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# Effect of origin and morphological characteristics of sessile oak (*Quercus petraea*) seedlings on the development of *Cryphonectria parasitica*

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**The presence of *Cryphonectria parasitica* on sessile oak (*Quercus petraea*) requires comprehensive research to understand how this fungus spreads. An experiment was conducted to investigate the impact of *C. parasitica* on different sessile oak stands. The experiment involved inoculating 2-year-old seedlings from 12 different half-sib lines. After 50 days, the results showed a significant difference in dieback, mortality, and dimensions of necrotic lesions among the 2-year-old sessile oak seedlings from different half-sib lines. Mortality dieback rates varied from 20% to 80% across the different half-sib lines. Seedlings from one half-sib line (8.33%) had the smallest length of necrotic lesions and the lowest dieback percentage, while seedlings from four half-sib lines (33.33%) had the narrowest width of necrotic lesions. Unlike other seedlings, the half-sib lines with smaller necrotic lesions did not experience mortality. Sessile oak seedlings' smaller diameter and height influenced the development of narrower necrotic lesions. For the first time, this study demonstrated the potential for selecting mother trees to produce sessile oak seedlings tolerant to *Cryphonectria parasitica*. It also suggested that seedling dimensions can be used to indicate their sensitivity to the fungus.**

**Keywords: Chestnut Blight Fungus, Different Hosts, Tolerance, Screening**

## Introduction

The invasive fungus *Cryphonectria parasitica* (Murr.) Barr, which originated in East Asia, is a bark pathogen affecting American chestnuts (*Castanea dentata* (Marsh.) Borkh.) and sweet chestnuts (*Castanea sativa Mill.*). This fungus causes one of the most significant diseases in forestry (Rigling & Prospero 2018). No specific ecological or climatic factors limit the spread of *Cryphonectria parasitica* to new areas within the EU (EFSA/PLH 2014). On the other hand, an increase in precipitation in spring and autumn is positively correlated with a more significant amount of inoculum formed by the fungus (Lione et al. 2022). Other species from the family *Cryphonectriaceae* have far less significance (Gryzenhout et al. 2009, Dennert et al. 2020).

The fungus *Cryphonectria parasitica* can be found in all known species of chestnut but causes the most significant damage to the American chestnut *Castanea dentata*. In addition to the American chestnut, other hosts of *Cryphonectria parasitica* include 33 species and 10 genera from the following families: *Aceraceae, Anacardiaceae, Betulaceae, Corylaceae, Fagaceae, Juglandaceae, Magnoliaceae* and *Myrtaceae* (Nash & Stambaugh 1982, Roane et al. 1986, Old & Kobayashi 1988, Turchetti et al. 1991, Dallavalle & Zambonelli 1999).

The development of *Cryphonectria parasitica* symptoms in American chestnuts and sweet chestnuts is characterized by necrotic lesions, which are bark cankers that ultimately lead to mortality (Rigling & Prospero 2018). Anatomical changes and reduced fiber cell traits were found in sweet chestnut trees infected with *Cryphonectria parasitica* compared to healthy trees (Özden Keleş et al. 2024). Sessile oak trees (*Quercus petraea* [Matt.] Liebl.) and pedunculate oak (*Quercus robur* L.) sustain less damage compared to sweet chestnut and northern red oak (*Quercus rubra* L.), but these tree species are still considered very sensitive (Tarcali et al. 2009, Radócz et al. 2010).

The high pathogenicity of *Cryphonectria parasitica* compared to other members of the *Cryphonectriaceae* family may be due to the lack of genes related to carbohydrate metabolism (Stauber et al. 2020). Oxalic acid, a key metabolite of *Cryphonectria parasitica*, degrades host cells (Havir & Anagnostakis 1983). During the initial stages of infection, certain enzymes known as tanases impact the host, while there is limited data on the roles of laccases and polygalacturonases (Lovat & Donnelly 2019). Also, cryparin, cutinases, cellulases, and endothiapepsin are potentially indirect pathogenesis factors (Lovat & Donnelly 2019). Changes in metabolism induced by vegetative incompatibility of different types of *Cryphonectria parasitica* can produce secondary metabolites (Witte et al. 2021).

The occurrence of hypovirulence is most often reflected in the slowing down of viru-

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**Tab. 1** - Geographic characteristics of analyzed population.

| | |
|---|---|
| Population | Avala |
| Exposure | S |
| Latitude | 44° 41′ 11″ |
| Longitude | 20° 30′ 51″ |
| Elevation (m a.s.l.) | 410 |

lence and phenotypic changes of *Cryphonectria parasitica*, *i.e.*, the weakening of the fungus, which leads to the reduction in the rate of canker spread (Rigling & Prospero 2018). The natural spread of hypovirulence is influenced by several factors, including the degree of host sensitivity, the expression of hypovirulence, the possibility of virus transmission, and the diversity of vegetatively compatible types of *C. parasitica* (Rigling & Prospero 2018). In addition to these factors, the orientation of the terrain (western exposure) and the appearance of other organisms, such as insects, crawlers, and mites, also contribute to the natural spread of hypovirulence (Çeliker et al. 2017, Rigling & Prospero 2018).

The viruses commonly used for controlling chestnut canker belong to the *Hypoviridae* family (Nuss et al. 2005). The known types include CHV-1, CHV-2, CHV-3, and CHV-4. Among these, the EP713 (CHV-1/EP713) isolate is the most investigated and utilized, serving as the foundation for *Cryphonectria parasitica* biocontrol in Europe. Aside from EP713, various CHV-1 subtypes with different levels of virulence have also been found (Allemann et al. 1999). In the early 1990s, two viruses of the new *Mycoreovirus* genus (*Reoviridae* family) were discovered, with *Cryphonectria* mycoreovirus 1, strain 9B21, causing reduced virulence without affecting pigmentation and sporulation processes (Enebak et al. 1994). Additionally, viruses from the *Partitiviridae* and *Crysoviridae* families have been identified in *Cryphonectria parasitica*, but their characteristics are still not fully understood (Hillman & Suzuki 2004).

When studying *Cryphonectria parasitica*, focusing on the sessile oak is essential. Sessile oak is one of the main species in mixed deciduous forests (Eaton et al. 2016), and its presence can lead to the potential spread of *Cryphonectria parasitica* into environmentally similar areas, increasing the infection pressure on sweet chestnuts, especially because the appearance of chestnut blight symptoms on sweet chestnuts can affect up to 98% of sampled trees in sweet chestnuts stands that are located near the sessile oak stands (Karadžić et al. 2019). Moreover, the successful restoration of sessile oak forests relies on the initial number of seedlings during the restoration process (Kanjevac et al. 2021).

In previous research on the development of *Cryphonectria parasitica* on sessile oak, variability in the pathogenicity of isolates was observed (Karadžić et al. 2019). As a continuation of this research, it is necessary to determine the role of the sessile oak origin and morphology, starting from early growth, on developing symptoms caused by *Cryphonectria parasitica*. The results will enable to obtain: (i) a more precise selection of sessile oak mother trees for natural regeneration or production of sessile oak reproductive material; (ii) a better prognosis of the development of *Cryphonectria parasitica* symptoms based on the observed dimensions of the sessile oak seedlings in the field. Tolerance individuals should be used to reduce the damage caused by *Cryphonectria parasitica* to sessile oak trees. It is essential to take all necessary measures to protect against the spread of *Cryphonectria parasitica*, including creating tolerant populations of different trees species. The null hypotheses tested in this study were: (i) mother trees do not influence the development of symptoms in two-year-old seedlings of sessile oak caused by *Cryphonectria parasitica*; (ii) the dimensions of the two-year-old sessile oak seedlings cannot be used as an indicator of their tolerance to *Cryphonectria parasitica*.

**Tab. 2** - Dimensions of two-year-old sessile oak seedlings inoculated with *Cryphonectria parasitica*. (H): average height; (σH): standard deviation for average height; (D): average diameter; (σD): standard deviation for average diameter.

| Half-sib line | H (mm) | $\sigma_H$ | D (mm) | $\sigma_D$ |
|---|---|---|---|---|
| 1 | 891.25 | 284.51 | 11.19 | 2.34 |
| 2 | 662.00 | 161.02 | 7.04 | 3.69 |
| 3 | 568.25 | 285.37 | 10.31 | 2.87 |
| 4 | 707.14 | 382.86 | 11.37 | 3.67 |
| 5 | 652.86 | 320.24 | 10.08 | 3.79 |
| 6 | 507.00 | 191.27 | 10.36 | 3.61 |
| 7 | 686.54 | 281.49 | 9.69 | 2.56 |
| 8 | 631.54 | 305.42 | 8.38 | 2.50 |
| 9 | 666.67 | 331.81 | 8.35 | 3.07 |
| 10 | 493.00 | 210.66 | 9.35 | 3.29 |
| 11 | 675.45 | 289.91 | 9.65 | 1.88 |
| 12 | 507.78 | 191.49 | 7.97 | 1.15 |

## Material and method

### Plant material

A total of 212 two-year-old sessile oak seedlings were used for the experiment. These seedlings were obtained from mother trees in the extraordinary landscape of "Avala" (see Tab. 1 for details). In July 2020, 12 superior genotype trees were chosen for the study. Subsequently, 1 kg of acorns was harvested from each tree in the fall of 2020. The collected acorns were then transported to the seedling nursery at the Institute of Forestry in Belgrade, Serbia. In the spring of 2021, the acorns were planted in 1.5-liter plastic pots filled with black and white peat (Freepeat, The Netherlands). The seedlings received regular care and protection measures throughout the first and second growing seasons. They were watered 2-3 times a week during the growing season, and weeds were removed manually. All seedlings' root collar diameter and height were measured before and after the experiment. The root collar diameter was measured using a vernier caliper with an accuracy of 0.1 mm, and the seedling height was measured using a ruler with an accuracy of 0.1 cm.

### Inoculation of sessile oak seedlings

In the summer of 2023, in the nursery of the Institute of Forestry in Belgrade, 128 sessile oak seedlings from 12 half-sib lines were inoculated with *Cryphonectria parasitica* (see Tab. 2), while 84 seedlings were used as control subjects. The inoculation process involved introducing *Cryphonectria parasitica* VC type EU-12 isolates under the bark of two-year-old sessile oak seedlings. This particular isolate was obtained from the mycological collection of the Institute of Forestry in Belgrade, and it was initially isolated from a sweet chestnut in the city of Vranje, Serbia. The tested strain of *Cryphonectria parasitica* was selected due to its natural occurrence on sessile oak and its expressed pathogenicity in earlier research (Karadžić et al. 2019). The cultures were grown for ten days on malt extract agar nutrient medium (MEA, 30 g $L^{-1}$ malt, Biolab, Hungary; 20 g $L^{-1}$ technical agar, Torlak, Serbia). Bark openings were made with a sterilized scalpel at the same height in all seedlings, and sections of cultures approximately 5 × 5 mm were placed. The control group was inoculated similarly, using a sterile MEA nutrient medium. All inoculation sites were wrapped with Parafilm® M and covered with aluminum foil.

The experiment lasted 50 days, during which all the inoculated seedlings developed necrotic lesions or dieback. At the end of the experiment, the seedlings were

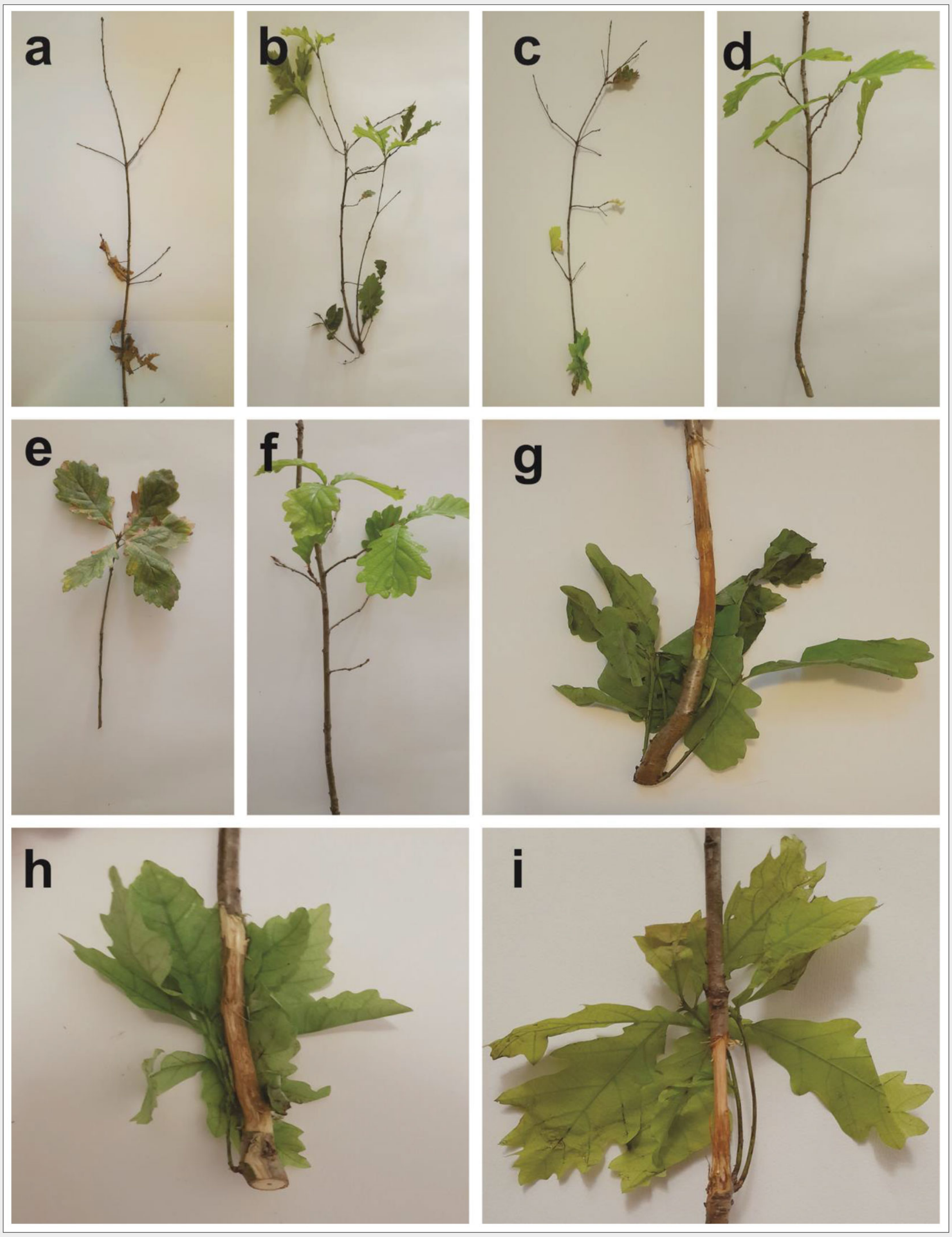


**Fig. 1** - Representative symptoms on two-year-old sessile oak (*Quercus petraea*) seedlings caused by *Cryphonectria parasitica.* (a,c): dieback and mortality of half-sib lines I and II; (b,d): control of half-sib lines I and II; (e): dieback of half-sib lines VI; (f): half-sib line control VI; (g): necrotic lesions and epicormic shoots of half-sib line I; (h): necrotic lesions and epicormic shoots of half-sib line II; (i): necrotic lesions and epicormic shoots of half-sib line VI.

cut at the level of the root collar. The bark was peeled, and the dimensions of the necrotic lesions were measured using a measuring tape. Necrotic lesions were identified by observing cambium discoloration, while dieback was determined by visually assessing canopy discoloration or defoliation. Mortality was defined as the significant loss of canopy and the presence of dry wood caused by pronounced necrotic lesions.

Re-isolations were attempted from all tested seedlings. The seedlings were cut into round sections and parts with conspicuous necrotic lesions were chosen. These parts were first sterilised on the surface by wiping with 96% alcohol and 3-5 s exposure to flame of tissue and remaining healthy bark that was not removed while measuring necroses. Then the bark was completely peeled off and the procedure was repeated again. Afterwards, these sections were placed on 3% MEA nutrient medium.

### *Statistical methods*

The effects of half-sib line, seedling height, and diameter on the length and width of necrotic lesions were tested using standard multiple regression. A general linear model (GLM) with Tukey's HSD *post hoc* test was used to compare the length and width of necrotic lesions across different half-sib lines. Homoscedasticity and linearity of the data for standard multiple regression and GLM were checked based on the dot plot. The normality of the residuals was determined using the Lilliefors-corrected Kolmogorov-Smirnov test.

The correlation between seedling and necrosis dimensions was analyzed using Spearman's rank correlation. This was done in cases where multiple regression indicated a significant influence of height and diameter on the development of the length or width of necrotic lesions. Additionally, binary logistic regression was employed to investigate the effects of half-sib line, seedling height, seedling diameter, and necrotic lesion dimensions on the occurrence of epicormic shoots.

The chi-square test for homogeneity and proportions was used to compare the occurrence of dieback and re-isolations among different half-sib lines. A chi-square test of independence was conducted to assess the relationship between mortality and tolerance of the half-sib lines. The fre-

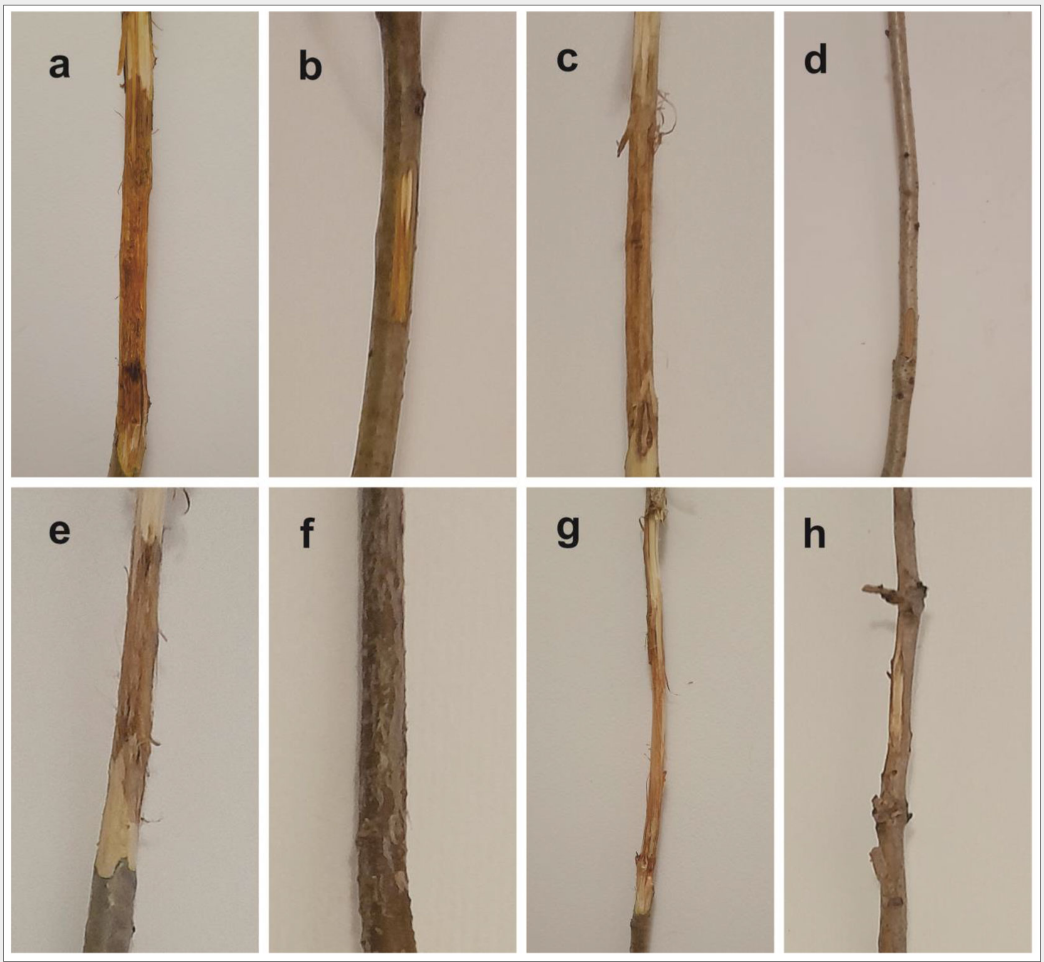


**Fig. 2** - Dimensions of necrotic lesions on two-year-old sessile oak (*Quercus petraea*) seedlings caused by *Cryphonectria parasitica*. (a): the greatest length of necrotic lesions on half-sib line I; (b): control of half-sib line I; (c): the greatest length of necrotic lesions on half-sib line II; (d): half-sib line control II; (e): the smallest length of necrotic lesions on the half-sib line VI; (f): half-sib line control VI; (g): the smallest width of necrotic lesions on the half-sib line VII; (h): control of the half-sib line VII.

quency of epicormic shoot occurrence among different half-sib lines was not analyzed due to its low percentage and absence in certain lines.

All statistical analyses were performed using the software package SPSS® ver. 27 (IBM, Armonk, NY, USA).

## Results

All seedlings inoculated with *Cryphonectria parasitica* developed necrotic lesions – cankers (Fig. 1, Fig. 2). However, among the twelve half-sib lines tested, the incidence of dieback was in the range 20% to 80% and the occurrence of epicormic shoots varied from 0% to 60% in seedlings inoculated with *Cryphonectria parasitica* (Fig. 1). The seedlings inoculated with *Cryphonectria parasitica* within seven half-sib lines (58.33%) exhibited mortality. The control group did not develop pronounced necrotic lesions, dieback, mortality or epicormic shoots (Fig. 1).

In the multiple regression analysis, we found that the length of the necrotic lesions was statistically significantly influenced by the seedling's affiliation to the specific half-sib line, as indicated in Tab. 3. Additionally, the width of the necrotic lesions was affected by the half-sib line, as well as the diameter and height of the seedlings, as shown in Tab. 4. The seedlings' diameter ($r_s$ = 0.500, $p < 0.001$) and height ($r_s$ = 0.498, $p < 0.001$) were positively correlated with the width of necrotic lesions. However, after conducting binary logistic regression, it was determined that the half-sib line, seedling dimensions, and necrotic lesion dimensions did not significantly influence the occurrence of epicormic shoots (Tab. 5).

According to the chi-square test results, a significant difference was found in the proportion of dieback among different half-sib lines (refer to Tab. 6). Half-sib line VI exhibited a smaller percentage of visible symptoms in the canopy, meaning it had a smaller proportion of dieback compared to the other half-sib lines (Tab. 6, Fig. 1a-f).

There were significant differences in the length and width of necrotic lesions among different half-sib lines as determined by the GLM (Tab. 6). According to Tukey's HSD test, half-sib line VI exhibited the smallest length of necrotic lesions, while half-sib lines I and II showed the most extensive lengths (see Tab. 6, Fig. 1g-i, Fig. 2a-f). Additionally, half-sib lines V, VII, IX, and X had the smallest widths of necrotic lesions (Tab. 6, Fig. 2g). The widths of necrotic lesions were similar in the other half-sib lines, as indicated by Tukey's HSD test (Tab. 6, Fig. 2a, Fig. 2c, Fig. 2e).

The experiment found a strong correlation between seedling tolerance and mortality (Chi-square = 25.377, $p < 0.001$). Tolerant half-sib lines (V, VI, VII, IX, and X) did not experience any mortality during the experiment. However, sensitive half-sib lines had mortality rates ranging from 10% to 50% (Fig. 1a, Fig. 1c).

**Tab. 3** - The effect of the half-sib line and morphological characteristics of sessile oak seedlings on the length of necrotic lesions caused by *Cryphonectria parasitica.*

| Model | Unstandardized Coefficients | | Standardized Coefficients | *t* | Prob. |
|---|---|---|---|---|---|
| | B | Std. Error | Beta | | |
| (Constant) | 54.465 | 4.931 | - | 11.045 | <0.001 |
| Half-sib line | -0.804 | 0.371 | -0.191 | -2.170 | 0.032 |
| Diameter | -0.311 | 0.510 | -0.069 | -0.609 | 0.544 |
| Height | 0.008 | 0.006 | 0.169 | 1.495 | 0.138 |

## Discussion

This study demonstrated how sessile oak seedlings' origin and size impact the *Cryphonectria parasitica's* development. As a result, the first and second null hypotheses were rejected, and the following alternative hypotheses were accepted: (i) mother trees influence the development of symptoms in two-year-old seedlings of sessile oak caused by *Cryphonectria parasitica*; (ii) the dimensions of two-year-old sessile oak seedlings can indicate their tolerance to *Cryphonectria parasitica*. The research on the *Cryphonectria parasitica* development was carried out on seedlings of the same age, which is why this factor is excluded as significant for the effects of inoculation, that is, the existence of differences between seedlings. The selected seedlings from tolerant sessile oak half-sib lines are a promising starting point for further research on their mass production and use in restoring sessile oak forests. Sessile oaks are vulnerable to numerous species of parasitic fungi (Fodor & Hâruţa 2016, Karadžić et al. 2017), as well as bacterial diseases, which can be mistaken for the effects of abiotic factors (Tkaczyk 2023). Additionally, several species of the *Phytophthora* genus can lead to decay in sessile oak trees (Milenković 2015, Jung et al. 2016, Tkaczyk et al. 2020). Minimizing damage to young sessile oak trees helps reduce the harm caused by mentioned organisms.

Using sessile oak seedlings with a smaller habitus has additional benefits in controlling *Cryphonectria parasitica*. More considerable hail-induced injuries have increased the likelihood of *Cryphonectria parasitica* infections (Lione et al. 2020). A smaller seedling habitus reduces the formation of large injury areas, making surface disinfection measures more effective, as demonstrated in sweet chestnuts (Akilli Şimşek et al. 2020). Also, drought conditions have an effect similar to hail, namely, they increase the occurrence of *Cryphonectria parasitic* infections (Prospero & Rigling 2013). In drought conditions, oak trees do not benefit from the slower growth of beech trees (*Fagus sylvatica* L.), often found in the same stands. Therefore, the smaller habit of the sessile oak may be adaptable to drought. In conditions where high productivity of this species is not expected, one should consider using seedlings of smaller diameter and height. This is crucial as significant water deficits can lead to more severe defoliation in sessile oak compared to other oak species in similar conditions (Kumbasli et al. 2017).

The health of sessile oak populations varies depending on their origin and geographic region (Trenyik et al. 2018). Therefore, it is necessary to influence the formation of healthy trees in the early stages of the stand development, which will have an appropriate lifespan in areas more at risk of decay. The study of the natural regeneration of sessile oak forests is faced with a lack of knowledge of all factors, which

**Tab. 4** - The effect of the half-sib line and morphological characteristics of sessile oak seedlings on the width of necrotic lesions caused by *Cryphonectria parasitica.*

| Model | Unstandardized Coefficients | | Standardized Coefficients | *t* | Prob. |
|---|---|---|---|---|---|
| | B | Std. Error | Beta | | |
| (Constant) | 9.897 | 1.658 | - | 5.968 | <0.001 |
| Half-sib line | -0.361 | 0.125 | -0.219 | -2.900 | 0.004 |
| Diameter | 0.517 | 0.172 | 0.290 | 3.012 | 0.003 |
| Height | 0.005 | 0.002 | 0.242 | 2.503 | 0.014 |

**Tab. 5** - The influence of half-sib line, morphological characteristics, and the development of necrosis of sessile oak seedlings on the occurrence of epicormic sprouts caused by *Cryphonectria parasitica.*

| Source | Wald Chi-Square | Type III df | Prob. |
|---|---|---|---|
| Intercept | 0.000005 | 1 | 0.998 |
| Half-sib line | 11.382 | 11 | 0.412 |
| Height | 0.633 | 1 | 0.426 |
| Diameter | 0.355 | 1 | 0.551 |
| Length of necrosis | 0.909 | 1 | 0.340 |
| Width of necrosis | 0.097 | 1 | 0.756 |

**Tab. 6** - Frequency and appearance of symptoms on sessile oak seedlings inoculated with *Cryphonectria parasitica* 50 days after inoculation. Significant differences between rows are marked with lowercase letters ($p < 0.05$). Statistically significant differences between columns are marked with capital letters ($p < 0.05$). (N): number of inoculated seedlings; ($L_{nec}$): length of the necrotic lesion (mm); ($W_{nec}$): width of the necrotic lesion (mm); ($S_{epi}$): percentage of seedlings with epicormic shoots (%);

| Half-sib line | *Cryphonectria parasitica* | | | | | | Control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Dieback (%) | $L_{nec}$ (mm) | $W_{nec}$ (mm) | $S_{epi}$ (%) | Re-isolation (%) | Dieback (%) | $L_{nec}$ (mm) | $W_{nec}$ (mm) | $S_{epi}$ (%) | Re-isolation (%) |
| 1 | 8 | 75.00 $^{a}$ | 64.13 ± 13.02 $^{aA}$ | 22.25 ± 5.04 $^{aA}$ | 25.00 | 87.50 $^{a}$ | 0.00 | 11.00 ± 2.16 $^{aB}$ | 5.71 ± 1.38 $^{aB}$ | 0.00 | 0.00 |
| 2 | 10 | 80.00 $^{a}$ | 61.20 ± 9.44 $^{aA}$ | 17.90 ± 4.89 $^{abA}$ | 60.00 | 100.00 $^{a}$ | 0.00 | 9.71 ± 6.92 $^{aB}$ | 4.29 ± 2.06 $^{aB}$ | 0.00 | 0.00 |
| 3 | 14 | 64.29 $^{a}$ | 52.00 ± 9.26 $^{abA}$ | 17.21 ± 6.15 $^{abA}$ | 50.00 | 85.71 $^{a}$ | 0.00 | 12.71 ± 2.63 $^{aB}$ | 6.71 ± 2.69 $^{aB}$ | 0.00 | 0.00 |
| 4 | 7 | 71.43 $^{a}$ | 52.57 ± 9.93 $^{abA}$ | 14.29 ± 4.79 $^{abA}$ | 0.00 | 85.71 $^{a}$ | 0.00 | 9.71 ± 5.12 $^{aB}$ | 4.57 ± 2.23 $^{aB}$ | 0.00 | 0.00 |
| 5 | 14 | 35.71 $^{a}$ | 51.64 ± 15.56 $^{abA}$ | 14.50 ± 5.54 $^{bA}$ | 0.00 | 78.57 $^{a}$ | 0.00 | 10.29 ± 7.04 $^{aB}$ | 4.43 ± 2.23 $^{aB}$ | 0.00 | 0.00 |
| 6 | 10 | 20.00 $^{b}$ | 37.75 ± 16.14 $^{bA}$ | 15.00 ± 5.06 $^{abA}$ | 10.00 | 80.00 $^{a}$ | 0.00 | 8.14 ± 6.18 $^{aB}$ | 3.57 ± 2.57 $^{aB}$ | 0.00 | 0.00 |
| 7 | 13 | 38.46 $^{a}$ | 50.38 ± 11.82 $^{abA}$ | 13.15 ± 4.28 $^{bA}$ | 7.69 | 84.61 $^{a}$ | 0.00 | 10.71 ± 4.54 $^{aB}$ | 6.00 ± 1.15 $^{aB}$ | 0.00 | 0.00 |
| 8 | 13 | 76.92 $^{a}$ | 49.46 ± 10.10 $^{abA}$ | 16.69 ± 5.98 $^{abA}$ | 7.69 | 92.30 $^{a}$ | 0.00 | 10.14 ± 5.61 $^{aB}$ | 4.43 ± 2.23 $^{aB}$ | 0.00 | 0.00 |
| 9 | 9 | 55.56 $^{a}$ | 57.22 ± 16.62 $^{abA}$ | 13.22 ± 6.12 $^{bA}$ | 44.44 | 88.89 $^{a}$ | 0.00 | 12.29 ± 3.25 $^{aB}$ | 6.14 ± 1.07 $^{aB}$ | 0.00 | 0.00 |
| 10 | 10 | 40.00 $^{a}$ | 50.60 ± 17.63 $^{abA}$ | 11.40 ± 4.79 $^{bA}$ | 30.00 | 90.00 $^{a}$ | 0.00 | 10.29 ± 5.71 $^{aB}$ | 5.57 ± 2.99 $^{aB}$ | 0.00 | 0.00 |
| 11 | 11 | 72.72 $^{a}$ | 45.45 ± 9.73 $^{abA}$ | 15.18 ± 3.66 $^{abA}$ | 0.00 | 90.91 $^{a}$ | 0.00 | 10.86 ± 5.73 $^{aB}$ | 5.43 ± 2.70 $^{aB}$ | 0.00 | 0.00 |
| 12 | 9 | 77.78 $^{a}$ | 51.33 ± 15.09 $^{abA}$ | 14.78 ± 3.23 $^{abA}$ | 33.33 | 77.78 $^{a}$ | 0.00 | 9.14 ± 5.52 $^{aB}$ | 4.14 ± 2.12 $^{aB}$ | 0.00 | 0.00 |

makes it difficult to provide general, widely applicable management recommendations (Kohler et al. 2020). We believe that improved sessile oak restoration strategies must include methods of stimulating the development of tolerant seedlings based on provenance and dimensions.

Many sessile oak stands have experienced damage from abiotic factors and competition from surrounding vegetation. In these cases, it has been suggested that restoring the stands through seed sowing is more effective than planting seedlings (Crainic et al. 2023). However, using sessile oak seedlings that are tolerant to *Cryphonectria parasitica* can help create a healthy habitat. In some situations, seedlings displaying intense symptoms should not be disregarded. For instance, a combination of less sensitive small-sized seedlings and more sensitive seedlings with epicormic shoots can be used to outcompete sessile oak in the surrounding vegetation. Epicormic shoots harm the quality of sessile oak trees, and their size and health depend on the condition of the crown (Spiecker 2021). Such seedlings can be beneficial when it is necessary to establish as many trees as possible in highly unsuitable habitats.

In continuation of the study on how the origin and morphology of sessile oak seedlings affect the development of *Cryphonectria parasitica*, it is essential to investigate how the habitat of sessile oak trees influences the development of this fungus. Fascinating is the observed difference in how *Cryphonectria parasitica* infects American chestnut trees in locations not associated with hypovirulence (Lawson et al. 2021). This observation highlights the significance of habitat conditions in developing *Cryphonectria parasitica*. Therefore, in the future, it will be necessary to study how tolerant half-sib lines interact with environmental conditions to enhance their positive effects.

In the end, tolerant half-sib lines and small-diameter and height-sized sessile oak seedlings should be combined with other protection measures against *Cryphonectria parasitica*. Past experiences with sweet chestnuts have proven that the wide variety of *Cryphonectria parasitica* populations makes biological control challenging (Ježić et al. 2012, Ježić et al. 2018). Therefore, phytosanitary measures should aim to prevent the introduction of new strains of *Cryphonectria parasitica* into Europe (Dennert et al. 2019). The successful use of the hypovirus CHV-1 as the primary biological control agent in Europe is related to the concentration of application (Romon-Ochoa et al. 2022), and the development of CHV-1 in mycelia is affected by temperature (Romon-Ochoa et al. 2023). In sweet chestnuts, hypovirulent strains are especially effective on young trees with visible cankers (Hoegger et al. 2003). However, the spread of hypovirulence is not always guaranteed (Milgroom & Cortesi 2004). Recent improvements have been made in the methods used to apply hypovirulence (Kunova et al. 2017). We believe that using tolerant sessile oak seedlings will enhance the positive effects of applying hypovirulence and thus provide better protection.

## Conclusion

In this study, we determined the impact of the mother tree and the size of sessile oak seedlings on the development of *Cryphonectria parasitica*. One half-sib line had a significantly smaller length of necrotic lesions. In contrast, four half-sib lines of sessile oak seedlings had a statistically significantly smaller width of necrotic lesions. The half-sib line with the smaller length of necrotic lesions also had a lower percentage of dieback. No mortality was observed in the tolerant sessile oak groups with smaller necrotic lesions. Smaller seedlings, in terms of diameter and height, were less likely to develop wider necrotic lesions. These findings will facilitate the selection of tolerant genotypes from specific mother trees and the use of seedling morphology as an indicator of tolerance.

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