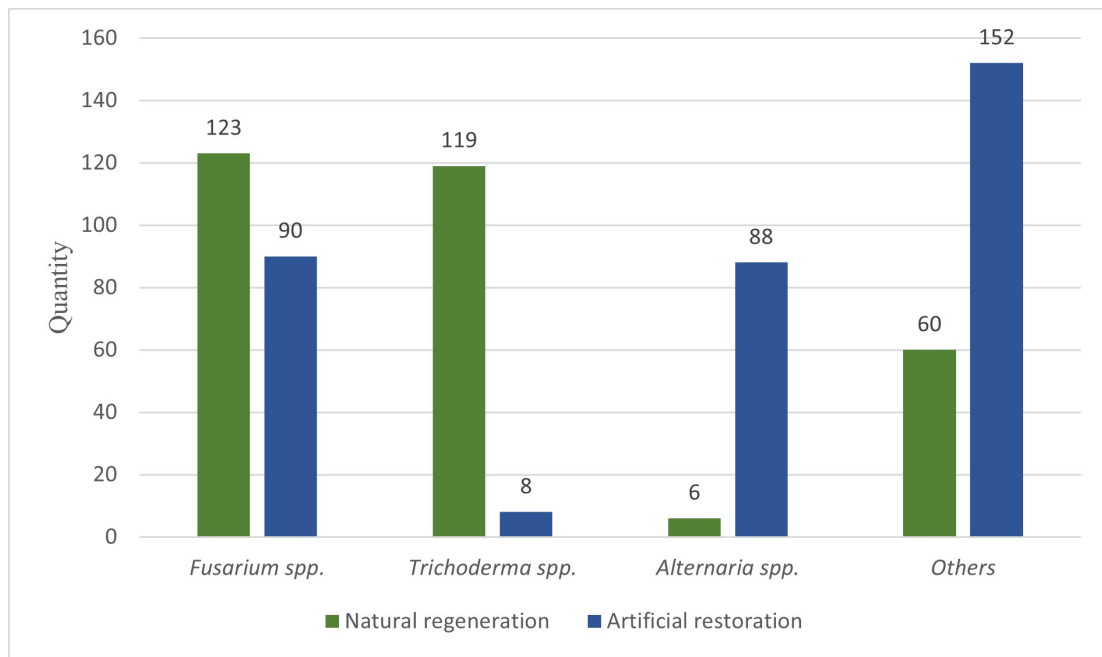


Figure 2 Numbers of fungal colonies isolated from oak shoots in natural and artificial restoration.

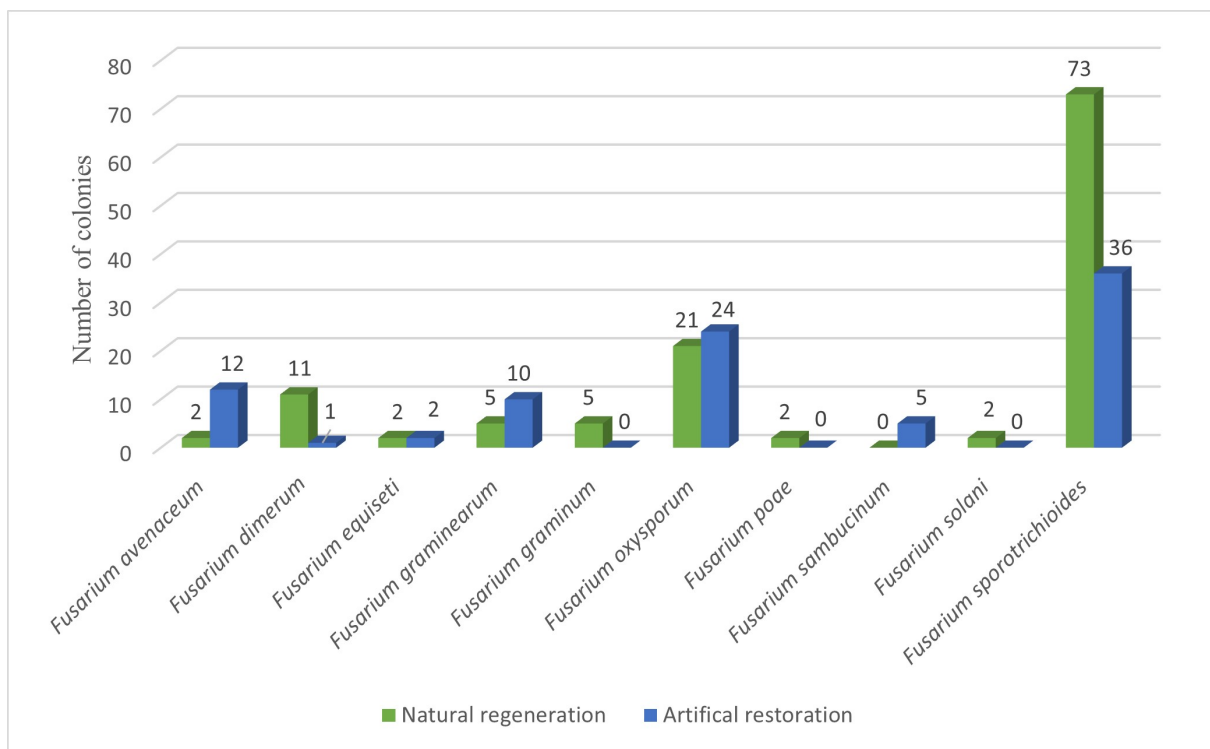


Figure 3 Abundance of individual *Fusarium* spp.

value is around 0.425, suggesting that the distribution of fungi is less even and the population is more concentrated in a few groups. The Berger–Parker index measures the evenness of the distribution of individuals between groups. The closer the value is to 1, the less even the distribution. In the case of the calculated values for the two restorations, the result for the natural restoration ($d = 0.708$) indicates a less even distribution compared to the artificial restoration ($d = 0.674$), mean-

ing that in the natural restoration, some groups may have a greater impact on species diversity than others (Table 1).

4. Discussion

Based on a statistical analysis of the Margalef index itself, we can see that it is slightly higher for artificial restoration than for natural restoration. This means that the artificial restora-

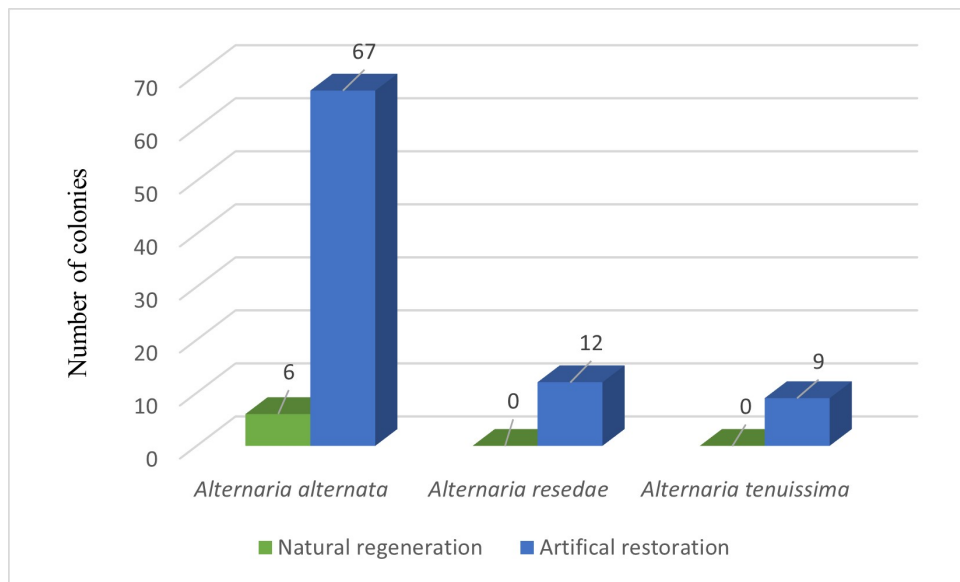


Figure 4 Abundance of individual species of the genus *Alternaria*.

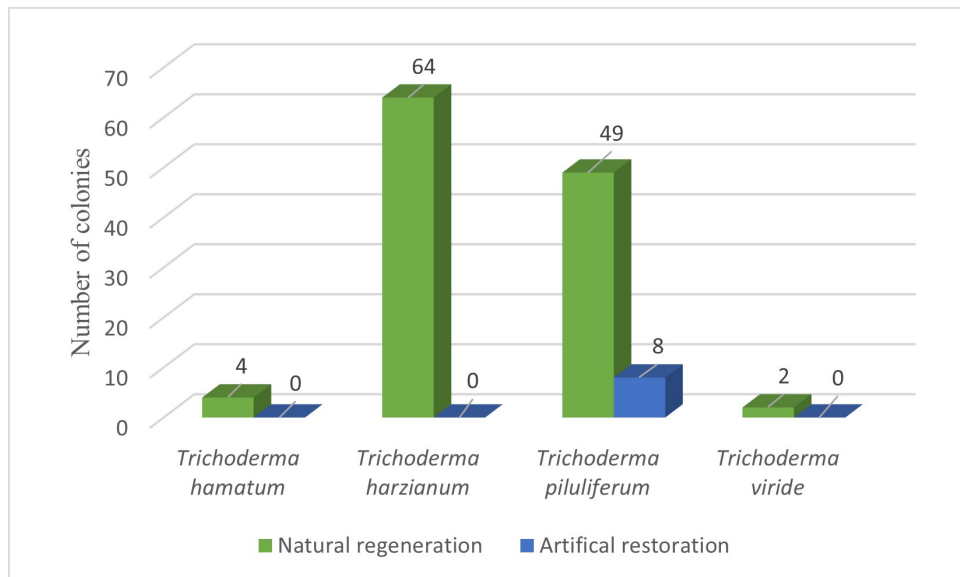


Figure 5 Abundance of individual species of the genus *Trichoderma*.

tion sample may have a slightly higher species diversity than the natural regeneration sample. However, it is important to note that the difference between the Margalef index results for the two groups is small and may not be of great biological significance. Further research and analysis are needed to further define biodiversity in both groups. When comparing the results of the Margalef and Shannon indices for the natural and artificial samples, the biodiversity was similar, and the difference between the Margalef index in the natural and artificial samples was small (2.68 vs. 2.71). The results for the Shannon index were also similar for the natural and artificial samples (1.35 vs. 1.36). This means that there is no significant difference in biodiversity between the natural and artificial samples as measured by the Margalef and Shannon indices. The reason for the lack of significant differences may be that artificial regeneration, like natural regeneration, is located in

the forest surrounded by large areas of the natural environment, which interacts in the same way with the regenerations studied. Magurran (1988) carried out a study comparing the value of biodiversity indices and found that deciduous oak forests had greater biodiversity than spruce forests. Due to the fact that the study in Bystrzyca was carried out only in oak plantations, there are no significant differences in the results of the indicators.

Trichoderma spp. has long been considered as a biological control agent (BCA) for plant diseases (Oszako et al., 2021). In the results from artificial restoration, a correlation can be observed as the number of *Trichoderma* spp. colonies increases the number of *Alternaria* spp. colonies decreases. The reason for this may be the antagonistic effect of *Trichoderma* spp., which in this case, blocks the growth of *Alternaria* spp. Scientific papers by researchers dealing with

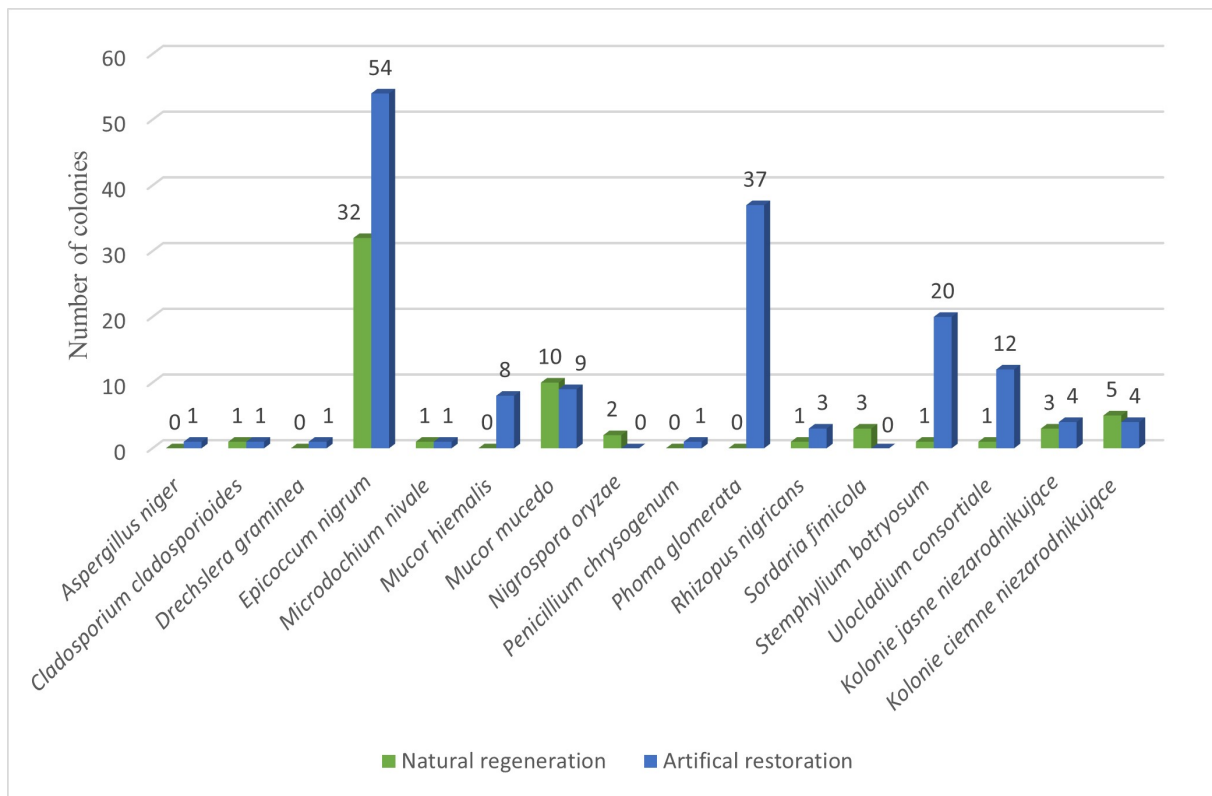


Figure 6 Abundance of other fungal species.

Table 1 Statistical analysis of biodiversity based on selected indicators.

	Margalef Index (MRI)	Shannon Index (SHDI)	Shannon Evenness Index (SEI)	Berger-Parker Index (BPI)
Natural regeneration	2.68	1.35	0.267	0.708
Artificial restoration	2.71	1.36	0.425	0.674

the antagonistic effect of *Trichoderma* spp. confirm this thesis (Smolińska et al., 2014; Smolińska & Kowalska, 2003). Studies on purple blotch of onions also confirmed the ability of *Trichoderma* spp. to compete with and ultimately restrict *Alternaria* spp. colonies (Abo-Elyousr et al., 2014). Gveroska and Ziberoski (2012), in a study on tobacco, showed that *Trichoderma* spp. has a strong reducing effect on *A. alternata*. The reason for this phenomenon may be that *Trichoderma* spp. colonies develop faster and more intensively. Harman (2006) found that *Trichoderma* spp. grows tropically towards the target fungi. The same relationship was confirmed in research by Pandey (2010). These studies too definitively showed that *Trichoderma* spp. is antagonistic to *Alternaria* spp. limiting and inhibiting the growth of these colonies by almost 67% (Pandey, 2010). The large number of *Trichoderma* spp. colonies seen in the study should be considered a positive phenomenon. *Trichoderma* spp. is described in the literature as a desirable antagonist. As a microorganism, it plays a significant role in the environment (Howell, 2003). Fungi of this species grow rapidly and also produce, metabolites that inhibit the growth of other pathogens, including fungi, and are thus referred to as mycoparasitic fungi (Mallams, 2004; Mańka et al., 1989, 2001; Mańka & Kacprzak, 1998;

Szwajkowska-Michalek et al., 2011). The above characteristics, as well as the ease of utilization of organic and inorganic compounds produced by *Trichoderma* spp., make it possess the characteristics of a good antagonist (Ropek et al., 2014).

The results from natural regeneration, illustrate more easily the antagonistic, positive effect of *Trichoderma* spp. As the number of colonies of this species increases, the number of *Fusarium* spp. and other, less numerous species decreases. The reduction in the growth of *Fusarium* spp. by *Trichoderma* spp. was confirmed in a study by Świerczyńska et al. (2011). Also, the greenhouse study by Mambaeva et al. (2019) confirms a strong inhibitory effect on other fungi, e.g., *Fusarium* spp. In both restorations, the high number of *Trichoderma* spp. left no space in the shoots for *Fusarium* spp. or *Alternaria* spp. to develop. The presence of *Trichoderma* spp. in such quantities means that there is no room in the environment for pathogenic fungi, indicating that the environment itself has characteristics that limit the growth of pathogens.

Fusarium spp. as pathogenic fungi leading to tree mortality co-occur on oak shoots. They are polyphages that infest oak trees ranging from annual to decades-old units. As pathogens, they are among the agents involved in the phenomenon of

oak dieback (Kowalski, 1996). *Fusarium oxysporum* is also considered the main culprit of seedling gangrene (Rudawska, 2000). *Fusarium* species classified as pathogens of *Quercus* spp. are mainly *F. oxysporum* (Gallego et al., 1999), *F. solani* (Bosso et al., 2012), and *F. avenaceum*. We can learn about the last of these on oaks from a study by Szynekiewicz and Kwaśna (2004), who isolated *F. avenaceum* from diseased shoots of *Q. rubra*, which showed signs of rot. The research in the Oława Forest District confirms the presence of the above *Fusarium* species on oaks, however, apart from these three, *F. sporotrichioides*, *F. dimerum*, *F. graminearum*, and four species: *F. equiseti*, *F. graminum*, *F. poae*, *F. sambucinum* in smaller quantities were the most numerous. Fungi of the type *Fusarium* were isolated, also from diseased oak shoots, in the study of Wit et al. (2015), confirming the presence of these pathogens on this tree species. Fungi of the genus *F. oxysporum* can also be found on other tree species. Their presence on the green Douglas fir *Pseudotsuga menziesii* was confirmed by Mousseaux et al. (1998). He also showed that *Trichoderma* spp. reduces mortality of Douglas-fir seedlings in container cultures. *F. oxysporum* can also infect tomatoes; again, inoculation with *Trichoderma* spp. can reduce the disease (Ślusarski, 2008).

Fungi of the genus *Alternaria* spp. are found worldwide. Many species are pathogens specialized to infect a variety of crops, orchards, ornamentals, or commercial plants (Ogórek et al., 2011). In Poland, the most common species is *Alternaria alternata* (Kimic et al., 2023). It is a polyphage that can be found on cereals and causes cereal black spot disease. It also appears on brassica and oilseed rape plantations (Ogórek et al., 2011). Research in the Oława Forest District has also confirmed its presence on oak shoots. *Alternaria* spp. colonize plants quite commonly, but their pathogenic properties are poor. *A. alternata*, *A. resedae* and *A. tenuissima* were observed in the experiment. The authors of the study also observed *Alternaria* spp. on oaks (Kowalski, 2006; Szynekiewicz & Kwaśna, 2004). Wit et al. (2015) confirmed with their study, data obtained from the Oława Forest District. Moreover, in their case, *Alternaria* spp. was also frequently found on oak shoots together with *Fusarium* spp. or *Cladosporium cladosporioides*. In a similar way, studies on ash by Kowalski and Łukomska (2005) showed the presence of *Alternaria* spp. on dead shoots. In a study by Szynekiewicz and Kwaśna (2004), *F. torulosum*, *F. avenaceum*, and *C. cladosporioides* were also present along with *A. alternata*. The last two also occurred with *A. alternata* in samples from the Oława Forest District. The study by Król et al. (2015) further mention the co-presence of *Epicoccum nigrum*, which was also present in samples from Bystrzyca.

It seems that further research into the correlation of co-occurrence between powdery mildew and other fungal species is necessary to gain an in-depth understanding of all species infecting oaks and to study their effects on this tree species.

5. Conclusion

Along with powdery mildew, fungi co-occur, which may contribute to oak mortality both in natural and artificial restoration.

Pathogenic fungi of the *Fusarium* spp. occurred in two regenerations, but were numerically dominant in the natural

restoration. Fungi of the type *Alternaria* occurred in two regenerations, but dominated the artificial restoration.

Antagonistic *Trichoderma* spp. fungi occurred in both study sites - natural and artificial restoration. However, they dominated the natural restoration. These fungi reduce the number of colonies of phytopathogenic fungi isolated from *Quercus* shoots.

The differences in the Margalef and Shannon index results for the two groups are small and from these results it can be concluded that the differences in biodiversity between natural and artificial restoration are imperceptible. Further research and analysis are needed to more accurately determine the biodiversity in both groups.

6. Supplementary material

The following supplementary material is available for this article:

Table S1. Number of fungi colonies isolated from oak shoots in natural and artificial regeneration in the Oława Forest District.

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