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Article

Interactions between *Phytophthora cactorum*, *Armillaria gallica* and *Betula pendula* seedlings subjected to defoliation

Justyna Anna Nowakowska[1]*, Marcin Stocki[2], Natalia Stocka[3], Sławomir Ślusarski[4], Miłosz Tkaczyk[4], João Maria Caetano[5], Mirela Tulik[6], Tom Hsiang[7], and Tomasz Oszako[2, 4]

[1] Institute of Biological Sciences, Faculty of Biology and Environmental Sciences, Cardinal Stefan Wyszynski University in Warsaw, Wóycickiego 1/3 Street, 01-938 Warsaw, Poland;
e-mail: j.nowakowska@uksw.edu.pl; orcid.org/0000-0001-5863-053X

[2] Institute of Forest Sciences, Faculty of Civil Engineering and Environmental Sciences, Białystok University of Technology, Wiejska 45E, 15-351 Białystok, Poland; e-mail: m.stocki@pb.edu.pl;
orcid.org/0000-0002-9430-7892;

[3] Faculty of Civil Engineering and Environmental Sciences, Białystok University of Technology, Wiejska 45E, 15-351 Białystok, Poland; e-mail: n.gierasimiuk@doktoranci.pb.edu.pl

[4] Forest Protection Department, Forest Research Institute, Braci Leśnej 3, 05-090 Sękocin Stary, Poland;
e-mail: S. Slusarski@ibles.waw.pl (S.S.);
e-mail: M.Tkaczyk@ibles.waw.pl (M.T.);
e-mail: T.Oszako@ibles.waw.pl; orcid.org/0000-0003-4688-2582 (T.O.)

[5] University of Aveiro, Department of Biology, University of Aveiro, Portugal; e-mail: joaomaria@ua.pt

[6] Department of Forest Botany, WULS-SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland; e-mail: mtulik@wl.sggw.pl; orcid.org/0000-0003-4021-6546

[7] Environmental Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1; e-mail: thsiang@uoguelph.ca

* Correspondence: j.nowakowska@uksw.edu.pl; Tel.: +48-22-569-6838

Abstract:

The purpose of this study was to better understand the interactive impact of two soil-borne pathogens, *Phytophthora cactorum* (as the primary pathogen) and *Armillaria gallica* (as secondary), on two-year-old seedlings of silver birch (*Betula pendula*) subjected to stress caused by mechanical defoliation simulating primary insect feeding. One year after treatments, the chlorophyll fluorescence measurement and gas chromatography coupled with mass spectrometry (GC-MS) were used to analyze the photosynthetic activity in leaves, the volatile organic compounds (VOCs) emitted by birch leaves and chemical compounds from roots. Only the infection of roots by *P. cactorum* increased photosynthetic rates in the leaves, which may suggest its cryptic development in contrast to fungi. The birch leaves in seedlings exposed to 50% defoliation, inoculation with *P. cactorum* and *A. gallica* emitted more aromatic carbonyls and alcohols, as well as half as much aliphatic esters, compared to untreated controls. In infected birch roots, the production of phenols, triterpenes and fatty alcohols increased, but fatty acids decreased. This was the first experimental

confirmation of the pathogenicity of *P. cactorum* on silver birch seedlings in Poland. The most severe damage to roots took place only in the case of two-way or three-way interactions. Higher levels of aromatic carbonyls and alcohols in leaves, as well as phenolic compounds in roots of stressed birches (compared to control) suggest an activation of plant systemic acquired resistance (SAR).

Keywords: birch; chlorophyll; leaves' damage; plants' pathogens; roots; secondary metabolites

1. Introduction

Silver birch (*Betula pendula* Roth.) is common throughout the lowlands and in lower mountainous regions in Europe and is subject to attack by *Phytophthora cactorum* (Lebert & Cohn) J. Schröt (as a primary pathogen) and *Armillaria gallica* Marxm. & Romagn. (as a secondary one) [1,2]. To date, over 150 *Phytophthora* species have been described from a broad range of hosts, including forest tree species and ornamental plants [3,4]. These groups of pathogens have led not only to an extensive mortality of different forest tree species but are also responsible for negative ecological impacts in many countries across the world. Moreover, climate warming and increased mean precipitation in the growing season have been often associated with increased invasiveness of phytophthoras in many plants [3,5]. In the last few decades, interest in *Phytophthora* species (Oomycetes) as plant pathogens, has been growing in Europe and on other continents, and also in forestry since these pathogens are causing emerging diseases of many forest tree species [6-9]. *Phytophthora ramorum* Werres, De Cock & Man in't Veld is an example of a harmful pathogen, which is responsible for Sudden Oak Death (SOD) in North America and Sudden Larch Death in Europe (SLD) [10-13]. Generally, the risk of transferring phytophthoras from nurseries to forest plantations is considered high, as *P. ramorum* was transmitted in seedlings from nurseries in California, and it affected production in over 20 US states [14].

In Europe, *P. cactorum* and a lot of other *Phytophthora* sp. are abundant in many local forest environments enduring different climatic conditions [15,16]. *P. cactorum* was isolated by Jung & Blaschke [15] from declining oaks stands (*Quercus robur* and *Q. petraea*), and by Lilja et al. [17] and Hantula et al. [18] from strawberry (*Fragaria* x *ananassa*) fruits and silver birch (*B. pendula*) roots. So far, in Poland, this fungus has only been reported in nurseries on sycamore maple (*Acer pseudoplatanus* L.), black alder (*Alnus glutinosa* Gaertn.), European beech (*Fagus sylvatica* L.) and silver birch (*B. pendula* Roth.) [19].

In the 1980's birch stand decline had been observed in Poland, but it was then considered as a complex disease involving water issues caused by drought. It is now known that species of *Phytophthora* causing damage to fine roots can mimic drought symptoms [20,21]. Some experiments demonstrated the possible susceptibility of one-year-old silver birch seedlings of Polish provenance by three species of *Phytophthora* i.e. *P. cinnamomi*, *P. citrophthora* and *P. plurivora* [22,23]. Nevertheless, the only published records of *P. cactorum* associated with birch in Poland are also of young birch seedling tissues [19], but not on adult trees [4].

Generally, tree damage and eventual mortality are caused not by a single pathogen, but a whole suite of disturbance agents, like harmful fungi and defoliator insects or unfavorable abiotic conditions. In Europe, among the root and butt rot fungi (*Armillaria* spp.), *A. gallica* is considered to be a weak pathogen of many forest tree species, increasing its activity after severe drought or

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defoliation [24-26]. However, *Armillaria* spp. are known to have growing destructive impact in European ecosystems due to climate changing conditions both in forest and in urban environments [27,28].

During last few years, abundant research has been devoted to the combined effects of pests and pathogens on forest ecosystems [29]. Additionally, along with phytopathogens, birch trees are often subjected to attack by several insect defoliators, such as *Deporaus betulae* (L.), *Phyllobius betulae* F. and *Ph. arborator* (Herbst.) leading to severe damage of the crown [30,31]. A tree's response to direct damage (e.g. defoliation) consists not only of a consecutive production of new leaves from dormant buds but also of physiological and biochemical reactions. Many plants, including forest tree species, change their photosynthetic activity and the level of secretion of VOC secretion in response to damage caused by external stress factors [32-34]. Plants weakened by partial defoliation very often become vulnerable to multi-pathogen infections [35].

The main purpose of this work was to better understand interactions between organisms which could be associated with birch decline syndrome. The interactions between soil borne pathogens on the production of secondary metabolites (as priming) by birch have not been sufficiently studied, and an objective was to examine the effects of the fungus (*A. gallica*) and oomycete (*P. cactorum*) on the chemical composition of birch volatiles secreted by young birch seedlings. To our knowledge there are no scientific reports in Poland about interactions between *P. cactorum* and *A. gallica*, which cohabitat in local forests in the same ecological niche, the soil [36]. Furthermore, since defoliation may predispose trees to infection, we hypothesized that combinations of partial defoliation and infection by *P. cactorum* and *A. gallica* would cause more mortality than each stress alone. We attempted to mimic insect defoliation by gradual cutting of leaves over time to generate a less abrupt physiological stress. We also analyzed photosynthetic activity via chlorophyll fluorescence and secondary metabolites content in leaves and roots to probe at the mechanism of physiological and biochemical plant response to separate and combined abiotic and biotic stresses

2. Material and Methods

2.1. Growth of Plant Material and General Experiment Design

In March 2017, two-year-old silver birch (*B. pendula* Roth.) plants were collected in Chojnów Forest District, Poland (52°02'20.3"N, 21°05'21.1"E), and re-planted singly in 15 L pots filled with a peat/perlite mixture 1:1 (v/v). Sixty four plants were grown in the controlled greenhouse conditions (ca. 22 °C and relative humidity 65% ± 5%) for a year, and then were used as 3-year-old seedlings in experiments in spring 2018. They were irrigated with tap water once a day and were not fertilized. Some pots were treated with inoculum of *P. cactorum* (in April 2018) or *A. gallica* (in the beginning of June 2018) or defoliated (in June 2018). For each treatment, eight replicate pots were used and the experiment was designed as follow: (1) untreated control, (2) *Armillaria gallica*; (3) *Phytophthora cactorum*; (4) Defoliation 50%; (5) *Armillaria gallica* + defoliation 50%; (6) *Phytophthora cactorum* + defoliation 50%; (7) *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; (8) *Armillaria gallica* + *Phytophthora cactorum*.

2.2. Inoculation of Plants with Pathogen Isolates

2.2.1. *Phytophthora cactorum*

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Liquid medium contained 250 cm^3 of vermiculite, 20 cm^3 of millet and 175 cm^3 of V8 medium (100 mL of multi vegetable juice, 2 g of calcium carbonate $CaCO_3$, 900 mL of distilled water) was dispensed into 1 L Erlenmeyer flasks (890 cm^3 of medium per each flask) and autoclaved for 15 minutes at 121 °C. An isolate of *P. cactorum* from the IBL collection (GenBank accession number NCBI KX242303) was placed into the flasks using hyphae grown on V8 agar (ten pieces of agar about 0.5x0.5 cm covered with mycelium). The cultures had been incubated for 7 days at room temperature [37]. They were also used to inoculate soil samples (prepared from a mixture of peat, sand and perlite in a 1:1:1 ratio) and added to each individual 1 L pot in the volume equal to 2% of the volume of the soil. The untreated check did not contain *P. cactorum* inoculum.

2.2.2. *Armillaria gallica*

To prepare the *A. gallica* inoculum, 1.5 - 2 cm thick stems of hazelnut (*Corylus avellana* L.) were first cut into 10 cm long sections, placed in a 630 cm^3 metal container, wrapped with aluminum, and autoclaved for 80 minutes at 121 °C. After cooling, hyphal plugs from 3 week old cultures of *A. gallica* (from the IBL collection originally isolated from European oak roots) on 2% malt agar were added to the sterilized stem containers, with the full contents of a 9-cm-diameter petri plate per container of hazelnut cuttings. The inoculated stem segments were then incubated at 25 °C for 3 months until segments were completely overgrown by mycelium. After this, the stem segments were placed in the appropriate birch seedling pots, with 2 segments per pot. Autoclaved hazelnut segments were placed in pots as controls.

2.3. Verification of the Seedlings Infection

The occurrence of inoculated pathogens in rhizosphere soil was checked as follows: (i) visually for *Armillaria* rhizomorphs attached to roots following Oszako et al. [38], or (ii) by baiting with leaves or (iii) culturing (*P. cactorum*) [39]. To meet Koch's postulates, the pathogen *P. cactorum* was re-isolated from infected leaves (during baiting). *P. cactorum* was isolated from rhizosphere soil also containing fine roots. For baiting, soil samples were taken from several pots of birch seedlings and were put into plastic boxes and watered (1 cm above soil level). On the surface of water, previously washed and dried 7-14-days-old oak and beech leaves were placed and incubated at room temperature. They were observed for discoloration daily for a week. When brownish spots appeared on the leaf surfaces (generally after 3–7 days of incubation at 20 °C), leaves were cut into small pieces (approx. 5 × 5 mm) and transferred to a selective medium: PARPNH – agar containing V8 - agar with 10 μg mL^{-1} pimaricin, 200 μm mL^{-1} ampicillin, 10 μm mL^{-1} rifampicin, 25 μg mL^{-1} pentachloronitrobenzene, 50 μg mL^{-1} nystatin and 50 μg mL^{-1} hymexazol [39]. These were incubated at 20 °C in the dark, and the hyphae emerging after 48 h were transferred to V8 - agar medium and checked for purity over the following days. Initial identification following morphological descriptions in Erwin & Ribeiro et al. [6] was confirmed molecularly (described below like in the *in planta* section).

To assess the presence of *P. cactorum in planta*, roots were collected a year after inoculation and washed thoroughly. Genomic DNA was extracted and purified using the NucleoSpin® Plant II Midi kit (Macherey-Nagel, Düren, Germany). PCR was done using species-specific primers designed for *P. cactorum* isolate JF300214.1 from GenBank, following Nowakowska et al. [40]. The TaqMan probe was labeled with the reporter dyes, i.e. JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein)

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at the 5′-end and HBQ1 quencher at the 3′-end (Sigma-Aldrich, MO, USA). PCR amplifications were performed in a total volume of 15 µL containing 1×Master Mix (Sigma-Aldrich), 2 µM of each primer, 0.2 µM of probe and 2 µL of diluted genomic DNA. PCR amplification included one cycle of denaturation at 95 °C for 3 min, 40 cycles of 95 °C for 30 s, 55-61 °C for 30 s, and 72 °C for 30 s. Amplifications were performed in a RotorGene 6000 (Qiagen, Hilden, Germany) apparatus and fluorescence of newly synthetized amplicons was monitored in each PCR cycle during the annealing phase following the manufacturer's instructions.

2.4. Defoliation of Birch Seedlings

To mimic progressive defoliation such as that caused by foliar insects and possibly foliar pathogens, 32 seedlings were partially and progressively defoliated over four weeks. In the first week 50% of the leaves had 25% of their area removed. In the successive three weeks, 25% more each week were removed from these leaves resulting in 50% defoliation after four weeks.

2.5. Evaluation of Birch Health Status

The last year of experiment, in July 2019 the health status of all 64 birch seedlings was visually assessed on the following scale: 1- healthy with 30%-50% of leaf loss; 2- diseased with 51%-75% leaf loss; 3- dying with more than 76% of leaf loss. Since the defoliation treatment occurred only in the first year, it was reasonable to assess defoliation in the succeeding years.

2.6. Measurement plant response to the stress factors

2.6.1. Biometric Parameters

After 13 months of growth, plant growth parameters were assessed. As soon as plants were removed from their pots and cleaned, height and collar butt diameter were measured, and fine root scanned on an EPSON Perfection V700 Photo Scanner, and image processed using WinRhizo® software (Regent Instruments, Canada). The number of living fine roots (< 2 mm) per length of mother roots (2–5 mm) was calculated and transferred to Excel for calculation of the following representative parameters: number of tips, fine root length, mother root length, fine root length per major root length, total root length, fine root surface area and fine root tips. After drying at 60 °C for 72 h (after which weight did not change anymore), dry biomass weight was measured in 8 replicates per treatment.

2.6.2. Fluorometric Analysis of Chlorophyll *a*

Fluorescence emitted by chlorophyll *a* is quantified by exposing a leaf (first kept in the dark) to light of a defined wavelength and measuring the amount of light re-emitted at longer wavelength [41]. Twelve months after the start of the experiment, chlorophyll fluorescence of the leaves was measured directly with a Handy PEA (Handy Plant Efficiency Analyser) fluorimeter following instructions from Hansatech Instruments Ltd. (King's Lynn, Nortfolk, Great Britain). In total, 10 measurements were made on each of three randomly chosen leaves for each of the eight treatments. Prior to measurements, leaves of 32 randomly chosen seedlings (half from each treatment) were kept for 30 min in the dark, where to the surface of each leaf, special clips were attached providing darkness in order to slow down photosynthetic activity. The measurements were done on the central

part of each leaf blade, on 4 mm^2 of each sample, i.e. in three replications per pot (from the top, middle and lower part of crown). The measurement conditions consisted of 1 s pulses of 3500 μmol $m^{-2}s^{-1}$ light. To measure the physiological stress at the level of photosystem II (PSII), several photosynthetic parameters were assessed: F_0 - initial fluorescence, Fm – maximal intensity of fluorescence; F_0/Fm - maximal photosynthetic activity, DI_0/CS_0 - photosynthetic efficiency measured as energy dissipation in form of heat, and PI total - total Performance Index of photosystem II (PSII) [42].

2.7. Secondary Metabolites Contained in Birch Leaves and Roots

To analyse VOCs in leaves, three leaf blades were harvested from each of the eight pots per treatment, and analysed with headspace solid phase microextraction and gas chromatography-mass spectrometry (HS-SPME/GC-MS) methods following Isidorov et al. [43] and Oszako et al. [38]. Each leaf (1 g) was placed into a 60 mL headspace screw-cap vial and heated for 30 min at 40 °C. The septum of the screw-cap was pierced by a needle protecting the adsorption fiber, and the DVB/CAR/PDMS fiber was exposed to the headspace gas phase. After 30 min of exposure, the SMPE fiber was introduced for 10 min into the injection port of the GC–MS apparatus. GC-MS analyses were done as stated above with the following differences: The injector worked in a splitless mode at temperature of 250 °C. The initial column temperature was 35 °C, rising to 250 °C at 5 °C min^{-1}. The detection was performed in a full scan mode from 29 to 600 atomic mass units (amu).

To assess the chemical composition of birch root extracts, soil was washed off roots of potted plant with tap water. This was done for 8 of 64 specimens randomly sampled. Metabolites extracted from were analysed with gas chromatography-mass spectrometry (GC-MS), a method previously developed by Stocki et al. [44,45]. For each sample, 1 g of cleaned roots was processed in diethyl ether (50 mL), and 10 mg of extract was dissolved in 1 mL of pyridine and 100 μL derivatization reagent - N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA; Sigma-Aldrich, Poznań, Poland). The mixture was heated for 30 min at 60 °C, and after reaction of derivatization, the chemical composition of each sample was analyzed using GC-MS apparatus (Agilent Technologies Inc., CA, USA). The samples were processed through an Agilent 7890A gas chromatograph with an Agilent 5975C mass spectrometer. Injection of each 1 μL sample was done using an Agilent 7693A autosampler. Chromatographic separation was performed on a capillary column HP-5MS (30 m x 0,25 mm x 0,25 μm) at a helium flow rate of 1 mL/min. The injector worked in a split (1:10) mode at temperature of 300 °C. The initial column temperature was 50 °C, rising to 325 °C at 3°C/min and the final temperature was held for 10 min. The ion source and quadruple temperatures were 230 °C and 150 °C, respectively. Electron ionization mass spectral (EIMS) was obtained at ionization energy 70 eV. The detection was performed in a full scan mode from 41 to 800 amu. For identification of extracted components, both mass spectra and retention indices were used. After integration, the content (%) of each component in the total ion current (TIC) was calculated. All measurements for leaves and root extracts were performed for each plant treatment in 3 replicates.

2.8. Statistical analysis

For health data, growth data, and fluorescence data, normality of distribution was tested using the Shapiro-Wilk test, and homogeneity of variance with the Levene's test. For data which did not satisfy the assumptions of ANOVA, and to enable parametric testing, data were subjected to

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Box-Cox transformation [46]. In order to assess which treatments differed significantly, ANOVA (analysis of variance) and Tukey post-hoc tests were used. All analyses were performed in the STATISTICA 13.1 package [StatSoft Polska] for $\alpha = 0.05$.

3. Results

In 2018, the 64 three-year-old potted birch seedlings were subjected to eight different treatments involving partial defoliation, inoculation with two different pathogens, and combinations thereof. In 2019, after a year of growth in the greenhouse, the 64 four-year-old seedlings were subjected to the following analyzes: (i) baiting-based and molecular detection of the *P. cactorum* and *A. gallica* in the soil and/or in plants; (ii) health state assessment of seedlings; (iii) evaluation of the response to stress factors via biometric and photosynthetic measurements; and iv) detection and measurement of volatiles emitted by leaves and compounds extracted from roots.

3.1. Detection of P. cactorum and A. gallica

P. cactorum was detected from rhizosphere soil of inoculated plants via baiting techniques. The isolates obtained were identified as *P. cactorum* following descriptions in Erwin & Ribeiro [6]. Real-time PCR reactions performed with the specific *P. cactorum* probes and primers yielded positive results for this pathogen presence in silver birch roots (Ct values ranged from 21.5 to 24.1).

Successful infection was observed a year after inoculation with *A. gallica.* Typical rhizomorphs of the fungus, attached to the root system of birch seedlings, were observed in all infected potted plants (i.e. in treatments 5 - 8 of the experiment).

3.2. Health Status Assessment of Plants

One year after the beginning of the experiment, the highest negative influence on birch health was noted in the treatment with both *Phytophthora* and *Armillaria* (Figure 1). The influence of partial defoliation stress on plant health status was more important than the influence of each pathogen separately. *A. gallica* + *P. cactorum* (8) caused the most severe damage (nearly reaching 75% leaf fall) finally leading to mortality.

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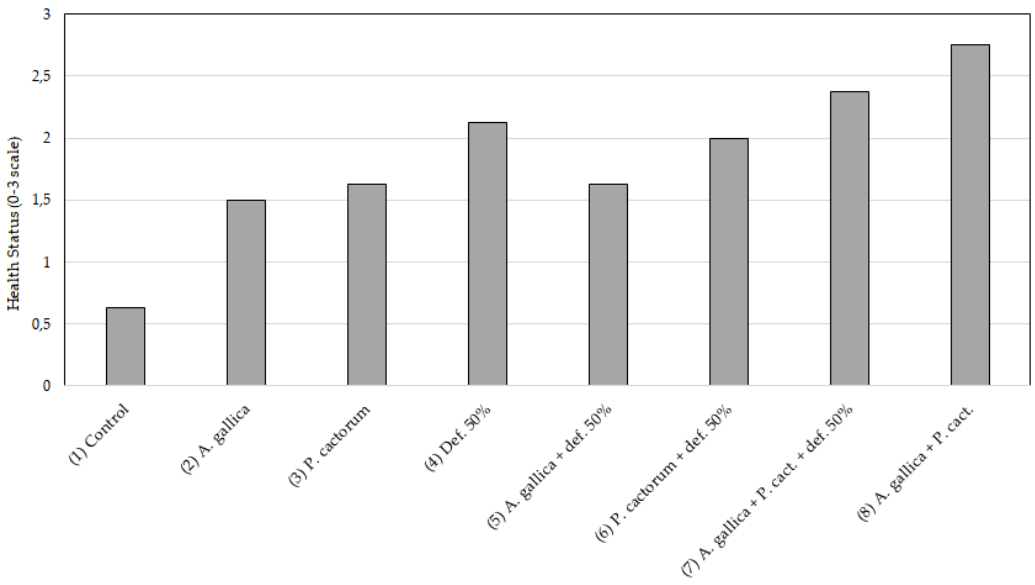

Figure 1. Health status of investigated birch seedlings in combination with 7 different stress conditions compared to the control. Scale of damage comprises: 1 – healthy plants with 30%-50% defoliation; 2 – diseased with 50%-75% defoliation; and 3 - dying with more than 75% defoliation. Each bar represents the mean of eight independent observations.

3.3. Plants Response to the Stress Factors

3.3.1. Growth parameters

The comparison of seedling height between different experimental treatments showed no statistically significant differences (data not shown). However, the comparison of root collar thickness indicated some differences between treatments (Figure 2). The combination of all three stress factors (two pathogens and defoliation, treatment 7) decreased significantly the development of root collar thickness compared to the control. Also, significant differences in this parameter were observed between the plants treated with *P. cactorum* (3). Other interactions (treatments 2, 4-6 and 8) were not statistically significant among themselves and the control (1).

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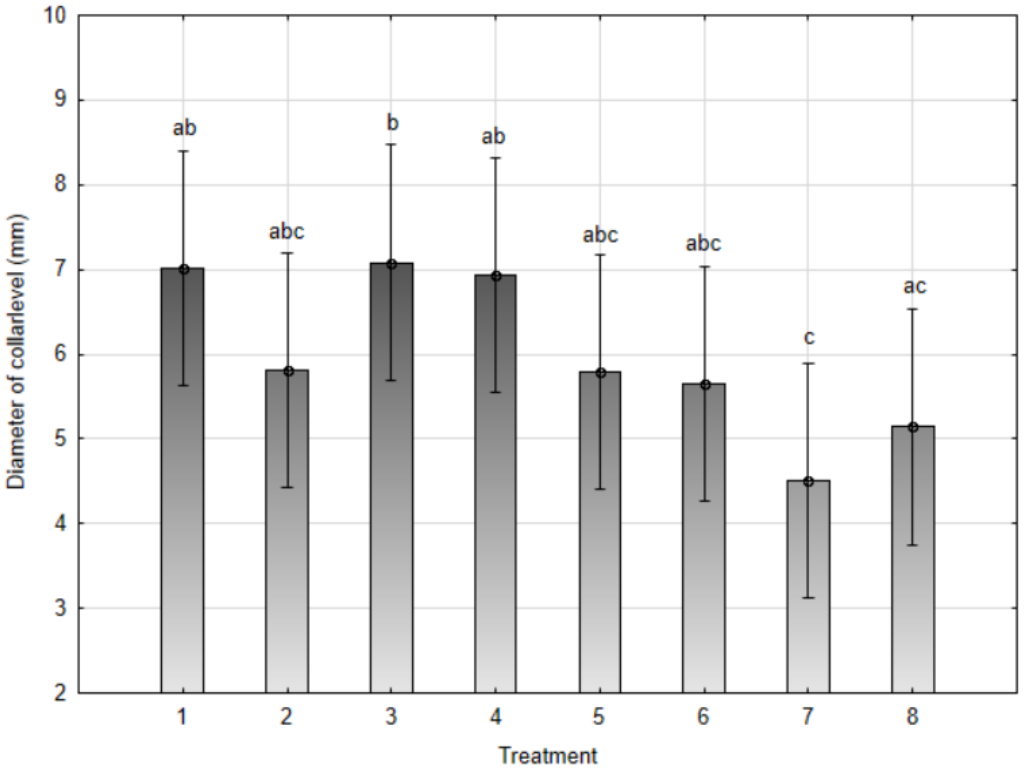

Figure 2. Comparison of diameter on collar level of birch seedlings between treatments: (1) – Control; (2) - *A. gallica*; (3) - *P. cactorum*; (4) - Defoliation 50%; (5) - *A. gallica* + defoliation 50%; (6) - *P. cactorum* + defoliation 50%; (7) - *A. gallica* + *P. cactorum* + defoliation 50%; (8) - *A. gallica* + *P. cactorum*. Letters above each Standard Error bar are based on Tukey post-hoc tests and letters in common indicate no significance difference; p = 0.043.

Among the investigated parameters, the number of root tips, fine root length, fine root surface area and fine root tips significantly diminished compared to the control (Table 1). The most pronounced decrease of most of the parameters observed for treatment 7, when seedlings were exposed to the interaction between two root pathogens and defoliation. In this case, fine root length decreased by 84%, total root length by 80%, and fine root surface area by 75%. In treatment 8 (*A. gallica* + *P. cactorum*), the same treatments showed respective decreases by 69, 70 and 72%. The harmful effects on root parameters were attenuated if pathogens or defoliation were tested separately. Dry biomass of the roots did not statistically differ from untreated plants in any experimental treatment.

Table 1. Influence of investigated interactions on root growth parameters (mean values ± S.D.; p-values are shown for each growth parameter in the bottom row).

Treatment[1]	Number of Tips	Fine root length (FRL)	Mother root length (MRL)	FRL/ MRL	Total root length (mm)	Fine root surface area (cm^2)	Fine root tips	Dry mass

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1	4891.4 ± 1533.1 a[2]	1485.1 ± 387.9 a	63.6 ± 21.7 a	31.1 ± 30.36 a	1575.8 ± 378.3 a	166.9 ± 44.5 a	4887.7 ± 1532.1 a	11.8 ± 5.9
2	3362.9 ± 1728.3 ab	905.2 ± 481.0 ab	56.1 ± 23.8 a	15.6 ± 4.5 a	987.9 ± 515.9 ab	110.1 ± 56.7 ab	3359.1 ± 1727.6 ab	13.3 ± 9.5
3	2659.9 ± 1239.3 ab	890.6 ± 514.1 ab	51.7 ± 34.1 a	18.1 ± 7.2 a	964.7 ± 552.3 ab	102.6 ± 64.8 ab	2656.7 ± 1238.7 ab	9.2 ± 5.7
4	2459.9 ± 1477.5 ab	725.4 ± 504.3 ab	49.6 ± 32.9 a	15.3 ± 8.4 a	798.6 ± 546.9 ab	84.1 ± 60.6 ab	2456.5 ± 1476.2 ab	9.8 ± 5.5
5	3740.4 ± 2304.7 ab	985.4 ± 667.7 ab	41.4 ± 23.9 ab	21.1 ± 10.9 a	1044.7 ± 692.1 ab	109.5 ± 73.7 ab	3738.1 ± 2303.7 ab	15.5 ± 13.8
6	3770.9 ± 2779.4 ab	1164.5 ± 932.2 ab	47.5 ± 34.2 a	20.2 ± 13.4 a	1226.0 ± 967.3 ab	130.4 ± 103.4 ab	3767.6 ± 2779.7 ab	9.0 ± 6.6
7	1379.2 ± 992.4 b	229.1 ± 182.9 b	5.6 ± 6.3 b	74.8 ± 46.9 b	314.5 ± 194.2 b	41.9 ± 15.9 b	1377.0 ± 997.0 b	5.9 ± 2.5
8	1670.9 ± 1376.2 b	446.6 ± 474.7 b	26.2 ± 25.6 ab	14.1 ± 11.7 a	493.1 ± 483.5 b	47.0 ± 45.7 b	1668.4 ± 1375.9 b	5.1 ± 3.2
p - value	0.007	0.01	0.01	0	0.011	0.006	0.01	0.108

[1] Treatments: (1) - control; (2) - *Armillaria gallica*; (3) - *Phytophthora cactorum*; (4) - Defoliation 50%; (5) - *Armillaria gallica* + defoliation 50%; (6) - *Phytophthora cactorum* + defoliation 50%; (7) - *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; (8) - *Armillaria gallica* + *Phytophthora cactorum*.

[2] Means are based on eight replicate observations. Values marked with the same letter do not differ significantly ($p < 0.05$) based on Tukey's post-doc test.

3.3.2. Chlorophyll fluorescence

Physiological status of birch seedlings was estimated based on selected chlorophyll fluorescence parameters. The initial fluorescence (F_0) in contrast to maximal fluorescence (Fm) were higher in all treatments compared to the control (Figure 3). The highest ratios F_0/Fm observed in *A. gallica* and *P. cactorum* alone (and in combinations with 50% of defoliation) indicate the highest level of chlorophyll fluorescence, which is inversely proportional to photosynthetic activity. This was in concordance with the highest ratios DI_0/CS_0 denoted in seedlings subjected to *A. gallica* and *P. cactorum* infections, showing the highest amount of dissipated energy in the form of heat (i.e. the highest loss of absorbed light energy). Defoliation 50% alone did not heavily affect the photosynthetic activity (F_0/Fm ~ 1 and DI_0/CS_0 ~ 1.2) but total photosynthetic performance index was low (PI total = 0.9).

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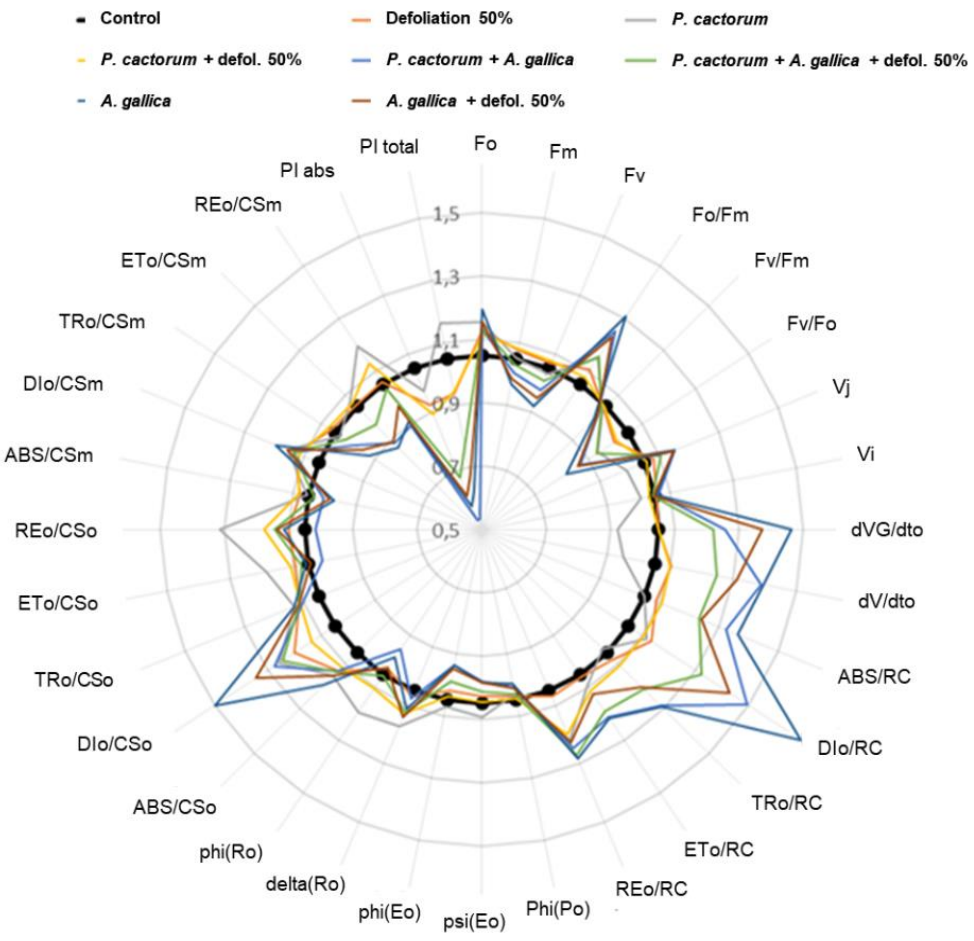

Figure 3. Comparison of chlorophyll fluorescence parameters in different treatments of experiment (colored lines). Description of the most important parameters (describing Clf and PI total) is given in the text.

In fact, all treatments showed reduced total photosynthetic activity (PI total < 1) in comparison to the control plants (PI total = 1) except the treatment with *P. cactorum* alone which did not adversely affect the photosynthetic activity, as PI total increased by 10% and F_0/Fm ~ 1 (Figure 3). Other interactions showed lower efficiency of photosynthetic apparatus compared to the control, however among all treatments, defoliation (50%) combined with *P. cactorum* resulted in slightly higher values of PI total (0.9) compared to the other treatments (mean PI total ~ 0.6). The interaction between two pathogens and partial defoliation reduced a PI total to 0.7, suggesting some compensation of the loss of activity in PSII due to the defoliation.

3.4. Secondary Metabolites of Leaves and Roots

Defoliation combined with inoculation with the two pathogens affected the chemical composition of the metabolites produced by leaves and roots of *B. pendula*. The content of VOCs emission by birch leaves varied between different treatments (Table 2). The amount of monoterpenes, e.g. citronellol, neral, (*E*)-geraniol, *α*-citral and eugenol increased in leaves under the influence of stress caused by *P. cactorum* and defoliation (Table 2, Table S1). Birch leaves infected by both pathogens (var. 7, 8) had significantly less sesquiterpenes compared to the control (Table 2),

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including α-copaene, β-bourbonene or β-caryophyllene (Table S1). The amount of aromatic esters (especially methyl salicylate) increased after infections by *P. cactorum* (3) and *A. gallica* + defoliation (5). The highest content of aromatic carbonyl compounds (7.12%) was found in the treatment with *P. cactorum* + defoliation (6) and the highest content of aromatic alcohols (12.47%) in the treatment with both *A. gallica* and *P. cactorum* (8) compared to the control (Table 2). Stressed birch leaves (treatments 7 and 8) emitted more aliphatic acids (9.68%) and aliphatic alcohols (21.41%), but remarkably reduced the emission of aliphatic esters, alkanes and alkenes (Table 2).

Table 2. The chemical composition of VOCs emitted by birch seedlings leaves in the treatments, 1 - control; 2 - *Armillaria gallica*; 3 - *Phytophthora cactorum*; 4 - Defoliation 50%; 5 - *Armillaria gallica* + defoliation 50%; 6 - *Phytophthora cactorum* + defoliation 50%; 7 - *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; 8 - *Armillaria gallica* + *Phytophthora cactorum*. More details are given in Table S1.

Group of Compounds	Chemical Content (%) by Treatment								LSD
	1	2	3	4	5	6	7	8	(p = 0.05)
Monoterpenes	15.95	19.23	17.42	18.50	15.19	21.59	15.46	14.22	0.333
Sesquiterpenes	7.57	6.16	18.34	19.20	14.95	11.31	1.49	1.48	0.004
Aromatic Esters	5.52	5.91	8.05	3.96	7.12	6.68	4.98	4.66	0.998
Aromatic Carbonyls	1.32	3.87	4.03	2.47	4.37	7.12	5.46	6.98	0.805
Aromatic Alcohols	5.64	8.35	8.82	7.60	7.02	10.62	11.75	12.47	0.986
Aliphatic Esters	14.41	6.27	3.75	6.97	5.11	3.86	2.85	3.31	0.154
Aliphatic Acids	1.12	-	-	0.38	-	0.17	11.85	9.68	0.154
Aliphatic Carbonyls	21.58	22.47	17.12	17.21	20.79	18.32	20.66	18.30	0.711
Aliphatic Alcohols	15.36	15.31	12.76	13.38	15.78	8.43	17.13	21.41	0.873
Alkanes and Alkenes	7.12	8.65	7.04	7.44	6.62	9.62	2.73	1.83	0.189
Other Compounds	1.78	1.62	1.27	1.53	1.56	1.38	3.88	4.31	0.522
Unidentified Compounds	2.63	2.17	1.40	1.38	1.49	0.89	1.76	1.35	0.917

The content of chemical compounds in root extracts also differed between experimental treatments (Table 3). The control roots (treatment 1) produced fewer phenolic compounds and triterpenes, compared to treatments 2-8 (Table 3). In particular, defoliation treatment (4) and *A. gallica* + *P. cactorum* (8) increased the level of phenolic compounds in birch roots from nearly 0.9% (control) to 12-13%. Also, the level of triterpenes showed the same tendency, being 7-8 times higher after defoliation (8.64%) and higher with *A. gallica* + *P. cactorum* infections (10.25%) than in the control (1.25%). The defoliated seedlings (4) also produced increased amount of sterols, i.e. campesterol, stigmasterol, *β*-sitosterol and stigmastanol compared to the control (Table S2). The opposite trend was observed in fatty acids content, with the lowest amount found in roots of defoliated plants (4) and in plants treated with *A. gallica* + *P. cactorum* (8). The highest content in fatty alcohols (e.g. 1-docosanol) was found in the treatment of *A. gallica* + defoliation. In the roots of plants

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infected with *A. gallica* (treatment 2) and *A. gallica* + *P. cactorum* with or without artificial defoliation (7, 8), the highest levels of phenolic compounds including phyto-flavonoids such as caffeic acid and catechin (Table S2), were recorded.

Table 3. The chemical composition of extracts from by birch seedlings roots in the experiment treatments, 1 - control; 2 - *Armillaria gallica*; 3 - *Phytophthora cactorum*; 4 - Defoliation 50%; 5 - *Armillaria gallica* + defoliation 50%; 6 - *Phytophthora cactorum* + defoliation 50%; 7 - *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; 8 - *Armillaria gallica* + *Phytophthora cactorum*. More details are given in Table S2.

Group of Compounds	Chemical Content (%) by Treatment								LSD
	1	2	3	4	5	6	7	8	(p = 0.05)
Phenolic Compounds	0.87	15.35	5.02	11.91	4.83	4.36	7.85	13.32	0.711
Triterpenes	1.25	3.63	2.73	8.64	4.46	3.69	4.82	10.25	0.864
Sterols	33.53	39.58	28.37	41.83	28.94	29.61	23.30	35.80	0.998
Fatty Acids	44.47	21.47	32.30	15.65	32.10	33.93	35.82	15.50	0.086
Fatty Alcohols	4.23	5.45	6.43	6.29	9.27	6.15	7.28	5.67	0.954
Other Compounds	8.33	8.17	12.01	8.31	8.79	15.00	9.03	7.83	0.479
Unidentified Compounds	7.30	6.35	13.14	7.39	11.60	7.26	11.90	11.64	0.751

4. Discussion

Many pests and fungal pathogens are responsible for biodiversity losses and reduction in yield and quality of wood production, so the understanding of underlying interactions between plants and different harmful biotic factors become a very important issue to maintain the sustainability of the forest ecosystem [47,48]. The main purpose of our investigation was to explore the interactions between two soil borne pathogens (*P. cactorum* and *A. gallica*) and *B. pendula* seedlings subjected to stress caused by partial mechanical defoliation simulating insect attack. We tried to determine the following: (i) whether birch is a potential host of *P. cactorum* and *A. gallica* pathogens in Poland; and (ii) whether stress from partial defoliation can stimulate enhanced root infection. We also attempted to figure out how combined effects caused by the aforementioned pathogens and partial defoliation influenced the photosynthetic activity of the treated plants, and what chemical compounds were produced in the stressed leaves and root of the birch seedlings.

4.1. Birch Damage Caused by P. cactorum and A. gallica

As far as we know, this is the first report from Poland of interactions between oomycete *P. cactorum* pathogenic to silver birch, and the fungus *A. gallica*. The pathogen *P. cactorum* was already previously recorded in Polish nurseries [49], and it was considered a primary agent responsible for destroying birch fine roots, while *A. gallica* followed the primary infection as an opportunistic pathogen. The later pathogens are often stimulated by root wounds and biochemical changes caused

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by defoliation [50]. Our previous investigations confirmed that *Armillaria gallica* prefers to attack roots, first affected by *Phytophthora* infection [38].

Our findings confirmed by qPCR assay that *P. cactorum*, a very dangerous oomycetes having a large host range, was indeed a pathogen of *B. pendula*. Despite some criticism that using ITS sequence alone is insufficient to allow specific identification of *Phytophthora* species [51], ITS-based TaqMan probes have been reported to successfully identify *P. megasperma, P. plurivora*, *P. pseudosyringae,* and *P. quercina*, present in soil material [40,52]. This oomycete is known as a pathogen causing disease of silver birch stems, easily isolated in forest nurseries using baiting (plant traps) from irrigation water ponds or directly from necrotic lesions on stems [53]. After detailed study by Rytkönen et al. [53], the pure cultures of *P. cactorum* isolated from water and birch seedlings showed a great morphological variability among isolates, but their genetic origin could not be determined despite RAMS analysis and the sequencing of the ITS1 and β-tubulin regions. In fact, two separate forms of the β-tubulin gene were detected in all Finnish isolates, suggesting that *P. cactorum* carries two loci for β-tubulin genes in its genome. Similar results were obtained from silver birch seedlings in Polish nurseries irrigated from nearby water sources [54]. Up to now, many *Phytophthora* species have been found in rivers as well as in water reservoirs, present in forest as well as in ornamental nurseries for plant irrigation, where microorganisms can overwinter for several years [55-60].

In general, plants grown in nurseries are exposed to infection via soil saturated with water-containing propagules of different pathogens. It is believed that the risk of infection occurs during each irrigation and often plants are asymptomatic when they are transferred to forest plantations. As a consequence, many infected seedlings leave nurseries unnoticed and develop disease symptoms after they are planted in favourable conditions (e.g. in wet stations) or after heavy rains or periodic flooding when soil is saturated with water. Before dying, infected plants become a source of infection for adult trees growing in the vicinity via soil and root contacts [61]. Many results indicate that the water used for irrigation is one of important sources of phytophthorosis (of roots and bases of trunks) in many plant species [62-65]. Observed since the end of the 1990s, death of black alders along the banks of rivers in Great Britain (but later also in many other European countries), was caused by *P. alni* Brasier & S.A. Kirk, *P. uniformis* Brasier & S.A. Kirk and *P. multiformis* Brasier & S.A. Kirk [66-69]. Spores of these species were spread with water, often hundreds of kilometers from where the disease appeared for the first time. When propagules of oomycetes are present in watercourses, their further dissemination is inevitable [70]. The combined system of rivers and watercourses means that they can move from country to country. Alders can also be affected by *P. plurivora* [71,72] but also it was found that an isolate of *P. plurivora* from Serbia could infect seedlings of Polish silver birch (*Betula pendula*) in the greenhouse [73]. In nurseries one can try to limit the disease by selection of healthy plants, in contrast to forest plantations where the pathogen can freely spread, causing economic losses. Pesticides used in forest nurseries, according only mask the diseases, and as a consequence, ca. 80% of the seedlings transferred from nurseries to forest plantations are probably infected but asymptomatic [4].

Silver birch decline in Poland became more prevalent starting in the 1980s, but phytophthorosis was not detected, although cankers on their stems and reddish exudates were commonly noticed then [74,75]. The mortality of birches was instead connected at that time with severe drought and

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water uptake problems by roots damaged by *Armillaria* fungi. Armillaria root disease caused by *A. ostoyae* (Romagn.) Herink is a common disease in many parts of the Northern hemisphere including western North America and Europe [27], and with changing climate the incidence of root disease and butt rot caused by *A. ostoyae* is likely to increase causing direct and indirect tree mortality [76]. Nevertheless, most *Armillaria* fungi existing in broad-leaved forests are considered to be opportunistic secondary pathogens (like *A. gallica*), so they usually act after primary pathogens such as *Phytophthora* species. The combination of oomycetes (*P. alni, P. plurivora, P. cambivora* and *P. quercina*) as primary pathogens and fungi (*Armillaria* spp.) as secondary ones was already noticed in alder, ash, beech, and oak stands in UK and Serbia [2,77]. It was confirmed that *A. gallica* mainly attacks fine roots, which start to rot and separate from main roots, while at the separation point, elongated wounds are followed by callus formation and characteristic scars [2]. Subsequently, the substances secreted from the scars stimulate the growth of rhizomorphs from the inoculum of *A. gallica*. The rhizomorphs usually attach to the surface of thicker roots, feeding saprophytically, but when plants are weakened, the fungus begins infection [78,79]. Based on our observations, we suggest that similar interactions take place in Polish birch stands. Although many birches died rapidly once the crown symptoms appeared (die-back), in other stands the disease took a chronic form with the loss of foliage and branch dieback increasing over time suggesting the activity of some resistance or tolerance mechanism [73]. Our research elucidated that *B. pendula* can be a host for *P. cactorum* and *A. gallica* in Poland, and our subsequent investigations focused on interactions between pathogen infections and defoliation of birch seedlings.

4.2. Birch Damage (Defoliation) Caused by Insect Pests

In general, insects responsible for the defoliation of birch generally belong into several groups, i.e. endophagous (mining insects), foliovores (eating whole leaves), aphids, skeletal, cambio and xylophages. Among others, the endophagous insects are mostly responsible for defoliation of birch trees. For instance, *Scolioneura betuleti* (Zdd.) is the most common mining insect on birch leaves in Poland [80]. This species usually has two generations a year, with adults of the first generation appearing in May and June, and of the second one in August and September. Their larvae feed inside the leaf, eating a layer between the lower and upper leaf blades, leading to browning and dying of leaves [81,82]. Another mining species of the genus *Scolioneura*, i.e. *Messa nana* (Kl.) mainly feeds on downy birch (*B. pubescens*), but it can also occur sporadically on silver birch. *Fenusa pusilla* (Lepel.) and *Agromyza alnibetulae* (Hend.) also occur on thirteen birch species, including silver and downy birch [83-85]. These insects have also two generations a year and mature individuals occur in June and at the end of August [85]. In our experimental design, the defoliation of birch seedlings was performed in June (similarly to insect attack in forest) and aimed to mimic the defoliation caused by endophagous and foliovore insects of birch with defoliation of 50% of the leaves gradually occurring over a 4 week period (25% of each leaf cut off per week).

Many foliovores can be observed on birch leaves, e.g. larva of *Anisostephus betulinum* (Kieff.) which feed on the galls, or the beetle *Agelastica alni* (L.), which prefers alders but during outbreaks also feeds on birch trees [85]. The endophagous and foliovorous species very often weaken trees but rarely cause mortality of deciduous trees species [86,87], and this was also observed in our experiments.

In our study, the partial defoliation of host (*B. pendula*) probably simulated damage by an aphid group of folivorous insects often occurring in broad-leaved forests, which do not cause direct total defoliation or immediate death of the host, but provoke weakening of the host through gradual and partial defoliation. Among the most common aphids in Poland are the following: *Glyphina betulae* L., *Euceraphis punctipennis* Zett. and *Callipterinella tuberculata* Heyden which all feed on young shoots or leaves of birch [88-90]. These aphids inhabit mostly birch, and feed in colonies on the lower side of the apical leaf, thereby limiting plant growth (primary stress). The honeydew excreted by aphids is very often a medium for fungi developing on the surface of leaves and young shoots [85].

One of the most common skeletal insects that feeds on different species of birch is *Deporaus betulae* (L.). Studies carried out in the Czech Republic by Urban [31] indicated that the insect was found on hornbeam (*Carpinus betulus* L.), black alder (*Alnus glutinosa* Geartn.), common hazel (*Corylus avellana* L.), and sporadically on beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* Matt.), small-leaved linden (*Tilia cordata* Mill.) or broad-leaved linden (*Tilia platyphyllos* Scop.). In our experiment we reduced the total foliar cover by 50%. The same authors [31] demonstrated that trees attacked by *D. betulae* compensated for the reduction of assimilative surface area by increasing the area of adjacent intact leaves by an average of 12.7%. A similar reaction was observed in formation of a greater number of leaves by *Betula pubescens* Ehrh. attacked by *Operophtera brumata* [91]. However, in our study we did not take this phenomenon into account as we did not measure the surface of leaves before and after defoliation, and we only tried to mimic natural feeding of insects by sequential removal of foliage (not 50% all at once).

4.3. Effects of Stress Factors on Physiological Response of Plants

The impact of harmful biotic factors on the forest tree species is often assessed by visual estimation of the general health condition of the crown [92,93]. *P. cactorum* and *A. gallica* directly destroy the plant root system, and in consequence influence the condition of foliage. After one year into our experiment, the influence of defoliation stress on plant health status was more pronounced than the influence of each pathogen alone. The lowest health status estimated was found with the treatment of both pathogens (*Phytophthora* and *Armillaria*) combined with defoliation. Perhaps the damage to roots caused by pathogens was masked by partial defoliation, which caused less visual disease symptoms. Because all investigated stress factors can affect birch root or foliage, we first attempted to examine plant general response at the level of fine root damage, and later - at the level of photosynthetic activity.

4.3.1. Root Status of Treated Plants

In our study, we placed *P. cactorum* directly into the soil to simulate infections in natural conditions in forests, without wounding because that is a very invasive method. After 72 hours of flooding, zoospores are released from sporangia of oomycetes and swim actively among water-saturated soil particles looking for fine roots of plants to be infected [39]. After inoculation and observation of disease symptoms, and through baiting techniques and observing infection of leaves (oak and beech), this demonstrated the viability and virulence of *P. cactorum* inoculum. By re-isolation on artificial media (PARPH), and obtaining pure cultures and comparing them with the original *P. cactorum* inoculum we also completed Koch's postulates. The observed increase of

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phenolic compounds in birch roots and development of rhizomorphs of *A. gallica* attached to roots demonstrated that the infection method we chose was successful [50].

The combination of the stress factors (two soil borne pathogens and defoliation), decreased significantly the development of root collar thickness in birch seedlings. Surprisingly, *P. cactorum* alone seemed not to play an important role in the development of this growth parameter, but its effect was more pronounced in the combination with *A. gallica* and artificial defoliation. Statistically significant decrease of birch fine root length by ca. 75% and fine root surface areas by ca. 73% took place only in the case of multi-interactions (*P. cactorum* + *A. gallica* + 50% defoliation, and *P. cactorum* + *A. gallica*). Lower level damage (15%) of the root system caused by rot has been observed by Mauer and Palátová [25] on root systems of 20-year-old birches affected by defoliation and on root systems of 15-year-old birches in the Ore Mountains (Czech Republic). Our findings were in agreement with their results showing that the defoliation damage was positively correlated with the degree of root rot and the depth of rooting. The proportion of roots affected mainly by root rot fungi increased with increasing levels of defoliation, which was also observed in our investigation. In the case of the Ore Mountains study, the dominant fungi developing on roots were *A. gallica* (like in Polish case) and *A. ostoyae* (also very common in Polish forests). Defoliated trees showed a clear loss of small roots, deteriorated longevity of small roots, and a switch from ectomycorrhizae to ectendomycorrhizae composition [25].

4.3.2. Photosynthetic Activity

The amount of fluorescence emitted by PSII directly enables the assessment of the physiological condition of the photosynthetic apparatus and allows the estimation of plant vitality [41, 94]. Defoliation caused by pests and pathogens are often reflected in changes in fluorescence parameters [95-97]. For instance, area- and mass-based (Asat) coefficients measured on *B. pendula* leaves grazed upon by beetle *Agelastica alni*, linearly declined with increasing amount of leaf perforation [95]. In our experiments, the interaction with two pathogens and partial defoliation diminished by 30% the value of the total performance index (PI total) compared to untreated plants. The only observed increase of total PI was in plants infected by *P. cactorum*, and this was probably due to the triggered natural mechanism of compensation as response of birch leaves to the primary agent of fine root infection. At least at the beginning, the strategy of plants fighting against a recognized pathogen could be increasing the efficiency of photosynthesis [98,99]. Similar decrease of the PI parameter was observed in wheat after infection by *Fusarium* sp. Because of the high sensitivity of the photosynthetic apparatus, chlorophyll fluorescence has been used for early detection of plant disease in asymptomatic plants, enabling prevention measures e.g. against the fusarioses in crops [100]. Physiological stress assessed by photosynthesis effectiveness resulting in PI total < 1 can be informative about weakened plants susceptible to natural infections by nearby pathogens.

4.4. Effects of Stress Factors on Host Chemical Compound Production

Plants respond to the harmful biotic factors by multiple mechanisms of defence, including induced systemic resistance (ISR) and systemic acquired resistance (SAR), both based on hormonal signaling and increased synthesis of secondary metabolites. Induced mechanisms of plant resistance can both act locally (at the site of infection) as well as throughout the plant by a cascade of signalling

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molecules (messengers) [101,102]. As a consequence, plants develop resistance mechanisms against subsequent insect attack or pathogen infection by producing the specific defense compounds, such as aromatic esters (benzyl acetate, methyl salicylate), aromatic alcohols (p-cresol), monoterpenes (neral, geraniol, eugenol), as well as sesquiterpenes (α–copaene, β-bourbonene), and oxygen derivatives of these two groups of chemicals [103-105]. Generally, the secondary metabolites have two major roles in stressed plants: (i) the development of ISR or SAR mechanisms, and (ii) the direct protection of plants against harmful pests and pathogens [106].

In our experiment, the signalling molecules secreted by birch leaves comprised molecules involved in SAR development, i.e. monoterpenes (geraniol and eugenol), triterpenes (lupeol, betulin) as well as aromatic esters including methyl salicylate. The amount of compounds known as important signalling molecules in SAR increased substantially in birch leaves and roots subjected to defoliation or pathogen infection coupled with defoliation treatment. The interactions between soil borne pathogens on the production of secondary metabolites by birch (such as leading to SAR development) have not been sufficiently studied, and therefore we focused on the effects of the fungus (*A. gallica*) and the oomycete (*P. cactorum*) on the chemical composition of compounds secreted by leaves and roots. The significantly lower content of sesquiterpenes in the leaves of the treated birches (especially during *A. gallica* infection) compared to the control may result from the redirection of these substances from leaves to the roots, where they can directly interact with the pathogen. Due to their lipophilic properties, sesquiterpenes and phyto-flavonoids produced after pathogen penetration into plant cells, take part in the direct inhibition of harmful microorganisms [107]. We contend that *A. gallica* benefited from the weakening of plants (after plant defoliation), and this was reflected in the level of defensive substances (phenolic compounds, triterpenes or sterols) which were reduced (compared to defoliation alone or *Armillaria* treatment alone), hence the infection process. *Phytophthora* could also mask its presence in plants because birch seedlings did not react like in the case of root infection by *Armillaria*, as the level of phenolic compounds in roots rose only about 5-6 times (not 15 times like in the case of the fungus). Keča et al. [108] showed that *P. plurivora* introduced into the soil two months before stem inoculation of ash seedlings with *Hymenoscyphus fraxineus* (T. Kowalski, Baral, Queloz, Hesoya, comb. nov.) increased plant tolerance to fungal disease. Treatment with *H. fraxineus* alone caused 100% mortality, while pre-inoculation with *P. plurivora* followed by *H. fraxineus* infections allowed 40% of the plants to survive.

Qualitative and quantitative compositions of extracts of naturally and artificially defoliated birch leaves revealed that the content of free sterols, triterpene compounds and flavones decreased one year after defoliation, but the amount of flavanones and flavanonols were increased [109]. The increased production of such secondary compounds has protective functions as a part of the plant's strategy in the fight against harmful insects [110,111]. The birch seedlings were also rich in numerous sterols in roots of defoliated plants and those infected by pathogens. It has been recently demonstrated that sterols (especially ergosterol) have antimicrobial function by binding with pathogenesis-related protein PR1a from tobacco infected by oomycetes [112]. The concentration of antimicrobial compounds increased in the leaves of defoliated red oaks in comparison to the non-defoliated plants, but the total amount of nitrogen, sugars, proteins, starch, lignin and hemicellulose was lower after one year of defoliation [113,114]. The defoliated trees had reduced

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amounts of nutrients, i.e. monosaccharides, amino acids or fatty acids, in order to reduce their attractiveness for herbivorous insects [115].

The content of secondary metabolites in birch tissues may be influenced by many other factors, such as: species, age, or health condition [116]. We only dealt with young *B. pendula* growing under different stress conditions and probably cannot fully extrapolate these results directly to adult trees. Nevertheless, defoliation can invoke the SAR mechanism of defence, especially if it occurs for the first time and is accompanied by simultaneous root infection [117]. Another aspect worth studying in the future is coordinated defence mechanisms by distance, e.g. several VOCs or terpenoids may be involved in signaling among neighbouring poplar species attacked by insects or herbivorous animals [102].

4.5. Genetic Plant Resistance, Variability of Pathogens and Their Pathogenicity Levels

Many trees, including birch, show certain levels of resistance to insect defoliation at the genetic level [113,118]. This is thanks to increment of other compounds like enzymes e.g. chitinase (in addition to phenols, terpenes and sterols), which can damage fungal cell walls. Phylogenesis of chitinase and its effect on the estimation of horizontal gene transfer from transgenic birch (*B. pendula*) has been the subject of Lohtander research group [119]. Chitinases are hydrolytic enzymes used in biotechnology in attempts to increase plant resistance to fungal pathogens [118]. Genetically modified plants usually raise concerns about the spread of transgenes into the environment through vertical or horizontal gene transfer (HGT). However, Lohtander et al. [119] identified chitinase-like sequences (from EST libraries) from birches and studied their phylogenetic relationships with other chitinases. Phylogenetic analyses were used to estimate the frequency of chitinase gene transfers between plants and other organisms, and the usefulness of phylogenetic analysis as a source of information to assess the risk of transgenic silver birch possessing the sugar beet chitinase gene. Thirteen partial chitin-like sequences with an approximate length of 600 base pairs were obtained from EST libraries. The sequences belonged to five classes of chitinase. Some bacterial chitinases from *Streptomyces* and the *Burkholderia* genus, as well as chitinase from *Phytophthora infestans*, grouped together with plant class IV chitinases, support the hypothesis that some class IV chitinases in bacteria evolved from eukaryotic chitinases through horizontal gene transfer. According to analyzes by Lohtander et al. [119], HGT gene from chitinase IV from eukaryotes to bacteria probably only occurred once. On this basis, the probability of HGT of the chitinase IV gene from transgenic birch to other organisms is very low.

The pathogenicity and genetic variability of *P. cactorum* originating from silver birch were investigated by Lilja et al. [120]. Inoculation of birch seedlings *B. pendula* with the pathogen *P. cactorum* caused necrotic lesions, and the spread of these changes around the stems led to their dying. Strains of the *P. cactorum* pathogen were pathogenic to host plants and were isolated from necrotic tissues at the base of birch seedlings *B. pendula* trunks. The same results we obtained in our experiment performed in 2018 [38]. However, in Lilja's experiment only include isolates derived from birch caused clear changes on unprotected birch bark, while in our experiment the *P. cactorum* (GenBank number KX242303) originated from oaks [37]. In Lilja et al. [120] experiment *P. cactorum* isolates derived from birch proved to be harmless to strawberries. Moreover, RAPD based analysis

revealed variability among *P. cactorum* species, isolates from silver birch differed in genetic profile from those of strawberries. UPGMA analysis grouped isolates obtained from birches and strawberry plants. These data proved that in Finland the recent outbreak of *P. cactorum* on birches could not have been caused by the import of strawberries affected by their rot [17]. In Poland we did not check this matter but *P. cactorum* was recorded as early as in 1940's [121] and often was considered as pathogen of European beech *Fagus sylvatica* (L.), causing characteristic discolorations and lesions on its cotyledons. It is very likely that the pathogen could spread from beech to birch seedlings and along with them was transferred to forest and now we can found it in diseased oak stands in Krotoszyn Plateau Forest District (data not published). The amount of oak stand decline was correlated with its genetic structure, as the more heterozygotes were found in the healthier stands [122].

In our study we focused only on species of *Armillaria* as fungi, and these often associate with trees (rhizomorphs are superficially attached to the external bark at the root collar); and when host plants are weakened, the infection process usually starts. However, the role of other fungi in this process needs more investigation. They may accelerate the speed of tree death (synergy) or slow it down if fungi show antagonistic interactions such as *Trichoderma* spp. killing rhizomorphs of *Armillaria* [123].

5. Conclusions

In summary, this research on birch seedlings subjected to partial defoliation, fungal infection by *A. gallica*, oomycete infection by *P. cactorum*, and combination thereof demonstrated the following:

1. Silver birch (*Betula pendula*) in Poland is a potential host for the oomycete pathogen *P. cactorum*.
2. Defoliation stress and infection by pathogens such as *A. gallica* and *P. cactorum* affect the chemical composition of metabolites produced by the leaves of *B. pendula*.
3. *P. cactorum* in interaction with *A. gallica* causes mortality of birch seedlings during one growing season.
4. *P. cactorum* increased efficiency of the photosynthetic apparatus suggesting not only oomycete cryptic development (in contrast to *A. gallica*) but also possible development of natural compensation mechanisms as responses of birch leaves to the primary agent of fine root infection.
5. *A. gallica* infection caused significant decrease of sesquiterpenes content in the leaves of the treated birches revealing possible redirection of these groups of chemical compounds from leaves to the roots, where they can directly interact with the pathogen during SAR.
6. The amount of fatty acids in roots of birches decreases under the imposed stresses (foliar insects and fungal infection); in contrast the amount of phenolic compounds, terpenes, sterols and alcohols increases. Fungal infection in birch roots causes more pronounced changes in the levels of phenolic compounds and triterpenes than infection caused by the oomycete.

Supplementary Materials: Table S1: Detailed chemical composition of VOC emitted from birch seedling leaves in the following experimental treatments: 1 – control; 2 - *Armillaria gallica*; 3 - *Phytophthora cactorum*; 4 - Defoliation 50%; 5 - *Armillaria gallica* + defoliation 50%; 6 - *Phytophthora cactorum* + defoliation 50%; 7 - *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; 8 - *Armillaria gallica* + *Phytophthora cactorum*., Table S2: Detailed chemical composition of extracts from birch seedling roots in the following experimental treatments: 1 – control; 2 - *Armillaria gallica*; 3 - *Phytophthora cactorum*; 4 - Defoliation 50%; 5 - *Armillaria gallica* + defoliation 50%; 6 - *Phytophthora cactorum* + defoliation 50%; 7 - *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; 8 - *Armillaria gallica* + *Phytophthora cactorum*.

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Table S1. Detailed chemical composition of extracts from birch seedling leaves in the following experimental treatments: 1 – control; 2 - *Armillaria gallica*; 3 - *Phytophthora cactorum*; 4 - Defoliation 50%; 5 - *Armillaria gallica* + defoliation 50%; 6 - *Phytophthora cactorum* + defoliation 50%; 7 - *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; 8 - *Armillaria gallica* + *Phytophthora cactorum*.

Group of Compounds	$t_{ret.}$[1]	Chemical Content (%) by Treatment							
		1	2	3	4	5	6	7	8
Monoterpenes, including:		**15.95**	**19.23**	**17.42**	**18.50**	**15.19**	**21.59**	**15.46**	**14.22**
Monoterpene $C_{10}H_{16}$[2]	10.44	-	1.00	0.69	-	0.62	0.81	-	0.59
Limonene	11.01	0.33	0.49	0.39	trace[5]	0.46	0.44	0.28	0.48
β-Ocimene	11.59	2.51	1.07	0.58	2.36	0.84	1.39	0.26	0.37
Monoterpene $C_{10}H_{16}$[2]	11.86	0.42	0.53	-	0.42	-	-	-	-
trans-Furanolinalool oxide	12.32	0.44	-	-	0.69	-	-	-	-
cis-Furanolinalool oxide	12.79	0.15	-	-	0.15	-	-	-	-
Terpinolene	12.80	-	-	0.16	-	0.19	0.20	-	-
p-Cymenene	12.80	-	0.18	-	-	-	-	-	-
Linalool	13.14	3.80	2.66	2.70	4.00	2.15	3.34	4.17	2.96
cis-allo-Ocimene	14.02	0.37	0.24	-	0.33	-	0.24	-	-
trans-allo-Ocimene	14.35	0.11	-	-	0.26	-	-	-	-
Lilac aldehyde B	14.38	-	-	-	-	-	0.11	-	-
Monoterpene $C_{10}H_{16}$[2]	14.39	0.16	0.15	-	-	-	-	-	-
Citronellal	14.67	0.25	0.30	0.40	0.36	0.33	0.56	0.41	0.36
cis-Pyranolinalool oxide	15.18	0.34	-	-	-	-	-	-	0.51
trans-Pyranolinalool oxide	15.29	0.29	0.31	0.28	0.28	0.33	0.37	-	-
α-Terpineol	15.76	-	-	-	-	-	-	0.24	-
Monoterpene $C_{10}H_{16}$[2]	15.79	-	-	0.22	-	-	0.29	-	-
β-Cyclocitral	16.64	0.10	0.13	-	0.07	0.14	-	-	-
Citronellol	16.81	0.92	1.72	1.93	1.36	1.33	2.40	1.44	1.46
Neral	17.21	0.79	1.32	1.22	1.12	1.02	1.31	-	-
(*E*)-Geraniol	17.56	2.44	4.70	3.83	3.03	3.50	4.08	3.94	4.62
α-Citral	18.00	0.94	1.91	1.65	1.51	1.66	2.30	0.90	0.95
Eugenol	20.35	1.46	2.29	3.17	2.54	2.32	3.31	3.82	1.92
(*Z*)-Geraniol	20.65	-	0.07	-	-	0.09	-	-	-
Geranyl acetate	20.99	-	-	-	-	-	0.09	-	-
(*E*)-Isoeugenol	22.69	0.13	0.16	0.23	-	0.21	0.33	-	-

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Sesquiterpenes, including:		**7.57**	**6.16**	**18.34**	**19.20**	**14.95**	**11.31**	**1.49**	**1.48**
δ-Elemene	19.86	-	-	0.05	-	-	-	-	-
α-Cubebene	20.18	0.21	0.21	0.51	0.59	0.56	0.42	-	-
Sesquiterpene $C_{15}H_{24}$ [2]	20.28	-	-	-	-	0.10	-	-	-
Cyclosativene	20.68	-	-	0.13	-	-	-	-	-
α-Ylangene	20.77	0.15	0.16	1.72	0.27	0.34	0.43	-	-
α -Copaene	20.89	1.42	1.32	2.27	3.36	3.64	2.10	0.26	0.51
β-Bourbonene	21.14	0.48	0.39	1.48	1.00	1.33	0.73	0.22	trace
Sesquiterpene $C_{15}H_{24}$ [2]	21.28	-	0.15	-	-	-	-	-	-
cis-α-Bergamotene	21.87	0.04	0.02	0.05	-	-	-	-	-
β-Ylangene	21.88	-	-	-	0.08	0.03	0.05	-	-
β-Caryophyllene	22.03	1.09	0.91	1.05	1.38	1.01	1.13	0.49	0.42
β-Copaene	22.26	0.15	0.13	0.35	0.57	0.39	0.25	-	-
trans-α-Bergamotene	22.38	0.18	0.14	0.30	0.30	0.18	0.25	-	-
Guaia-6,9-diene	22.60	0.23	0.25	3.62	0.45	0.35	0.84	0.20	0.25
cis-Muurola-3,5-diene	22.70	-	-	-	0.37	-	-	-	-
Sesquiterpene $C_{15}H_{24}$ [2]	22.76	-	0.11	0.75	0.11	0.19	0.31	-	-
trans-Muurola-3,5-diene	22.80	-	-	-	0.15	-	-	-	-
α-Humulene	22.88	0.17	0.16	0.28	0.72	0.30	0.30	-	-
Sesquiterpene $C_{15}H_{24}$ [2]	22.95	-	-	0.41	-	0.09	0.12	-	-
allo-Aromadendrene	23.07	0.30	0.25	0.61	0.66	0.71	0.36	-	-
cis-Muurola-4(14),5-diene	23.12	-	-	-	0.50	-	0.22	-	-
trans-Cadina-1(6),4-diene	23.37	-	-	-	0.09	-	-	-	-
γ-Muurolene	23.43	0.29	0.30	1.37	1.17	1.28	0.67	-	-
α-Amorphene	23.53	-	-	-	-	0.13	-	-	-
Germacrene D	23.56	0.19	0.17	0.41	1.56	0.13	0.51	-	-
(*Z*,*E*)-α-Farnesene	23.80	0.15	0.07	0.09	-	-	-	-	-
α-Muurolene	24.00	0.16	0.16	0.40	0.66	0.50	0.29	-	-
(*E*,*E*)-α-Farnesene	24.13	1.49	0.55	0.23	1.26	0.71	0.57	0.32	0.30
Sesquiterpene $C_{15}H_{24}$ [2]	24.19	-	-	0.16	-	-	-	-	-
γ-Cadinene	24.35	0.28	0.26	0.72	1.19	1.06	0.52	-	-
δ-Cadinene	24.56	0.41	0.37	0.85	1.96	1.05	0.83	-	-
trans-Cadina-1,4-diene	24.79	-	-	0.04	0.09	0.04	0.04	-	-
α-Cadinene	24.90	0.03	0.03	0.10	0.18	0.09	0.07	-	-
α-Calacorene	25.05	0.03	0.03	0.14	0.15	0.08	0.08	-	-
Salviadienol	25.30	-	-	0.03	-	0.03	-	-	-
(*E*)-Nerolidol	25.45	-	-	-	-	-	0.08	-	-
β-Calacorene	25.53	-	-	0.03	0.03	0.42	-	-	-
Humulene epoxide II	26.63	-	-	-	0.05	0.04	-	-	-
Sesquiterpene $C_{15}H_{24}$ [2]	26.78	0.14	-	-	0.13	0.07	-	-	-
α-Corocalene	26.89	-	-	0.03	0.04	-	0.03	-	-
τ-Muurolol	27.31	-	-	0.05	0.05	0.05	-	-	-

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Cadalene	28.07	-	-	0.04	0.04	0.06	0.04	-	-
Guaiazulene	30.27	-	-	0.08	-	-	0.06	-	-
Aromatic Esters, including:		**5.52**	**5.91**	**8.05**	**3.96**	**7.12**	**6.68**	**4.98**	**4.66**
Benzyl acetate	14.99	0.14	0.17	0.21	-	-	0.25	-	-
Methyl salicylate	15.90	4.97	5.27	7.46	3.26	6.87	5.91	4.67	4.40
(*Z*)-3-Hexenyl benzoate	25.64	0.36	0.30	0.23	0.42	0.14	0.33	0.32	0.26
Hexanyl benzoate	25.78	-	0.12	0.10	0.19	0.05	0.12	-	-
(*E*)-2-Hexenyl benzoate	25.95	0.05	0.04	0.05	0.09	0.06	0.06	-	-
Aromatic Carbonyls, including:		**1.32**	**3.87**	**4.03**	**2.47**	**4.37**	**7.12**	**5.46**	**6.98**
Benzaldehyde	8.98	0.68	3.03	3.20	2.03	3.66	6.28	4.49	5.44
Benzene acetaldehyde	11.43	0.64	0.84	0.83	0.44	0.71	0.84	0.97	1.54
Aromatic alcohols, including:		**5.64**	**8.35**	**8.82**	**7.60**	**7.02**	**10.62**	**11.75**	**12.47**
Benzyl alcohol	11.16	4.14	5.92	5.96	5.55	4.76	7.60	7.26	8.76
p-Cresol	12.35	-	0.63	0.77	-	0.42	0.90	0.41	0.48
2-Phenylethyl alcohol	13.51	1.50	1.80	2.09	2.04	1.85	2.12	4.08	3.23
Aliphatic Esters, including:		**14.41**	**6.27**	**3.75**	**6.97**	**5.11**	**3.86**	**2.85**	**3.31**
(*Z*)-3-Hexenyl acetate	10.37	3.42	1.17	0.80	1.53	0.87	1.07	trace	0.77
Hexyl acetate	10.55	1.62	0.30	0.59	0.78	0.36	0.36	0.24	0.26
(*E*)-3-Hexenyl acetate	10.64	4.23	0.77	0.56	0.77	-	0.43	-	0.69
(*Z*)-2-Hexenyl acetate	10.64	-	-	-	-	0.56	-	-	-
(*Z*)-3-Hexenyl butanoate	15.64	1.42	0.89	0.36	0.65	0.65	0.41	0.55	0.33
Hexyl butanoate	15.79	0.57	0.33	-	0.42	0.29	-	-	-
(*Z*)-3-Hexenyl 2-methylbutanoate	16.94	0.79	0.79	0.37	0.47	0.84	0.32	0.57	0.45
(*Z*)-3-Hexenyl (*E*)-2-butenoate	17.03	0.84	0.64	0.36	0.67	0.41	0.50	0.44	0.36
(*Z*)-2-Hexenyl isovalerate	17.10	0.41	0.35	0.13	0.30	0.31	0.12	-	-
(*Z*)-3-Hexenyl pentanoate	18.35	0.04	-	-	-	-	-	-	-
Ethyl nonanoate	18.69	-	-	-	-	-	0.04	-	-
(*E*)-3-Hexenyl tiglate	19.16	0.03	-	-	0.02	-	-	-	-
(*Z*)-3-Hexenyl tiglate	19.45	0.48	0.45	0.28	0.59	0.27	0.33	0.51	0.45
Hexyl tiglate	19.60	0.14	0.11	-	0.19	0.12	-	-	-
(*E*)-2-Hexenyl tiglate	19.80	0.28	0.23	0.14	0.40	0.21	0.15	-	-
(*Z*)-3-Hexenyl hexanoate	20.93	-	-	-	-	-	-	0.31	-
(*Z*)-3-Hexenyl (*Z*)-3-hexenoate	21.06	0.15	0.23	0.15	0.18	0.21	0.13	0.23	trace
Aliphatic Acids, including:		**1.12**	**-**	**-**	**0.38**	**-**	**0.17**	**11.85**	**9.68**
Acetic acid	2.13	-	-	-	-	-	-	4.06	4.25
Hexanoic acid	9.60	0.57	-	-	-	-	-	2.05	1.60
3-Hexenoic acid [3]	10.20	-	-	-	-	-	-	2.13	2.76

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3-Hexenoic acid [3]	10.32	-	-	-	-	-	-	2.57	-
2-Hexenoic acid [3]	10.76	-	-	-	-	-	-	1.03	1.07
2-Hexenoic acid [3]	12.57	0.11	-	-	-	-	0.13	-	-
Heptanoic acid	13.84	0.43	-	-	0.38	-	-	-	-
Dodecanoic acid	25.33	-	-	-	-	-	0.04	-	-
Aliphatic Carbonyls, including:		**21.58**	**22.47**	**17.12**	**17.21**	**20.79**	**18.32**	**20.66**	**18.30**
Acetone	1.73	-	-	-	-	-	-	0.81	1.70
2-Butenal	2.58	-	-	-	-	-	-	0.45	0.33
Acetoin	3.22	-	-	-	-	-	-	0.26	-
(*E*)-2-Pentenal	3.95	-	-	-	-	-	-	0.46	0.42
Hexanal	4.87	0.80	0.69	0.16	0.24	0.10	trace*	0.56	0.44
(*Z*)-2-Hexenal	5.86	-	-	-	-	-	-	0.13	-
(*E*)-2-Hexenal	6.15	16.78	15.97	12.19	13.48	15.18	12.24	11.70	9.27
Heptanal	7.29	-	-	-	-	-	-	-	0.24
(*E*,*E*)-2,4-Hexadienal	7.55	0.14	0.51	0.16	0.07	0.42	0.30	0.78	0.37
(*Z*)-2-Heptenal	8.87	-	-	0.03	-	-	-	0.19	-
2-Methyl-3-octanone	9.65	-	-	-	-	-	-	-	0.25
6-Methyl-5-hepten-2-one	9.77	1.51	1.85	1.28	1.16	1.31	1.34	0.84	0.95
(*E*,*E*)-2,4-Heptadienal	10.02	-	-	-	-	-	-	0.36	0.47
Octanal	10.23	-	0.47	-	-	0.49	0.59	-	-
(E,E)-2,4-Heptadienal	10.41	-	-	-	-	-	-	0.66	-
(*E*)-2-Octenal	11.84	-	-	-	-	-	-	0.37	-
(*Z*)-2-Octenal	11.87	-	-	0.42	-	0.48	0.49	-	0.47
Nonanal	13.25	1.18	1.38	1.35	1.16	1.30	1.59	2.28	2.86
(*E*)-2-Nonenal	14.85	0.12	0.15	0.14	0.14	0.15	0.18	0.23	trace
Decanal	16.17	0.51	0.71	0.69	0.36	0.65	0.74	0.38	0.54
(*E*)-2-Decenal	17.74	0.12	0.20	0.16	0.11	0.16	0.21	0.18	trace
Undecanal	18.98	0.13	0.15	0.14	0.11	0.14	0.16	-	-
Dodecanal	21.64	0.14	0.16	0.18	0.16	0.18	0.19	-	-
Tetradecanal	26.54	0.04	0.06	0.05	0.06	0.06	0.07	-	-
Pentadecanal	28.81	0.07	0.10	0.08	0.10	0.10	0.12	-	-
Hexadecanal	30.96	0.03	0.03	0.03	0.03	0.04	0.05	-	-
Hexahydrofarnesyl acetone	31.55	0.03	0.04	0.04	0.04	0.04	0.05	-	-
Aliphatic Alcohols, including:		**15.36**	**15.31**	**12.76**	**13.38**	**15.78**	**8.43**	**17.13**	**21.41**
(*Z*)-2-Penten-1-ol	4.22	-	-	-	-	-	-	0.73	0.70
(*Z*)-3-Hexen-1-ol	6.09	-	-	-	-	-	-	10.02	11.91
(*Z*)-2-Hexenol	6.46	12.54	10.58	7.79	9.45	11.68	2.26	1.85	2.17
1-Hexanol	6.48	-	-	-	-	-	-	1.77	2.08
Hexylene glycol	7.83	0.06	trace	0.17	0.28	0.25	0.21	0.42	1.14
6-Hepten-3-ol	7.84	-	0.40	-	-	-	-	-	-
1-Heptanol	9.25	-	-	0.77	-	-	1.24	-	0.36

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6-Methyl-5-hepten-2-ol	9.93	2.53	3.54	3.15	2.76	3.01	3.55	1.53	2.51
(*E*)-2-Octen-1-ol	12.17	-	-	-	-	0.10	-	-	-
(*Z*)-2-Octen-1-ol	12.25	-	-	0.44	-	-	-	-	-
1-Octanol	12.25	0.22	0.46	trace	0.51	0.41	0.55	0.47	0.56
1-Nonanol	15.19	-	0.32	0.44	0.37	0.34	0.62	0.34	-
Alkanes and Alkenes, including:		**7.12**	**8.65**	**7.04**	**7.44**	**6.62**	**9.62**	**2.73**	**1.83**
1-Undecene	12.85	0.26	0.20	0.17	0.20	0.17	-	-	-
(*E*)-4,8-Dimethyl-1,3,7-nonatriene	13.61	1.26	1.14	0.86	0.48	1.36	1.78	0.34	0.31
n-Dodecane	16.00	1.04	1.18	-	0.73	-	1.26	0.32	-
1-Tridecene	18.55	0.36	0.44	0.38	0.33	0.36	0.40	-	-
n-Tridecane	18.78	0.67	1.34	0.52	0.65	0.57	0.72	0.42	0.77
1-Tetradecene	21.20	1.00	1.44	1.12	1.49	1.26	1.61	0.45	0.24
n-Tetradecane	21.40	0.59	0.82	0.72	0.71	0.70	0.85	0.34	0.27
1-Pentadecene	23.70	0.24	0.29	0.77	0.28	0.28	0.38	-	-
n-Pentadecane	23.89	0.51	0.66	1.01	1.05	0.82	0.93	0.33	0.25
1-Hexadecene	26.07	0.51	0.56	0.91	0.79	0.53	0.88	0.30	trace
n-Hexadecane	26.24	0.45	0.32	0.34	0.46	0.35	0.41	0.23	trace
1-Heptadecene	28.33	0.05	0.05	0.06	0.05	0.05	0.08	-	-
n-Heptadecane	28.48	0.16	0.16	0.16	0.16	0.15	0.22	-	-
1-Octadecene	30.47	-	-	-	0.02	-	0.03	-	-
n-Octadecane	30.61	0.03	0.04	0.03	0.04	0.04	0.05	-	-
n-Nonadecane	32.64	-	-	-	-	-	0.01	-	-
Other Compounds, including:		**1.78**	**1.62**	**1.27**	**1.53**	**1.56**	**1.38**	**3.88**	**4.31**
2-Ethylfuran	3.13	-	-	-	-	-	-	0.17	-
γ-Hexalactone	11.73	0.87	0.64	0.41	0.72	0.72	0.44	1.64	1.84
Naphtalene	15.54	0.06	-	-	-	-	-	-	-
1-(2-Butoxy-1-methoxy)-2-propanol, isomer 1 [4]	17.19	-	-	-	-	-	-	1.17	1.34
1-(2-Butoxy-1-methoxy)-2-propanol, isomer 2 [4]	17.33	0.71	0.80	0.74	0.81	0.68	0.81	0.89	1.13
(*E*)-*β*-Ionone	23.62	0.14	0.18	0.11	-	0.15	0.12	-	-
Unidentified Compounds		**2.63**	**2.17**	**1.40**	**1.38**	**1.49**	**0.89**	**1.76**	**1.35**

[1] Retention time (min); [2] Based on the GC-MS analysis, the compound was identified as monoterpene/sesquiterpene with the chemical formula, but the chemical structure of compound was not specified; [3] isomer (*E*) or (*Z*); [4] *n*-butyl or *iso*-butyl; [5] trace – below 0.01%.

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Table S2. The detailed chemical composition of extracts from birch seedling roots in the following experimental treatments: 1 – control; 2 - *Armillaria gallica*; 3 - *Phytophthora cactorum*; 4 - Defoliation 50%; 5 - *Armillaria gallica* + defoliation 50%; 6 - *Phytophthora cactorum* + defoliation 50%; 7 - *Armillaria gallica* + *Phytophthora cactorum* + defoliation 50%; 8 - *Armillaria gallica* + *Phytophthora cactorum*.

Group of Compounds	$t_{ret.}$ [1]	Chemical Content (%) by Treatment							
		1	2	3	4	5	6	7	8
Phenolic Compounds, including:		**0.87**	**15.35**	**5.02**	**11.91**	**4.83**	**4.36**	**7.85**	**13.32**
(*E*)-p-Coumaric acid	46.38	-	-	-	-	-	-	-	0.12
(*Z*)-Caffeic acid	48.12	-	9.51	-	0.42	-	-	0.18	0.48
(*E*)-Caffeic acid	52.74	-	1.92	1.89	1.95	1.75	2.08	2.46	2.59
Catechin	72.47	0.87	1.34	0.98	0.89	0.98	0.87	3.35	2.70
Cirsimaritin	76.74	-	-	-	-	-	-	-	0.16
α-Tocopherol	77.01	-	0.44	0.31	0.65	0.31	0.31	0.23	0.51
Flavonoid glucoside [2]	86.00	-	1.68	1.83	5.03	1.41	0.77	1.11	5.23
1-Eicosyl p-coumarate	88.19	-	-	-	0.24	-	-	-	-
1-Docosyl caffeate, isomer 1 [3]	90.97	-	-	-	-	-	-	-	0.11
1-Docosyl caffeate, isomer 2 [3]	94.24	-	-	-	0.88	-	-	-	0.75
1-Tetracosyl p-coumarate	95.07	-	-	-	0.26	-	-	-	-
Triterpenes, including:		**1.25**	**3.63**	**2.73**	**8.64**	**4.46**	**3.69**	**4.82**	**10.25**
Triterpene $C_{30}H_{50}$ [4]	74.28	0.72	-	-	-	-	-	-	-
Triterpenoid $C_{30}H_{48}O$ [4]	78.79	-	0.71	0.77	0.94	0.57	0.59	0.78	0.93
Triterpenoid $C_{30}H_{48}O$ [4]	78.95	-	0.36	-	0.44	-	0.33	0.38	0.42
Triterpenoid $C_{30}H_{48}O_2$ [4]	79.37	0.53	1.33	-	1.30	1.49	1.00	1.15	1.00
Triterpenoid $C_{30}H_{46}O_2$ [4]	79.52	-	0.48	1.02	0.57	1.17	0.31	0.39	0.41
Triterpenoid [2]	80.22	-	-	-	-	-	-	0.75	-
Triterpenoid $C_{30}H_{46}O_2$ [4]	80.72	-	-	-	-	-	-	0.46	-
Lupeol	81.89	-	-	-	0.81	0.35	0.54	-	0.39
Triterpenoid [2]	84.09	-	-	-	-	-	-	-	0.27
Triterpenoid $C_{30}H_{46}O_3$ [4]	84.42	-	-	-	-	-	0.27	-	-
Triterpenoid [2]	84.54	-	-	-	-	-	-	-	0.47
Triterpenoid [2]	84.93	-	-	-	0.68	-	-	-	-
Betulin	85.01	-	-	-	0.93	0.38	-	-	1.95
Oleanolic acid	85.26	-	-	-	0.49	-	-	-	-
Betulinic acid	85.56	-	-	-	0.91	-	-	-	1.39
Triterpenoid [2]	86.39	-	-	-	0.74	-	-	-	-
Triterpenoid acetate [2]	86.83	-	-	-	0.83	-	-	-	-
Methyl acetylbetulinate	86.84	-	0.75	0.93	-	0.51	0.65	0.90	3.03
Sterols,		**33.53**	**39.58**	**28.37**	**41.83**	**28.94**	**29.61**	**23.30**	**35.80**

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including:									
Campesterol	79.18	1.00	1.61	1.13	1.88	1.31	1.18	0.95	1.50
Stigmasterol	79.81	3.51	4.87	2.53	4.81	3.02	3.28	2.72	3.41
β-Sitosterol	80.96	28.54	31.46	23.57	32.15	24.07	24.04	18.76	28.22
Stigmastanol	81.14	0.48	1.29	1.15	1.89	0.54	1.12	0.86	1.60
Avenasterol	81.27	-	-	-	-	-	-	-	0.32
Steroid $C_{29}H_{48}O$ [4]	82.73	-	0.35	-	1.09	-	-	-	0.75
Fatty Acids, including:		**44.47**	**21.47**	**32.30**	**15.65**	**32.10**	**33.93**	**35.82**	**15.50**
Hexanoic acid	12.73	-	-	-	0.10	0.21	0.20	-	0.10
Dodecanoic acid	36.53	-	-	-	-	-	-	-	0.18
Tetradecanoic acid	43.39	-	0.28	0.33	0.20	0.32	0.43	0.33	0.22
Pentadecanoic acid	46.59	-	-	-	-	-	0.27	0.27	0.13
Palmitelaidic acid	49.33	-	-	0.24	-	-	0.17	-	-
Palmitic acid	49.69	16.30	0.61	12.16	4.98	11.27	11.04	11.13	4.64
Heptadecanoic acid	52.64	-	-	0.20	-	-	0.16	0.24	0.08
Linoleic acid	54.52	15.04	10.22	9.32	3.52	9.53	10.73	11.14	4.00
Oleic acid	54.69	7.76	4.95	3.76	2.31	4.49	4.75	5.14	1.92
(*E*)-9-Octadecenoic acid	54.89	0.59	0.28	0.31	-	0.29	0.37	0.35	0.14
Stearic acid	55.47	3.48	2.96	3.46	1.68	3.31	3.33	3.24	1.53
Fatty acid [2]	60.45	-	-	0.61	-	0.38	0.34	1.17	0.47
Eicosanoic acid	60.80	0.79	-	-	-	-	-	-	-
Docosanoic acid	65.79	0.51	0.76	0.67	0.77	0.79	0.59	0.57	0.41
Tricosanoic acid	68.15	-	1.07	0.92	0.81	1.14	1.20	2.00	1.18
Tetracosanoic acid	70.43	-	0.34	0.31	0.80	0.35	0.36	0.25	0.37
Hexacosanoic acid	74.78	-	-	-	0.46	-	-	-	0.13
Fatty Alcohols, including:		**4.23**	**5.45**	**6.43**	**6.29**	**9.27**	**6.15**	**7.28**	**5.67**
1-Hexadecanol	47.03	-	0.38	0.29	0.17	0.38	0.43	0.30	0.14
1-Octadecanol	53.00	-	0.36	-	0.15	0.32	0.79	-	0.19
1-Docosanol	63.61	3.09	3.09	2.68	3.65	5.38	3.30	3.99	2.32
1-Tricosanol	66.03	-	-	-	-	0.31	0.24	0.29	0.19
1-Tetracosanol	68.36	1.14	1.62	3.46	2.16	2.88	1.39	2.70	2.82
1-Hexacosanol	72.81	-	-	-	0.16	-	-	-	-
Other Compounds, including:		**8.33**	**8.17**	**12.01**	**8.31**	**8.79**	**15.00**	**9.03**	**7.83**
6-Methyl-5-hepten-2-one	9.39	0.48	-	-	0.43	-	0.33	-	-
Ethylene glycol	9.47	0.53	0.29	0.25	0.22	0.33	0.32	0.36	0.19
Boric acid	9.83	-	-	0.34	0.14	0.21	0.21	0.32	0.22
Lactic acid	12.43	0.63	0.28	0.38	0.17	0.50	0.29	0.29	0.20
Phosphotic acid	21.76	-	-	-	-	-	-	-	0.12
Glycerol	21.96	0.80	0.46	0.50	0.48	0.58	0.57	0.58	0.52
2-(2-Butoxyethoxy)ethyl	24.18	2.20	2.42	4.25	3.16	2.93	7.38	1.23	2.81

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acetate, isomer 1 [5]									
2-(2-Butoxyethoxy)ethyl acetate, isomer 2 [5]	25.12	0.52	-	0.27	0.18	0.37	0.47	0.24	0.31
n-Tetradecane	26.46	-	-	-	0.13	-	-	-	0.17
n-Pentdecane	30.36	-	-	-	-	-	-	0.21	0.10
2-Butoxyethoxyethanol	34.92	-	-	-	-	-	-	0.42	-
n-Heptadecane	38.42	-	-	-	-	-	0.23	-	-
Methylbenzene-sulfamide	40.26	1.04	0.85	0.24	0.15	0.00	0.30	0.54	0.11
Azelaic acid	41.87	-	-	0.25	-	-	0.24	0.28	0.23
Octadecanenitrile	51.28	0.71	-	0.89	0.36	0.67	0.64	0.73	0.12
Hexadecanamide	55.32	0.34	0.44	0.55	0.40	0.40	0.38	0.43	0.21
Oleamide	59.99	-	-	0.39	-	-	-	-	0.17
Oleanitrile	59.99	-	0.33	-	-	-	0.27	-	-
Octadecanamide	60.77	0.56	1.72	1.93	1.53	1.63	1.52	1.60	0.96
1-Monopalmtin	64.94	0.53	0.84	1.21	0.37	0.75	1.11	1.26	0.42
1-Monolinolein	68.86	-	0.54	0.56	0.22	0.43	0.74	0.54	0.47
Heneicosanedioic acid	73.71	-	-	-	0.38	-	-	-	0.51
Unidentified Compounds		**7.30**	**6.35**	**13.14**	**7.39**	**11.60**	**7.26**	**11.90**	**11.64**

[1] Retention time (min).; [2] Based on the GC-MS analysis, the compound was identified as flavonoid glucoside/triterpenoid/triterpenoid acetate/fatty acid, but the chemical formula and the chemical structure of compound were not specified; [3] isomer (*E*) or (*Z*); [4] Based on the GC-MS analysis, the compound was identified as triterpene/triterpenoid/steroid with the chemical formula, but the chemical structure of compound was not specified; [5] *n*-butyl or *iso*-butyl.