Control of Pathogen *Erysiphe alphitoides* Present in Forest Crops in Current Climatic Conditions

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Abstract: The production of oak seedlings in intensive crops involves the modification of natural conditions, namely the degree of humidity, through artificial irrigation, which favors the appearance of the pathogen *Erysiphe alphitoides*, responsible for the Oak Powdery Mildew (OPM) disease. Thus, it is necessary to identify new substances and technologies to control OPM. In this sense, new products approved by the European Union (EU) and Forest Stewardship Council (FSC) were identified, both synthetic and, a great novelty, biological (based on chito-oligosaccharides-oligogalacturonans: COS-OGA). In order to quantify the results, a correlation was made with climatic factors, by sampling data related to temperature and relative humidity with Data Logger devices. The obtained results suggest that OPM has a high virulence in the temperature range of 20 to 30 °C; at a relative humidity above 75%. The data obtained from the field experiments show that the synthetic products controlled OPM with an effectiveness between 70% and 95%, and the biological product behaved almost similarly, between 60% and 90%, which creates high opportunities for environmentally friendly control of forest pathogens.

Keywords: cryptogamic agents; fungicides; sessile oak; seedlings

1. Introduction

Phytopathology is the branch of plant biology that requires continuous improvement, even more so the forestry one, which has as its object the study of the diseases of forest plants. These, having a long life cycle, are constantly threatened by pathogens, which evolve and constantly adapt to environmental factors, plant immunity, as well as substances used for control [1,2].

The implementation of sustainable forestry, a wish of the current forestry policies, requires the production of a healthy and vigorous planting material, in the necessary assortment, quantity, and quality [3], which will grow well under the conditions of the current climate dynamics. Climate change affects temperatures, precipitation both as a temporal distribution and annual average values, as well as the frequency of extreme weather events, so they can have a significant effect on the production of seedlings, but also on the spatial distribution of diseases [4].

In the last two decades, due to climate change, which is a consequence of anthropogenic activities, diseases occurring in forest plant species have become more frequent, causing considerable damage [5–8].
Ghelardini et al. [8] suggest that in the future, the continuing large-scale perturbations due to human activities, including the compounded effects of different anthropogenic stressors, will probably have large and difficult-to-predict impacts on forest ecosystems, changing species composition and altering biogeochemical cycles and ecosystem dynamics. Under such uncertain conditions, other drivers of forest disease emergence, known or unknown at the present time, might become prevalent or arise in a new or different way.

Global trade has also acted as a vector for the transmission of pathogens to new locations, while changing land use and intensive forestry practices have had a large impact on increasing disease severity and even led to the awakening of some latent and invasive pathogens such as Hymenoscyphus fraxineus, Ophiostoma novo-ulmi, Cryphontectria parasitica, Neonectria faginata, Erysiphe alphitoides, etc. [9].

Meanwhile, introduced species can invade new areas, especially in the case of pathogens, and can cause significant damage to native species [10]. Diseases caused by invasive pathogens have detrimental effects on ecosystem functions and production through damage to each infected tree [11,12].

The spectrum of pathogens, being subject to evolution, as a result of adaptations to natural environmental conditions or with clearly anthropic influences, although it is mostly known, can never be limited to a certain moment, and its knowledge requires a permanent follow-up. But, parallel to this spectrum of diseases, there is a permanent development of the diversity of technical solutions, on the one hand, based on new, non-polluting, and non-toxic products, and on the other hand, through the use of cultural methods, advantageous from an economic point of view and effective in the fight against diseases [13].

Li et al. [14] suggest that in the theory of invasion ecology, invasiveness has been associated both with the biological traits of the invasive species and with environmental and community features in the naturalized range that render an ecosystem prone to invasion and define invasibility. Social and economic factors are crucial for species introduction, whereas biogeographical and ecological factors are important for naturalization, with evolutionary forces being key mediators of invasiveness.

The species-energy theory suggests that, in regions with higher biomass productivity, host–pathogen system stability and the pathogen-carrying capacity of an ecosystem are higher. Host species persistence is higher in areas with varied topographical and edaphic conditions, and large ecosystem diversification [15].

Most pathogens were introduced into new areas accidentally through imported infected plant material. Since the elimination of an introduced species is impossible once it has become acclimatized, it is imperative to take measures to control it [12].

One such pathogen is Erysiphe alphitoides (Griffon and Maubl.), which is of North American origin and was reported in Europe in 1905 in the Iberian Peninsula, and in 1907 in France [10].

The pathogen had a very severe impact, especially on seedlings, the degree of severity being different depending on the oak species. The most severely affected species was the so-called Pyrenean oak, Quercus pyrenaica (Quercus toza), for which high mortality rates were observed in southwestern and western France. Quercus robur was then reported to be more sensitive than Quercus petraea, while Mediterranean evergreen oaks, Quercus cerris, Quercus rubra, and Quercus palustris were considered resistant. After a few years, the disease caused by Erysiphe alphitoides was considered less dramatic and is now well established. Currently, the disease has taken over the entire territory occupied by oaks in the northern hemisphere.

The disease manifests itself mainly on shoots and leaves, on which typical symptoms of powdery mildew appear, from spring to late autumn. The attacked leaves are deformed, asymmetric, brittle, and covered by the ectoparasite mycelium. Towards autumn, on both sides of the leaf, cleistothecia appear in the form of small, black, grouped dots. The attacked shoots do not develop completely and freeze in winter [10,16].
In Romania, the pathogen *Erysiphe alphitoides* was reported in 1908, initially in the Teleajen district (today Prahova county), expanding in a short time to all oak cultures in Romania. Today, it is found in nurseries and forests, on all species of the *Quercus* genus, especially on young seedlings, where it can cause significant damage, through necrosis of young organs (leaves, stems), leading to drying of the plants if the attack is very strong. It also settles on mature trees, where damage is visible after successive long-term attacks. [17].

Long-term studies are needed to determine how plant pathogens evolve in response to the dynamics of environmental factors and human activities, and such studies are particularly important for managing the well-being of affected forests [18].

Complex research of a fundamental or applied nature regarding the production of seedlings has been carried out in most of the countries that produce forest seedlings, to respond to production requirements [9]. The aspects of the research aim to identify the factors that favor the production of the disease: causal pathogens, the environmental conditions that favor their appearance and spread, the susceptibility of forest species to pathogenic microorganisms in the soil, how the losses caused by the disease can be avoided or limited, through what means can increasing the resistance of the crops be achieved [19].

The negative impact of environmental factors on Romanian forests is similar to that described in most European countries. There are numerous situations in which the deterioration of the vegetation state of forest cultures, especially that caused by diseases in general and *Erysiphe alphitoides* in particular, has started to become frequent [20–24].

The production of forest seedlings is a constant need in forestry production to regenerate the forest fund both through plantations and by complementing natural regeneration. In intensive cultures from nurseries, where forest seedlings are produced, with the ensuring of optimal conditions for development, premises are also created for the development of infections with different pathogens, or infestations with various pests. It is thus necessary to apply both preventive (essential in the case of pathogens) and curative treatments [25,26].

Since certain products used to control pathogens no longer correspond to the new trends for environmental protection, it is necessary to carry out studies on the adaptability of pathogens to new climatic trends as well as the adaptation of new fungicides to the needs of forestry. These involve the combination of laboratory studies with those regarding the observation of pathogen behavior in the field [27,28].

The planting material must meet high quality standards to be able to be used in the development of quality, stable stands and to fulfill ecological and production functions. In the current context of international environmental protection policy, a wide range of chemical products intended to control and combat different pathogens in forest crops have been withdrawn due to the persistence of substances in the soil, or have been restricted by forest certification rules [29,30]. For this reason, for dangerous pathogens to be kept under control, it is necessary to test new products, already approved in the agricultural field, and to extend their approval for the control of diseases in the forestry field [31,32].

For example, the fungicides approved in agriculture for the control of biotic agents that produce powdery mildew are applied and act in different ways (demethylation inhibitors, carboxamides, aniline-pyrimidines, external quinone inhibitors, phthalimides, and inorganic sulfur, etc.) in forest crops; these fungicides encounter difficulties associated with the development of resistant fungal strains [33–35].

The aim of this study is to identify and promote in forestry practice some last-generation, synthetic, but especially biological, antifungal substances with high efficiency in the control of oak powdery mildew (OPM) in oak crops in nurseries in relation to the maximum efficiency from a phytosanitary, ecological, and economic point of view.

2. Materials and Methods

The experiments were located in the following:
(a) the Valea Iusului Nursery within the Lechinta Experimental Base;
(b) the Voivodeni Forestry Nursery, within the Reghin Forest District (Table 1, Figures 1 and 2).
2. Materials and Methods

The experiments were carried out in the following:

(a) the Valea Iușului Nursery within the Lechința Experimental Base;

(b) the Voivodeni Forestry Nursery, within the Reghin Forest District (Table 1, Figures 1 and 2).

Table 1. Geographical characterization elements of the experimental surfaces.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Coordinates</th>
<th>Altitude (masl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Valea Iusului Nursery</td>
<td>46.947683° N 24.243044° E</td>
<td>467</td>
</tr>
<tr>
<td>2</td>
<td>Voivodeni nursery</td>
<td>46.725072° N 24.605525° E</td>
<td>378</td>
</tr>
</tbody>
</table>

The experiments in the nurseries were carried out according to a methodology developed by the Forest Protection Collective of the National Institute for Research and Development in Forestry during the project PN190710205 (BIOSERV) and adapted in PN23090102 (FORCLIMSOC) in order to obtain results that can be applied in production [33,36]. The trials were based on the method of experimental blocks, in which the block is represented by repetition (4 repetitions being placed), and within each repetition seven variants were placed:

(i) a sessile oak culture (year 1), in the nursery Valea Iușului—INCDS Experimental Base of Lechința,

(ii) a pedunculate oak culture (year 2), in the nursery of Voivodeni—Forest District of Reghin (RNP-ROMSILVA).
Figure 2. Details of experimental surfaces (above satellite location, below ground details): (a), Valea Iușului nursery, (b), Voivodeni nursery, (source: Google Earth and original photo).

Three treatments in each culture were applied in the period 8 June–6 August 2023. Disease severity (DS) was estimated before and after the treatments, using an interval 0–4 scale system:
- **ua**—uninfected,
- **w**—low: small, isolated yellow–green to yellow spots on 1–24% of leaf surface,
- **m**—medium: large yellow spots, confluent on 25–74% of the leaf surface; these can also appear on non-lignified stems,
- **h**—high over 75% generalized infections on the leaves and on the tips of the stems in the form of a white powder, which lead to their deformation and drying [33].

The experimental variants for testing antifungal substances are the following:
- **V1**—proquinazid 20%, zero point one hundred twelve milliliters per zero point five liters of water (0.112 mL/0.5 L water);
- **V2**—azoxistrobin 200 g/L + difeconazol 125 g/L, zero point five milliliters per zero point five liters of water (0.5 mL/0.5 L water);
V3—12.5 g/L COS-OGA (chito-oligosaccharide-oligogalacturonan) one point thirty-three millilitres to zero point five liters of water (1.33 mL/0.5 L water);  
V4—proquinazid 20%, zero point zero eighty-four millilitres to zero point five liters of water (0.084 mL/0.5 L water);  
V5—azoxistrobin 200 g/L + difeconazole 125 g/L, zero point three hundred seventy-five millilitres to zero point five liters of water (0.375 mL/0.5 L water);  
V6—12.5 g/L COS-OGA (chito-oligosaccharide-oligogalacturonan) one milliliter to zero point five liters of water (1 mL/0.5 L water);  
V7M—control (untreated variant).

Proquinazid is a new active substance from the group of quinazolines acting specifically against powdery mildew on vines and cereals. This inhibits the formation of apressors and the germination of spores, also inducing the plant’s defense reaction. This action is long-lasting and residual, with the culture being protected for a period of 14–21 days (the persistence of the treatment is 6–8 weeks).

Azoxystrobin is an active substance that acts as an inhibitor of mitochondrial respiration, blocking the energy cycle of the pathogen. It intervenes in the biological cycle of the fungus, mainly during the germination of the spores and during the initiation of penetration into the plant tissues. It shows gradual uptake and local (translaminar) systemic activity in the leaves. Its activity is predominantly preventive.

Difenconazole, the other compound of the fungicide, has preventive and curative actions. It acts by blocking the biosynthesis of steroids in the cell membranes of fungi. Local systemic action and slight acropetal translocation.

The biological fungicide based on chito-oligosaccharides-oligogalacturonans acts by increasing the amounts of PR-proteins (peroxidases, chitinases, gluconases) and PAL (phenylaniline ammonium lyase). PAL is a key enzyme involved in the production of phytoalexins. Thus, cell walls are strengthened, proteins are synthesized and they help destroy pathogen cells, producing antimicrobial components (antibiotics produced by plants) [37–39].

After establishing the severity of the disease on the aforementioned scale, the degrees of attack were determined for each individual variant according to the Townsend–Heuberger formula: DS(%) = (Σ(n × v))/(N × V) × 100, where: n: the degree of infection established according to a certain scale; v: the number of individuals in the respective category; N: the total number of classes; V: the total number of individuals (Abbreviations) [40].

Based on disease severity calculated using the formula above, the effectiveness of the treatments was evaluated according to the Abbot formula E(%) = 100 − Z, Z = DSv × 100/DSc, where E: effectiveness; DSv: degree of attack, in the treated variants; DSc: degree of attack, in the control variants; Z: the ratio between the degree of attack in the treated and control variants [33].

For the processing and interpretation of the data, the analysis of variance (Anova: Two-Factor without Replication) and the limit difference test (DL) LSD—Least Significant Difference were used.

Data logger devices -Trotec BL 30 (Figure 3) were installed in the experimental plots, with the help of which hourly data on air humidity and temperature were recorded. In the nurseries, the data were recorded between 16 May and 5 September 2023.
3. Results

Control of Oak Powdery Mildew (OPM)

The initial application of the treatments was conducted in accordance with the climatic factors and the periodicity recommended by the producers, 7–10 days, the interval between them being adjusted according to the climatic conditions in the field, established with the help of Data Logger devices.

(a) In the Valea Iușului nursery, OPM disease severity was very low; in 21 June, medium-sized seedlings were observed infected in the control variant; in August, the disease progressed; and in September, medium and highly affected seedlings were observed in the V7M control, V6—12.5 g/L COS-OGA (chito-oligozaharide-oligogalacturonane) 1 mL/0.5 L water and V3—12.5 g/L COS-OGA (chito-oligozaharide-oligogalacturonane) 1.33 mL/0.5 L water (Figure 4).

The incidence of the disease was reported in all experimental variants, starting with the constant achievement of an average daytime temperature between 20 °C and 30 °C, respectively, with the occurrence of fluctuations in relative humidity between 70 and 90%.

Thus, from the moment of the first determination of the severity of the disease in June, until the one in August, the advance of the infection was observed, with the month of September being the peak of the severity of the disease.

In the period from June to September, the average temperature was over 23.4 °C degrees Celsius, and the relative humidity was 75.7%, the optimal conditions for the development of the pathogen being met (Figure 4).

Disease severity is well controlled by the treatments used; the least effective treatment options are those with lower doses: V4—proquinazid 20%, 0.084 mL/0.5 L water, V5—afoxystrobin 200 g/L + difeconazole 125 g/L, 0.375 mL/0.5 L water, and V6—12.5 g/L COS-OGA (chito-oligosaccharides-oligogalacturonans) 1 mL/0.5 L water, but these are also close in efficiency to the variants with the doses recommended by the manufacturers. The lowest efficiency was recorded in the V6 variant—12.5 g/L COS-OGA (chito-oligosaccharides-oligogalacturonans) 1 mL/0.5 L water, from repetition 3 (Figure 5).
**Figure 4.** OPM disease severity by daily air temperature and humidity in the Valea Iușului nursery (ua—uninfected, w—low: 1–24%, m—medium 25–74%, and h—high over 75%; the percentages mean the degree of mycelial coverage of the leaves).

**Figure 5.** Establishing disease severity and the effectiveness of treatments by variant in the Valea Iușului nursery.
After performing the analysis of variance and the LSD test, significant differences at the 0.05 level and significant differences at the 0.01 level were observed between the V6 variants and the rest of the variants (Table 2).

Table 2. Analysis of variance and the significance of the disease severity in treated variants on sessile oak culture from the Valea Iusului nursery.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-Value</th>
<th>F Crit</th>
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<table>
<thead>
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<th>Means</th>
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<th>V4</th>
<th>V5</th>
<th>V3</th>
<th>V1</th>
<th>V2</th>
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<tbody>
<tr>
<td>V6</td>
<td>4.25</td>
<td>1.10*</td>
<td>1.72**</td>
<td>1.77**</td>
<td>2.00**</td>
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<tr>
<td>V4</td>
<td>3.59</td>
<td>0.62</td>
<td>0.67</td>
<td>0.90</td>
<td>1.69**</td>
<td></td>
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<tr>
<td>V5</td>
<td>3.03</td>
<td>0.05</td>
<td>0.28</td>
<td>1.07*</td>
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<td>V3</td>
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<td>0.23</td>
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<td>1.02</td>
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<tr>
<td>V2</td>
<td>1.83</td>
<td></td>
<td></td>
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DL 5%—1.068, DL 1%—1.675, DL 0.1%—2.854. * significant differences at the 0.05 level; ** significant differences at the 0.01 level.

As was observed in the disease severity, and in the case of the effectiveness of the treatments, significant differences at the 0.05 level and significant differences at the 0.01 level were observed between the V6 variants and the rest of the variants (Table 3), this variant being treated with 12.5 g/L COS-OGA (chito-oligosaccharides-oligogalacturonans) 1 mL/0.5 L water, biological fungicides in the smallest dose.

Table 3. Analysis of variance on the effectiveness of treatments determined using the Abbot formula in sessile oak culture from the Valea Iusului nursery.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
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<td>Error</td>
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<td>Total</td>
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<table>
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<td>V2</td>
<td>92.01</td>
<td>3.19</td>
<td>3.77</td>
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<td>V3</td>
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<td>V5</td>
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<td>7.66**</td>
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<td>V4</td>
<td>85.33</td>
<td>5.19</td>
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DL 5%—4.488, DL 1%—7.039, DL 0.1%—11.992. * significant differences at the 0.05 level; ** significant differences at the 0.01 level.

In the nursery Valea Iusului the attack of the pathogen recorded a maximum after the middle of August (Figures 6 and 7).
Table 3. Analysis of variance on the effectiveness of treatments determined using the Abbot formula in sessile oak culture from the Valea Iușului nursery.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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V2: *Significant differences at the 0.05 level; **Significant differences at the 0.01 level.

In the nursery Valea Iușului, the attack of the pathogen recorded a maximum after the middle of August (Figures 6 and 7).

Figure 6. Uninfected sessile oak seedlings during May 2023 in the Valea Iușului Nursery.

Figure 7. Medium and high affected seedlings during late August 2023 in the Valea Iușului Nursery.
(b) In the Voivodeni nursery, infected seedlings have been observed since June, the disease severity being an average one. On 6 June, seedlings from low to highly affected were identified in all variants, with V7M control being most medium and highly infected. In August, the disease progressed, and in September, a maximum percentage of moderate seedlings was recorded, and in variants Vb3—12.5 g/L COS-OGA (chito-oligozaharide-oligogalacturonane), V4—proquinazid 20%, Vb6—12.5 g/L COS-OGA (chito-oligozaharide-oligogalacturonane), and V7M control, highly affected seedlings were also observed (Figure 8).

As in the case of the Valea Iușului nursery, in the oak plot of the Voivodeni nursery, the occurrence of the disease was favored by the average temperature above 20 °C and the relative humidity which is maintained above 75% starting from June until the middle of the month August (Figure 8).

As in the case of the sessile oak culture in the Valea Iușului nursery and in the Voivodeni nursery, disease severity is kept well under control by the treatments used; the least effective treatment options are those with lower doses V4—proquinazid 20%, 0.084 mL/0.5 L water, V5—azoxystrobin 200 g/L + difeconazole 125 g/L, 0.375 mL/0.5 L water, and V6—12.5 g/L COS-OGA (chito-oligosaccharides-oligogalacturonans) 1 mL/0.5 L water, the percentage difference in the efficiency of the treatments compared to the versions with the doses recommended by the manufacturers reaching up to 30%. The lowest efficiency was recorded in variant V4—proquinazid 20%, 0.084 mL/0.5 L water, from repetition 3 (Figure 9).

After performing the analysis of variance and the LSD test, significant differences at the 0.05 level and significant differences at the 0.001 level were observed between variants V2, V3, and V6 and the rest of the variants (Table 4).
After performing the analysis of variance and the LSD test, significant differences at the 0.05 level and significant differences at the 0.001 level were observed between variants V2, V3, and V6 and the rest of the variants (Table 4).

Table 4. Analysis of variance on the disease severity in treated variants on oak culture from the Voivodeni nursery.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-Value</th>
<th>F Crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rows</td>
<td>417.47</td>
<td>5</td>
<td>83.49</td>
<td>13.96</td>
<td>0.000034</td>
<td>2.90</td>
</tr>
<tr>
<td>Columns</td>
<td>228.27</td>
<td>3</td>
<td>76.09</td>
<td>12.72</td>
<td>0.000212</td>
<td>3.29</td>
</tr>
<tr>
<td>Error</td>
<td>89.69</td>
<td>15</td>
<td>5.98</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>735.44</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variants</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>V6</td>
<td>13.40</td>
</tr>
<tr>
<td>V4</td>
<td>11.81</td>
</tr>
<tr>
<td>V5</td>
<td>11.21</td>
</tr>
<tr>
<td>V1</td>
<td>4.21</td>
</tr>
<tr>
<td>V3</td>
<td>3.90</td>
</tr>
<tr>
<td>V2</td>
<td>3.64</td>
</tr>
</tbody>
</table>

DL 5%—3.629, DL 1%—5.692, DL 0.1%—9.697. ** significant differences at the 0.01 level; *** significant differences at the 0.001 level.

As was observed in the disease severity, and in the case of the effectiveness of the treatments, significant differences at the 0.05 level and significant differences at the 0.001 level were observed between variants V4, V5, and V6 and the rest of the variants (Table 5).

The main cause of the differences recorded between the variants in the Voivodeni nursery is due to the pedunculate oak, which, according to the specialized literature, is more sensitive to OPM.

In the nursery Voivodeni, the strong attack manifested itself from the beginning of August (Figures 10 and 11).
Table 5. Analysis of variance on the effectiveness of treatments determined using the Abbot formula in oak culture from the Voivodeni nursery.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-Value</th>
<th>F Crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rows</td>
<td>3972.08</td>
<td>5</td>
<td>794.42</td>
<td>62.66</td>
<td>1.62378 × 10⁻⁹</td>
<td>2.90</td>
</tr>
<tr>
<td>Columns</td>
<td>140.35</td>
<td>3</td>
<td>46.78</td>
<td>3.69</td>
<td>0.035942983</td>
<td>3.29</td>
</tr>
<tr>
<td>Error</td>
<td>190.16</td>
<td>15</td>
<td>12.68</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4302.59</td>
<td>23</td>
<td>140.35</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Variants Means**

<table>
<thead>
<tr>
<th>Variants</th>
<th>Means</th>
<th>V2</th>
<th>V3</th>
<th>V1</th>
<th>V5</th>
<th>V4</th>
<th>V6</th>
</tr>
</thead>
<tbody>
<tr>
<td>V2</td>
<td>88.78</td>
<td>87.04</td>
<td>1.74</td>
<td>1.90</td>
<td>22.20*</td>
<td>27.21***</td>
<td>30.08***</td>
</tr>
<tr>
<td>V3</td>
<td>87.04</td>
<td>0.16</td>
<td>20.46***</td>
<td>25.47***</td>
<td>28.34***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V1</td>
<td>86.88</td>
<td></td>
<td>20.3***</td>
<td>25.31***</td>
<td>28.18***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V5</td>
<td>66.58</td>
<td>5.01</td>
<td></td>
<td>7.88*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V4</td>
<td>61.57</td>
<td></td>
<td></td>
<td>2.87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V6</td>
<td>58.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DL 5%—5.285, DL 1%—8.288, DL 0.1%—14.120. * significant differences at the 0.05 level; *** significant differences at the 0.001 level.

Figure 10. Uninfected oak seedlings during May 2023 in the Voivodeni nursery.

Figure 11. Medium and high affected oak seedlings at the beginning of August 2023 in the Voivodeni nursery.
4. Discussion

The nurseries are located in different natural areas, with varying influences on the development of the seedlings. The pathogens are directly influenced by the microclimates found in the nursery locations.

If the Voivodeni nursery is located in a meadow area, where the atmospheric humidity is relatively uniform throughout the growing season, the Valea Iusului nursery is located in a wooded area, where the relative humidity is quite varied, and some trees in proximity can be considered vectors of transmission of pathogens.

Seedlings obtained in nurseries are affected by pathogens due to the large number per surface unit; therefore, they must be constantly monitored and phytosanitary control measures applied to obtain the necessary quality and quantity [41–43].

The control measures used in the past involved the use of products that had good efficiency, but had a high retention in the soil. For this reason, at present, they are being withdrawn from forestry production; therefore, it is necessary to test new substances to obtain seedlings in good quality and in the quantities necessary to ensure the continuity of the forest fund [44].

Oak forest crops in nurseries are annually affected by the pathogen *Erysiphe alphitoides* that causes the “oak powdery mildew” disease, especially at the beginning of the growing season, when the leaves of the plants are in the development period, and the tissues directly exposed to the external environment are in the forming phase [10,45].

In the available specialized literature, starting with Hewitt in 1974, the correlation between the effects of temperature and relative humidity on mycelial development is described, with 25 °C and 96% relative humidity. Mycelial growth was observed at low relative humidity values of only 32% in the temperature range of 15–25 °C [46]. Pap et al. [47] obtained results similar to those obtained by Hewitt [46], concluding that a combination of favorable temperature and optimal humidity is of great importance in the epidemiology of the pathogen.

Also, Karadžić and Milijašević [48] observed that the strongest development is in the range of 20–30 °C and a relative humidity of 76–96%, and Glavaš [49] pointed out that powdery mildew spread requires moderate temperature, favorable relative humidity, light, and developing leaves.

According to Ramut and Pusz [12], rain is another factor that, on the one hand, favors the development of powdery mildew and, on the other hand, 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. Various studies 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 [12].

The use of fungicides to control the pathogen *Erysiphe alphitoides* has been limited in the last decade by the new EU and FSC environmental regulations; therefore, it is necessary to test and use approved fungicides used in other fields, such as agriculture, fruit growing, or viticulture [50], which, on the one hand, should be effective in combating “oak powdery mildew”, and on the other hand, have a reduced impact on the environment [51–55].

At this moment, the synthetic active substances azoxystrobin, difenoconazole, and proquinazid [56,57] are used worldwide in powdery mildew control, and COS-OGA (chitos-oligosaccharides-oligogalacturonans) is among the non-polluting, biological fungicides [58] that can be used in the future in accordance with new regulations of environmental protection. Little is known about the effects of the organic fungicides applied to forest seedling production. The most common products that come close to biological ones are based on copper (Cu) and sulfur (S). Definitely, the replacement or reduction in chemical inputs by using sustainable and eco-friendly compounds might lead to benefits for the microbial communities in soils and for future seedling generations [59–61].
The use of different preparations called biostimulants against oak powdery mildew has been reported with different effects. Buraczyk et al. [62], used a preparation based on humic acids and chitosan polymers to reduce oak powdery mildew infestation but did not affect the growth and development of the seedling itself. In another study, Tkaczyk et al. [63] confirmed that the use of phosphite-based preparations also reduced the infestation of oak seedlings by *Erysiphe alphitoides*, although it was a side effect of the research conducted.

In this regard, as demonstrated by the present study, COS-OGA is able to reduce production losses and at the same time maintain the main characteristics of seedlings. According to the available information, this is the first use of COS-OGA to control powdery mildew or any other diseases in forestry. Its biological formulation and large spectrum against pathogens open new ways into the forest protection system.

5. Conclusions

The analyzed climatic factors, namely temperature and relative humidity, directly influence plant growth, but at the same time, influence and trigger the virulence of pathogens.

The experimental variants were treated when the initial situation was very favorable and the application of the first treatments took place when the infection did not appear. The infections caused by *Erysiphe alphitoides*, evaluated after the application of the last treatment, in the oak culture are weak, the percentages of medium and heavily attacked saplings being below 10%, compared to the control variants, where the percentage is over 50%, and in the case of oak are medium, 20–40%, compared to the control variant, where this percentage is over 70%.

Data obtained on the initiation of infection with the pathogen *Erysiphe alphitoides*, correlated with climatic factors, show that the infection spreads at a temperature in the range of 20 and 30 °C and a relative humidity above 75%, when it is necessary to apply the treatments at an interval of 7–10 days.

All tested fungicides registered significant differences compared to the untreated control, the best results being recorded in the V2 variant—azoxystrobin 200 g/L + difeconazole 125 g/L, 0.5 mL/0.5 L water. Also, the biological fungicide COS-OGA had a very close efficiency to that of the synthetic fungicides in the case of the treatments on both oak and sessile oak seedlings.

The use of synthetic fungal products and, in particular, biological ones, will have a direct impact on the production of oak seedlings from forest crops, which is reflected in avoiding losses due to the inability of seedlings to lignify.

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**Abbreviations**

EU European Union
FSC Forest Stewardship Council
OPM oak powdery mildew
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