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Effect of *Prunus serotina* Ehrh. Volatile Compounds on Germination and Seedling Growth of *Pinus sylvestris* L.

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Abstract: In temperate European forests invaded by *Prunus serotina* Ehrh. (black cherry), a reduction in the spontaneous regeneration capacity of *Pinus sylvestris* L. (Scots pine) is observed. It could be caused by various factors, including allelopathic properties of this invasive plant. In this study the phytotoxic effect of *P. serotina* volatile compounds on *P. sylvestris* and the seasonal variation in this effect were assessed. Simple assays showed that volatiles emitted from *P. serotina* leaves significantly inhibited root growth of *P. sylvestris* seedlings. Their negative effect on stem growth was much weaker. The strongest phytotoxic effect on Scots pine seedlings was caused by the volatiles emitted from the youngest black cherry leaves. In fresh foliage of *P. serotina*, nineteen volatile organic compounds were identified by gas chromatography–mass spectrometry (GC–MS). The dominant compound was benzaldehyde. On the basis of tests of linalool alone, it was found that this monoterpene present in the volatile fraction has a strong allelopathic potential and inhibits germination, root elongation and shoot elongation of pine seedlings. The results of our research suggest that volatile compounds from *P. serotina* leaves could limited survival of *P. sylvestris* individuals in the seedling phase.

Keywords: allelopathy; volatile compounds; linalool; germination; seedling growth; *Prunus serotina*; *Pinus sylvestris*

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1. Introduction

Due to the wide geographical range and small habitat requirements the Scots pine, *Pinus sylvestris* L., is one of the most important forest species in Europe and occurs in many different types of forest communities [1]. The natural Scots pine regeneration depends on many factors, starting with the abundance of seeds in the sowing year and choice of soil preparation method after clear-cutting [2]. Seed germination in the first growth phase depends strongly on the depth and variable humidity of the humus layer, until seedling radicles reach more water-stable mineral soil [3,4]. Moreover, allelochemicals released from coexisting plant species affect the germination and development of young seedlings [5]. Further successful pine recruitment depends on the course of competition for resources with shrubs and trees [6] and the presence of natural enemies [7].

The introduction of invasive tree species reduced the extent of natural Scots pine renewal [8,9]. One of the most important invader plants in European forests is black cherry, *Prunus serotina* Ehrh. This alien species grows in a wide range of habitat conditions, has a high ability of generative reproduction and vegetative sprouting, its numerous seeds are dispersed by birds and mammals and it establishes a long-living shade-tolerant seedling bank [10–12].

In forests dominated by *P. serotina*, alterations of the biotic and abiotic ecosystem properties are observed. The dense shrub of black cherry with its litter layer negatively affects the understory species through mechanical impediment and shading [13–15]. In the case of

Scots pine, it is assumed that light is the factor that most limits sapling growth [16]. Previous research indicates that *P. serotina* could also reduce the growth of *P. sylvestris* seedlings due to the release of phytotoxic compounds during leaf decomposition [17]. Such allelopathic interactions appear to be more intense in soils with low resource availability [18]. At the same time the fast decay of *P. serotina* leaf litter and humus formation accelerate nutrient circulation [19–22] and soil microbial activity [23]. Moreover, self-sowing of black cherry results in increased competition with pine saplings in the regeneration layer [9].

It cannot be ruled out that the emergence of new *P. sylvestris* generations may be also affected by exudates or volatiles released directly from fresh *P. serotina* leaves, which remain on the plant. Drogoszewski and Barzdajn [24] showed in ex situ experiments that water extracts from fresh black cherry leaves inhibit the germination process of Scots pine. No research studies have examined the influence of *P. serotina* volatile organic compounds (VOCs) on natural pine regeneration. We performed an experiment, under laboratory conditions, to evaluate the phytotoxic effects of *P. serotina* volatiles released from fresh leaves on *P. sylvestris* seeds and seedlings, and the seasonal variation of these effects. We tested the following hypotheses: (1) *P. serotina* volatiles affect germination and seedling growth of *P. sylvestris*, (2) radicles and stems of Scots pine seedlings differ in the sensitivity to black cherry volatiles and (3) volatile compound production varies during the growing season.

2. Materials and Methods

2.1. Plant Material

Seeds of *P. sylvestris* used in bioassays were certified material supplied by Kluczbork Forest Division (SW Poland, national seed lot code PL/4228/6/2014).

The sampling site was located in a stand of two- and three-year natural regeneration of Scots pine with self-sowing black cherry in Wołów Forest Division (SW Poland; 51°20′ N, 16°31′ E and 110 m a.s.l.). Scots pine renewal was obtained on a fresh coniferous habitat located on rusty podzolized soil (Polish soil classification), equivalent to Dystric Brunic Folic Arenosol (Ochric) (FAO WRB classification). These soils are sandy, resulting in low water retention and nutrient availability. The cutting area was characterized by a uniform coastal border, without an old forest stand (clear-cut in 2011 after 80 years of cultivation), with soil prepared with tiller and plough. There has been massive planting of black cherry in Wołów FD since 1950, mainly for soil improvement. Since its introduction, it has spread spontaneously and profusely in all forest habitats.

In the area chosen, five saplings of *P. serotina* (up to 0.5 m high, with numerous suckers sprouting) were randomly selected and marked. The plant material was collected in April, June and August 2018. In each of these months, six leafy twigs (about 30 cm long, growing in the lowest part of the sapling, with no signs of any damage) were cut from each sapling, secured in separate zip-lock plastic bags and transported in a cooler.

2.2. Germination and Growth Assays

The biological activity of organic volatile compounds was determined for each of five selected saplings of *P. serotina*, taking into account the three uptake periods. The germination test was conducted in transparent plastic containers (diameter 24 cm, height 13 cm). To begin, ten seeds of *P. sylvestris* were sown in a Petri dish (9 cm diameter) with a layer of filter paper (0.16 mm thick) and moistened with 5 mL of distilled water. Each Petri dish was deposited in the center of the container. Next, fresh leaves of the black cherry were placed with gloved hands around the Petri dish, taking into account three variants of weight: 10, 20 and 40 g. The leaves were without direct contact with the seeds, but allowing the compounds to volatilize into the airspace within the container. The containers were sealed with plastic film to minimize loss of the volatiles. Three replicates were designed for each combination. Seeds germinating on Petri dishes in containers without leaves were used as a control. The experiment was carried out in a germination room with a daily light/dark cycle, day 16 h = 10 klx, night 8 h, 24 °C/14 °C, 60% relative humidity. After

two weeks the germination rate and the seedling growth parameters (length of radicle and stem) were determined. Seeds with minimal root length of 1 mm were considered as germinated seeds.

2.3. Chromatographical Analysis

The composition of volatiles emitted by the black cherry leaves was determined by the solid phase microextraction (SPME) method for sampling volatile organic compounds. The analyses were performed separately for plant material from each sapling at three collection dates. Fresh leaves of *P. serotina* were cut in pieces about 2 cm long, then 1.5 g of plant material was inserted into a test tube and it was tightly closed with a rubber membrane. The membrane was pierced by the stainless steel SPME (DVB/CAR/PDMS 50/30 µm, 2 cm) needle protecting the fiber (Supelco Co., Bellefonte, PA, USA). The volatile compounds were exposed to fiber for about 30 min at 23 °C. After this period the fiber was retracted into the needle. The chemical composition of the volatile compounds absorbed on the fiber was analyzed using a gas chromatograph (GC) coupled to a mass spectrometer (MS) Saturn 2000 (GC–MS system, Varian, Walnut Creek, CA, USA) with a ZB-5 (Phenomenex, Shim-Pol, Warsaw, Poland) column (30 m × 0.25 mm film × 0.25 µm film thickness), conducting desorption for 5 min. The MS was equipped with an ion-trap analyzer set at 1508 for all analyses with an electron multiplier voltage of 1350 V. Scanning (1 scan/s) was performed in the range of 39–400 m/z using electron impact ionization at 70 eV. The analyses were carried out using helium as a carrier gas at a flow rate of 1.0 mL min^{-1} in a split ratio of 1:10 and the following program: 40 °C at the beginning and hold 3 min; 5 °C min^{-1} to 110 °C; 20 °C min^{-1} to 300 °C. The injector and detector were held at 200 and 300 °C, respectively. Identification of compounds were based on three methods: (i) comparison of obtained spectra with databases NIST 17 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and FFNSC [25], (ii) comparison of calculated retention indices using a retention indices calculator [26] with values presented in NIST 17 and FFNSC and (iii) comparison of retention times of unknown compounds with authentic standards. For comparison of mass spectra we used the AMDIS (v. 2.73) (provided by Phenomenex, Shim-Pol, Warsaw, Poland) and GCMS solution (v. 4.20) (provided by Phenomenex, Shimadzu, Kyoto, Japan) programs.

2.4. Allelopathic Bioassay with Linalool

One selected volatile organic compound was tested separately on germination and seedling growth of *P. sylvestris* under the same conditions as before for all volatiles from *P. serotina* leaves. We chose the compound linalool for its potential allelopathic possibilities [27]. Pure linalool (97%) obtained from Sigma-Aldrich (Saint Louis, MO, USA) was dissolved in ethanol. Four different dilutions (5, 10, 25 and 50 µg/mL) were prepared and then a piece of filter paper (2 cm × 1 cm) was impregnated with 1 mL of each solution, kept a few seconds outside to evaporate the solvent and placed in a container next to a Petri dish with pine seeds [28]. Germination rate and the growth parameters of seedlings were obtained as previously described.

2.5. Statistical Analyses

Statistical analysis was conducted using STATISTICA software (v. 13) (TIBCO Software Inc., Palo Alto, CA, USA). The compliance of data with the normal distribution was assessed using the Shapiro–Wilk W test, and the homogeneity of variance was checked by Levene's test. Significance was evaluated in all cases at $p < 0.05$. One-way analyses of variance (ANOVA) followed by Tukey's HSD post hoc tests (for data with normal distribution and homogeneity of variance in the group), or the Kruskal–Wallis tests followed by post hoc tests (for data in which normal distribution or homogeneity of variances was not obtained) were used to determine the differences between the germination capacity, root and stem growth of *P. sylvestris* seedlings, and the different leaf weight of *P. serotina*. The tests and analyses were conducted separately for each month. The response of *P. sylvestris* seeds and

seedlings to the VOC from *P. serotina* leaves—with predetermined weight (10, 20 or 40 g) and depending on leaf collection month—were determined by one-way ANOVAs with Tukey's HSD tests. The differences between the content of compounds in volatile fraction from *P. serotina* fresh leaves between the months were assessed using the Kruskal–Wallis tests or ANOVAs with Tukey's HSD tests. The same tests were also used to determine the effects of linalool concentration on the seed germination and growth parameters of Scots pine seedlings.

3. Results

3.1. Influence of Black Cherry Volatiles on Pine Seed Germination and Seedling Growth

In the germination bioassays, the volatiles from *P. serotina* fresh leaves did not cause any significant effect on *P. sylvestris* germination (Table 1). The strongest phytotoxic effect on Scots pine seedlings was caused by volatiles emitted from leaves collected in April compared to the response of seedlings to VOCs from *P. serotina* leaves collected in June and August (Figure 1). In addition, the growth of seedlings decreased with increasing April leaf biomass of the black cherry. With 40 g of *P. serotina* leaves, the reduction of root length and stem height of *P. sylvestris* young plants was 48.3% and 19.5%, respectively, compared to the same growth parameters for the control. Black cherry leaves collected in June showed a weaker but still significant inhibitory effect both on roots (20.4%–25.7%) and on stems (4.1%–9.5%) of Scots pine seedlings. In the case of volatiles emitted from August leaves only, a reduction of seedling root growth was observed (11.1%–12.6%).

The growth response of the root of *P. sylvestris* seedlings differed significantly depending on the month of *P. serotina* leaf collection; it was the strongest in the presence of leaves collected in April and the weakest for August leaves (Figure 1). Moreover, root growth inhibition increased with leaf weight from 10 g and 20 g to 40 g. Differences in pine stem growth were found only for the 40 g weight of black cherry leaves. Volatiles from black cherry leaves collected in April caused more negative effect on seedling height than leaves from other collection dates.

Table 1. Effect of volatiles released by *Prunus serotina* leaves (10, 20 and 40 g), collected in three months of the growing season (April, June and August), on germination and growth parameters of *Pinus sylvestris* seedlings. Data are mean ± SE. Different letters (a, b, c) following values in rows indicate significant differences for different weight of *P. serotina* leaves determined in Tukey HSD or Kruskal–Wallis tests, with $p \leq 0.05$; n = 15.

Parameter	Control	April		
		10 (g)	20 (g)	40 (g)
Germination capacity (%)	95.3 ± 0.16 a	90 ± 0.26 a	90.7 ± 0.28 a	88 ± 0.22 a
Root length (mm)	42.8 ± 0.89 a	28.3 ± 0.81 b	24.07 ± 1.29 c	22.13 ± 1.16 c
Stem height (mm)	50.9 ± 0.80 a	49.73 ± 0.88 a	44.73 ± 1.28 b	41 ± 1.49 b
		June		
		10 (g)	20 (g)	40 (g)
Germination capacity (%)	95.3 ± 0.16 a	92.7 ± 0.25 a	90 ± 0.24 a	90 ± 0.24 a
Root length (mm)	42.8 ± 0.89 a	34.07 ± 0.98 b	32.8 ± 0.95 b	31.8 ± 1.38 b
Stem height (mm)	50.9 ± 0.80 a	48.8 ± 0.55 ab	46.07 ± 0.97 b	47.07 ± 0.86 b
		August		
		10 (g)	20 (g)	40 (g)
Germination capacity (%)	95.3 ± 0.16 a	94 ± 0.19 a	92 ± 0.24 a	91.3 ± 0.16 a
Root length (mm)	42.8 ± 0.89 a	38.07 ± 1.13 b	40.73 ± 1.13 ab	37.4 ± 0.9 b
Stem height (mm)	50.9 ± 0.80 a	49.2 ± 0.54 a	48.47 ± 1.01 a	47.2 ± 1.4 a

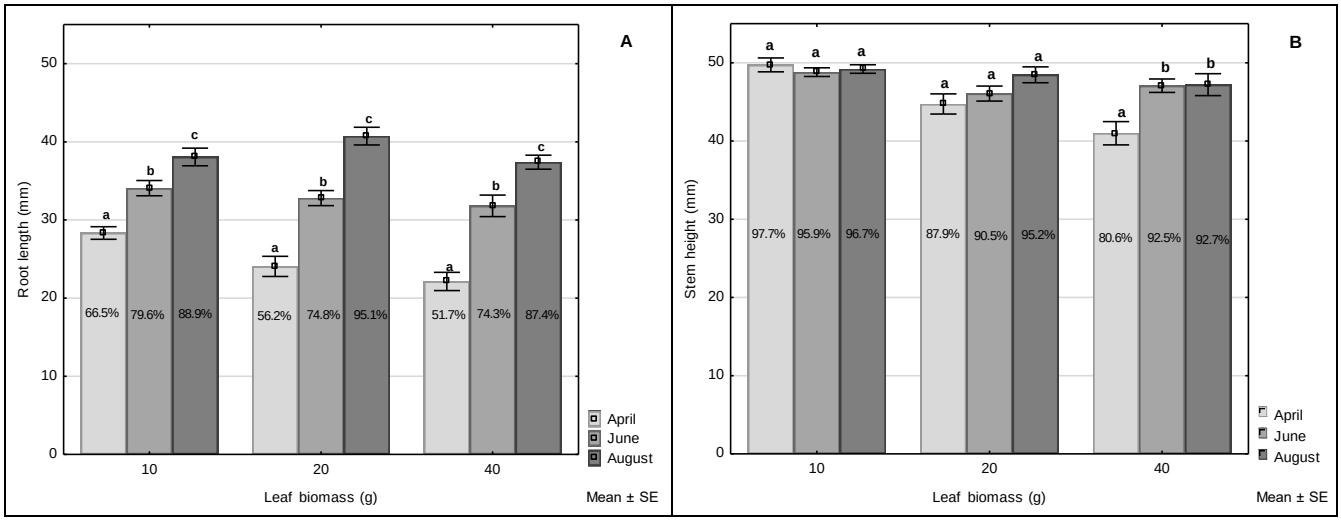

Figure 1. Effects of volatiles released by *Prunus serotina* leaves, collected in three months of the growing season (April, June and August), on root length (**A**) and stem height (**B**) of *Pinus sylvestris* seedlings. Data are mean ± SE. Numbers in bars represent % of control. Significant differences between collected dates for each leaf biomass (marked with letters a, b, c) were estimated by the Tukey HSD test at $p \leq 0.05$; n = 15.

3.2. Composition of Volatile Organic Compounds of Black Cherry Leaves

Analyses using GC–MS led to the identification of 19 compounds of the total organic volatile fraction from *P. serotina* fresh leaves and revealed the presence of aldehydes, alcohols, ketones, terpenoids and ester (Table 2). In the case of seven compounds, seasonal variations in their content in volatile fraction were found. The volatiles were exclusively dominated by benzaldehyde. The content of this compound in the total volatile fraction from leaves collected in April and June was comparable, 99.02% and 99.05%, respectively. In aging foliage of black cherry, a significant decrease in content of benzaldehyde to 97.22% was observed.

Table 2. Composition of volatile organic compounds (%) identified in fresh *Prunus serotina* leaves collected in three months of growing season (April, June and August). Data are mean ± SD. KI exp.—retention index calculated according to n-alkanes; KI lit.—retention index obtained from NIST17 and Adams [29] database. Significant differences between months marked in rows by different letters (a, b) were estimated by HSD of the Tukey test or Kruskal–Wallis test, $p \leq 0.05$; n = 3.

Compound	KI exp.	KI lit.	April (%)	June (%)	August (%)
2-ethyl furan	705	703	0.0527 ± 0.0488 a	0.0180 ± 0.0091 a	0.0145 ± 0.0005 a
(E)-2-pentenal	752	755	0.0034 ± 0.0006 a	0.0043 ± 0.0006 a	0.0040 ± 0.0010 a
(E)-3-hexenal	814	811	0.3277 ± 0.0396 ab	0.5290 ± 0.1837 b	0.1750 ± 0.0320 a
(E)-2-hexenal	857	854	0.0020 ± 0.0020 a	0.0034 ± 0.0032 a	0.6600 ± 0.0330 b
1-hexanol	867	868	0.0023 ± 0.0006 a	0.0060 ± 0.0056 a	0.0155 ± 0.0025 a
(Z)-2-hexen-1-ol	872	870	0.0867 ± 0.0532 a	0.0070 ± 0.0043 a	0.3345 ± 0.0945 b
(*E, E*)-2,4-hexadienal	915	911	0.0343 ± 0.0396 a	0.0480 ± 0.0745 a	0.1665 ± 0.0115 a
benzaldehyde	965	962	99.0227 ± 0.4016 b	99.048 ± 0.3233 b	97.2275 ± 0.2375 a
2,4-heptadienal	1016	1013	0.0263 ± 0.0093 b	0.0080 ± 0.0026 a	0.0405± 0.0065 b
benzyl alcohol	1035	1036	0.0347 ± 0.0574 a	0.0067 ± 0.0015 a	0.3003 ± 0.0630 a
linalool	1097	1099	0.0140 ± 0.0017 a	0.0043 ± 0.0038 a	0.0150 ± 0.0050 a
n-nonanal	1103	1102	0.0013 ± 0.0006 a	0.0043 ± 0.0049 a	0.0660 ± 0.0080 a
phenyl ethyl alcohol	1106	1108	0.0050 ± 0.0026 a	0.0050 ± 0.0010 a	0.0955 ± 0.0175 b
(*E*)-4-decenal	1194	1196	0.0050 ± 0.0026 a	0.0067 ± 0.0046 a	0.4650 ± 0.0770 a
estragole	1198	1196	0.2190 ± 0.3776 a	0.0103 ± 0.0066 a	0.0000 ± 0.0000 a
α-cyanobenzyl alcohol	1305	1302	0.0087 ± 0.0057 a	0.0073 ± 0.0050 a	0.1430 ± 0.0320 b
cis-jasmone	1392	1397	0.0083 ± 0.0035 a	0.0370 ± 0.0225 a	0.0125 ± 0.0025 a
(*E*)-β-caryophyllene	1413	1417	0.0710 ± 0.0358 a	0.0787 ± 0.0772 a	0.0240 ± 0.0070 a
nerylacetone	1437	1435	0.0187 ± 0.0064 a	0.1377 ± 0.1220 a	0.0050 ± 0.0040 a

3.3. Influence of Linalool on Pine Seed Germination and Seedling Growth

The analyses showed that linalool had a significant inhibitory effect on Scot pine seeds at a concentration of 50 µg/mL, the highest used in our laboratory tests (Table 3). The germination capacity of *P. sylvestris* decreased by 17% as compared to the value of this parameter for seeds in the control. With the increase of the concentration of linalool (from 10 to 50 µg/mL) seedlings developed shorter roots. A negative effect of linalool on the growth of seedling stems was observed at concentrations of 25 and 50 µg/mL. The roots were the most sensitive to the effects of the tested monoterpene. At a concentration of 50 µg/mL of linalool, the pine root length was shortened to 44% and stem height to 79% in relation to the parameters of seedlings growing under control conditions.

Table 3. Effect of linalool used at different concentrations (5, 10, 25 and 50 µg/mL) on germination and growth parameters of *Pinus sylvestris* seedlings. Data are mean ± SE, numbers in brackets represent % of control. Different letters (a, b, c) following values in rows indicate significant differences for the tested linalool solutions determined in the Tukey HSD test or Kruskal–Wallis test with $p \leq 0.05$; n = 15.

Parameter	Control	Linalool			
		5 (µg mL^{-1})	10 (µg mL^{-1})	25 (µg mL^{-1})	50 (µg mL^{-1})
Germination capacity (%)	95.3 ± 0.16 a (100%)	96 ± 0.16 a (101%)	91.3 ± 0.24 ab (96%)	89.3 ± 0.27 ab (94%)	78.7 ± 0.32 b (83%)
Root length (mm)	42.8 ± 0.89 a (100%)	42.5 ± 1.3 a (99%)	29.2 ± 1.05 b (68%)	25.9 ± 1.36 bc (61%)	18.7 ± 0.51 c (44%)
Stem height (mm)	50.9 ± 0.80 a (100%)	50.4 ± 0.90 a (99%)	50.7 ± 0.76 a (100%)	45.1 ± 1.14 b (89%)	40.3 ± 1.37 c (79%)

4. Discussion

In the natural environment, plant volatile organic compounds (VOCs) released into the atmosphere are directly absorbed on the plant surface and taken up into the leaf via stomatal openings or cuticle diffusion [30]. They also might be transferred to the soil and undergo processes of adsorption, dissolution and degradation, before being taken up by the plant roots [31]. Many volatile compounds emitted by native and alien species have phytotoxic properties and inhibit the growth of coexisting species in their habitat [32]. The results of our laboratory study confirm that this type of interaction is possible in the case of black cherry and Scots pine. The study of Robakowski and Bielinis [33] indicated that fresh leaves of *P. serotina* added to the substrate showed a positive allelopathic effect on the growth of one-year-old *Quercus petraea* seedlings, another tree species that naturally regenerates in pine forests. Orr et al. [34] also demonstrated that allelopathy may be one of the mechanisms underlying the negative impact of invasive tree species on native trees. However, the strongest effect is observed during germination and early seedling growth rather than in the next stages of tree life.

In our study, the presence of *P. serotina* volatile compounds did not have an inhibitory effect on *P. sylvestris* germination capacity, but they strongly limited the length of seedling roots and contributed slightly to the inhibition of growth of pine seedling stems compared to the control. For comparison, water extracts from black cherry fresh [24] or decomposed leaves [17] marginally affect the germination process, but they strongly inhibit the radicle elongation of newly germinated Scots pine [17]. The work of other authors also indicates that in the case of allelopathic interactions, germination generally is less sensitive than growth of seedlings [35]. In the case of water solutions with potential allelopathic properties, the radicles of the tested species remain in contact with the filter paper, leading to constant absorption of the solution. This may contribute to the greater sensitivity of the seedling root compared to the shoot [36]. Volatiles released from fresh black cherry leaves could be absorbed at the same time by roots and the shoots, which may explain the simultaneous growth response of both parts of the seedlings to the presence of allelopathic

compounds. The most observed effect of most allelochemicals on seedling growth appears to be mediated through a disruption of photosynthesis or respiration [33,37].

Our laboratory study shows that benzaldehyde is the main component of VOCs emitted by fresh black cherry leaves and its content ranged from 99% of the fraction in spring to 97% at the end of summer. Verma and coauthors [38] reported that benzaldehyde and/or its derivatives also predominate in the leaf essential oil compositions of other *Prunus* species. The lower benzaldehyde emission from senescing leaves demonstrated by the authors mentioned is consistent with the results of our analyses. The allelochemical content may vary during the growing season, which is reflected by seasonal changes in plant phytotoxicity [39]. Benzaldehyde is formed following the enzymatic hydrolysis of prunasin, a cyanogenic glycoside present in *Prunus* sp., which is a well-known defense compound produced as a chemical defense against herbivores [40,41]. In the intact plant, prunasin is stored separately from hydrolytic enzymes. As a result of tissue disruption it is degraded to benzaldehyde, glucose and hydrogen cyanide (HCN) by the sequential action of the β-glucosidase prunasin hydrolase and mandelonitrile lyase [42]. Additionally, the research of Del Cuoeto [43] indicates that enzymatic hydrolysis of prunasin, with HCN emission, occurs not only in response to damage by external factors, but also as a result of naturally occurring processes controlling flower development.

During the preparation of our experiment, separation of the petioles from black cherry twigs probably triggered the activation of several plant defense mechanisms simultaneously. This is evidenced by the noticeable presence in volatile compound profile of jasmone and green leaf volatiles (GLVs) such as 1-hexenol, 2-hexen-1-ol, 2-hexenal and 3-hexenal, which are emitted only in trace amounts in unstressed plants [44]. In addition, damage to the plant caused prunasin degradation, resulting ultimately in the simultaneous formation of benzaldehyde, present in the composition of VOCs and HCN, was not tested by us. Hydrogen cyanide impedes seed germination and the plant growth rate due to inhibition of respiratory functions in cells [45]. The negative effect of benzaldehyde, manifested in limiting the root growth, was previously observed in *Brassica campestris* seedlings [46], which was associated with a disturbance in absorption of available nitrogen forms [47].

In the composition of *P. serotina* volatile organic compounds, we also detected linalool and β-caryophyllene, two compounds with confirmed allelopathic effects that are emitted in low quantities from undamaged leaves [48]. The phytotoxic effect of β-caryophyllene has been observed in relation to seeds and seedlings of cultivated plants such as *Brassica rapa* L., *Raphanus sativus* L., *Solanum lycopersicum* L. [49,50] and *Lactuca sativa* L. [28]. Due to the confirmed presence of this compound, both in the sesquiterpene fraction from foliage of *P. serotina* [51] and *P. sylvestris* needles [52,53], it was not included in our study research. Linalool, one of the major compounds in essential oils of numerous spice plants, is a monoterpene with significant phytotoxicity for several crop species (*Lactuca sativa*, *Allium cepa* L., *Hordeum spontaneum* K. Koch, *Secale cereale* L.) and weeds (*Sinapis arvensis* L., *Amaranthus retroflexus* L., *Chenopodium album* L., *Rumex crispus* L.) [27]. Detailed research by Singh et al. [54] showed the possible mechanism of the negative effect of synthetic linalool on germination and growth of *Cassia occidentalis* (L.) Link seedlings. Treatment with this monoterpene causes a reduction in the chlorophyll content of the cotyledonary leaves and a weakening of the respiratory ability of growing seeds. Our results demonstrated that linalool adversely affects the germination and growth of *P. sylvestris* seedlings and therefore could account for the observed phytotoxicity imparted by total leaf volatiles from *P. serotina* leaves. However, it should be noted that allelopathic interactions are not due to the presence of a single compound, but rather several allelochemicals acting synergistically [55].

The methods used in this study were effective in determining the phytotoxicity of the volatiles of black cherry in relation to Scots pine on the basis of a small amount of leaves. However, the research also had some limitations. The concentrations of VOCs from fresh leaves of *P. serotina* used in the tests were probably much greater than those that are naturally emitted by plants. The VOCs identified included both those that are

continuously released by the plants and those whose emission was induced by stress (mechanical damage of leaf blades). It should also be taken into account that allelopathic interactions between species in the same plant community occur simultaneously with the phenomenon of competition and are subject to certain seasonal dynamics, and the phytotoxic effects observed in laboratory conditions may not be relevant in nature [39]. Furthermore, the activity of allelopathic compounds in phytocenoses is modified by the influence of several biotic and abiotic factors [56]. Laboratory allelopathic assays using filter paper do not take into account the presence of soil. Studies that assess the actual role of soil environment in plant VOCs degradation are scarce [31], probably because the vast majority of soil microorganisms cannot be cultured with current cultivation techniques [57]. Meanwhile, soils have the capacity to uptake VOCs and this flow depends on the tree types and soil temperature [58]. Furthermore, VOCs transformations depend on the type of litter and soil, and the communities of soil microorganisms [59]. Trowbridge et al. [58] observed in temperate mixed-forest that the higher VOCs degradation in ectomycorrhizal fungi soil as compared to arbuscular mycorrhizal soil was related to differences in the activity of soil microbial communities. The complex relationships and processes to which VOCs are subject in soil environment are still waiting to be recognized.

5. Conclusions

Our laboratory study revealed that volatile organic compounds emitted from fresh leaves of *P. serotina* do not affect the germination process, but limit the growth of roots and stems of *P. sylvestris* seedlings. The growth reaction of the radicles proves their greater sensitivity to phytotoxic compounds compared to the stem. Among the volatile compounds released by the fresh leaves of *P. serotina*, benzaldehyde predominated. Its content in the volatile fraction of leaves collected in April and June was comparable and significantly higher compared to the material obtained in August. Seasonal variation of volatile phytotoxicity was observed—volatiles emitted from the youngest leaves of the black cherry had the strongest negative impact on Scots pine seedlings. Additional analyses carried out for one selected volatile compound, linalool, showed that it has a strong phytotoxic potential and inhibits both germination and elongation of roots and shoots of pine seedlings. The results of our research suggest that VOCs from *P. serotina* leaves could limit the survival of *P. sylvestris* individuals in the seedling phase.

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