

# Identification of *Pinus sylvestris* Clones with the Highest and Lowest Allelopathic Potentials

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## Abstract

Allelopathy is a kind of interaction between plants in which the inhibitory effect on growth and germination can play an important role during the struggle for existence in interspecific competition. The species (or clone of one species) with a higher allelopathic potential might win the competition and place themselves in a better biosocial position for growth. Also, the clones with a lower allelopathic potential might be useful in agroforestry, e.g. as trees useful for shading the crops. The allelopathic potential of Scots pine (*Pinus sylvestris* L.), the tree species with a wide range of distribution in Europe and of great economic importance, was estimated in this study. To this end, needles from trees growing in a clonal seed orchard were collected and used as a material to obtain water leachates. The leachates were diluted to 25 and 50% and used in allelopathy tests. The differences between potentials of leachates from Scots pine clones to inhibit germination and growth of the test plant (*Sinapis alba* L. cv. Borowska) were assessed. All leachates significantly affected the germination and hypocotyl length in comparison to the control, also, there were significant differences between allelopathic effects of leachates from different clones. The clones 1702 and 1703 were characterized as highly allelopathic, with the potential confirmed in two independent allelopathic tests. The clones 355 and 2209 were characterized as clones with a moderate allelopathic potential. Also, leachates derived from needles collected from the upper part of the crown exerted a significantly stronger influence on the germination of the test plant than the leachates derived from the lower part of the crown. The possibility of selecting clones with the known allelopathic potential for the successful use in forestry was discussed.

**Keywords:** allelopathic potential, clones, germination, hypocotyl length, leachates, Scots pine, *Pinus sylvestris*, test plant, *Sinapis alba*

## Introduction

Scots pine (*Pinus sylvestris* L.) is the dominant tree species in Poland and in many other countries of the north-east Europe. Ecotypical variation within the species of Scots pine has been studied by many scientists (Steinbeck 1966, Wright et al. 1966, Chmura 2000, Oleksyn et al. 2002). Polish provenances of Scots pine often show the best incremental ability (Hebda et al. 2017). A particularly valuable type of this species is Taborz Scots pine in Puszcza Taborska (Taborska Forest). The quality of timber of Taborz pine has been recognized in Europe since the 16th century. This means that it is a unique on the global scale tree with a great potential for reforestation, but it is worth noticing that allelopathic properties of this type of pine have never been investigated before.

Allelopathy (biochemical interactions among plants) can determine the intensity of vegetation (Rice 1974),

the speed and intensity of the process of natural succession, and success of the natural regeneration of forest tree species. The importance of allelopathy in plant-plant interactions has been rarely tested (Ridenour and Callaway 2001). According to some scientists, one of the likely causes of sparse vegetation under the pine tree stands can be the allelopathic impact of various pine species (Rice 1984; Fernandez et al. 2006, 2008; Kato-Noguchi et al. 2009, Aklibasinda et al. 2017). The allelopathic potential and the strongest inhibitory effect on herbaceous plants germination was confirmed for the needles of the red pine (*Pinus densiflora*) by Kato-Noguchi et al. (2009), for Aleppo pine (*Pinus halepensis*) by Fernandez et al. (2006), for Chinese pine (*Pinus tabulaeformis*) by Jia et al. (2003), and finally, for the Scots pine (*Pinus sylvestris*) by Bulut and Demir (2007). In turn, inhibitory effects of some grasses on various species of the genus *Pinus* were confirmed by Peterson (1965),

Rietveld (1975), Priester and Pennington (1978) and Fisher and Adrian (1981). Substances with the known allelopathic potential had occurred in the needles of Scots pine, but some of them might have been removed from needles by elution throughout many years in the natural environment (Kainulainen and Holopainen 2002). Therefore, this is the reason why Scots pine is known as a species with an allelopathic potential (Bulut and Demir 2007). The term “allelopathic potential” also needs explanation here. Whenever this term is in use, it means that germination/growth of the test plant is inhibited by leachates collected from the donor plant (Oleszek and Jurzysta 1987).

The aim of the research described in this paper was to assess the extent of the allelopathic effect of Scots pine on the test plant. The plant selected for tests was white mustard (*Sinapsis alba* L.), proposed previously by others authors as a receiver species (or acceptor, or test plant) (Csiszár 2009; Csiszár et al. 2013, Tunaitienė et al. 2017). The current research compared the impact of leachates obtained from different Scots pine clones on the test plant. This study was possible because ‘Taborska’ Scots pine clones were planted in three clonal seed orchards in Poland for the production of Scots pine seedling material for reforestation purposes. Clones originating from the mature Taborska pine trees were named with special numbers, e.g. 335. The clonal seed orchard plantations are the ideal scientific test system, which affords the possibility of comparing different clones growing in one locality and under the same environmental conditions, and enables assessing the allelopathic potential and competitive abilities of these clones. The mechanism by which plants increase their allelopathic capability in competition has been previously investigated (Uesugi and Kessler 2013), hence the meaning of allelopathic potential in competition will be discussed as well.

## Materials and Methods

### Collecting material for the allelopathic tests

Plant material was collected from the Taborska Scots pine trees growing in clonal seed orchards located in Poland. Description of these orchards was presented in Table 1. In each of these three orchards grafts were planted at 5 × 5 m spacing. The orchard was arranged in 6 or 30 blocks (see Table 1). Each block contained clones randomly distributed in different proportions.

There were three experiments. Scots pine needles were collected from the lower part of the crown of standing trees from each of 5 different clones. In experiment 1, five clones (335, 351, 1702, 1703, 2209) were selected from two different blocks for allelopathic tests. In experiment 2, needles were collected from one block from the lower

**Table 1.** Characteristics of Scots pine seed orchards

Characteristic	Number of clonal seed orchard		
	1	2	3
Number of experiments	1	2	3
Year of experiment	2015	2015	2016
Name of nearest village	Klenica	Giedyle	Bukownica
Localization of village	51°59'34.6"N 15°47'02.3"E	54°14'34.0"N 19°56'34.0"E	53°43'55.0"N 19°24'49.4"E
Regional State Forest Administration	Zielona Góra	Olsztyn	Olsztyn
Forest District	Sulechów	Orneta	Susz
Area, ha	5.82	21.63	32.25
Year of establishment	1991	1975 – 1978	1977 – 1985
Number of clones/blocks/grafts	56/6/1269	173/30/9000	175/30/9838
Habitat type of forest	Mixed fresh coniferous	Fresh deciduous	Mixed fresh coniferous
Mean height of tree, m	13	20 – 21	16
Mean diameter of tree, cm	25	33 – 34	28
Soil subtype	Haplic Brunic Arenosol	Haplic Brunic Arenosol	Haplic Brunic Arenosol
Mean annual temperature, °C	8.9	7.6	7.5
Mean annual precipitation, mm	584.2	625.8	642.8

part of the crown but also from the upper part of the crown (the trees were cut to allow needle collection), and the same clones as clones in experiment 1 were selected. In experiment 3, samples of clones (335, 355, 1321, 1704, 2208) were selected from five blocks and also needles from five young seedlings growing in five different blocks were collected. The day of collecting material for experiment 1, 2 and 3 was as follows: the 5<sup>th</sup> of December 2014, the 8<sup>th</sup> of December 2014 and the 15<sup>th</sup> of December 2015, respectively. The needles were collected during winter rest. The one-year needles were removed from trees with shoots and transported to the laboratory in paper bags. They were used as a plant material of a donor plant in the allelopathy testing procedure. Donor plant material for allelopathic tests was collected, transported and used within 24 hours in each experimental procedure (drying).

### Laboratory procedure

In a laboratory of the Department of Plant Breeding and Seed Production, the University of Warmia and Mazury in Olsztyn, the needles were pulled from the stem and dried in an oven at 45 °C. Dried needles were cut into 1-cm pieces and placed in a beaker, then hot (96 °C) deionized water was poured into the beaker in the following proportion: 125 ml of water for each 25g of dried needles. Afterwards the beaker was plumbed with paraffin and left at 20 °C for 24 hours. This procedure was similar to the procedure described by Blum (2014) with slight modifications. After 24 hours of maceration, the leachate was used as a base for dilution. Dilution with deionized water was applied to obtain 25 and 50% leachate. Each leachate corresponded to a respective clone and block. Thereafter, the allelopathic potential was analyzed with white mustard (*Sinapsis alba* L.) cv. ‘Borowska’ as a test plant. Hundred seeds of the test plant were

put into Petri dishes (Ø 10 cm) on two layers of a filter paper (Munktell) that was watered with 7 ml of leachate or 7 ml of pure deionized water (control). Therefore, prepared Petri dishes were put into a growth chamber (Sanyo MLR-350T, Sanyo Electric Co., Ltd., Osaka, Japan) with constant temperature of 20 °C. That test was repeated four times for each clone and localization. The percentage of seeds germinating in dishes was observed after 24 and 72 hours and hypocotyl length of seedlings (in mm) was measured after 72 hours. Germination was measured twice to observe the resistance of the test plant to allelopathy. Hypocotyl length measurements were made with using faster and easier method of assessing the effect of allelopathy.

**Statistical analysis**

To determine statistical differences between mean values, one-way ANOVA was conducted. The conservative *post-hoc* Bonferroni test was used to avoid false-positive test results during means comparison (set alpha = 0.05). All analyses were conducted with the use of Statistica 12.0 analytics software package (StatSoft 2013).

**Results**

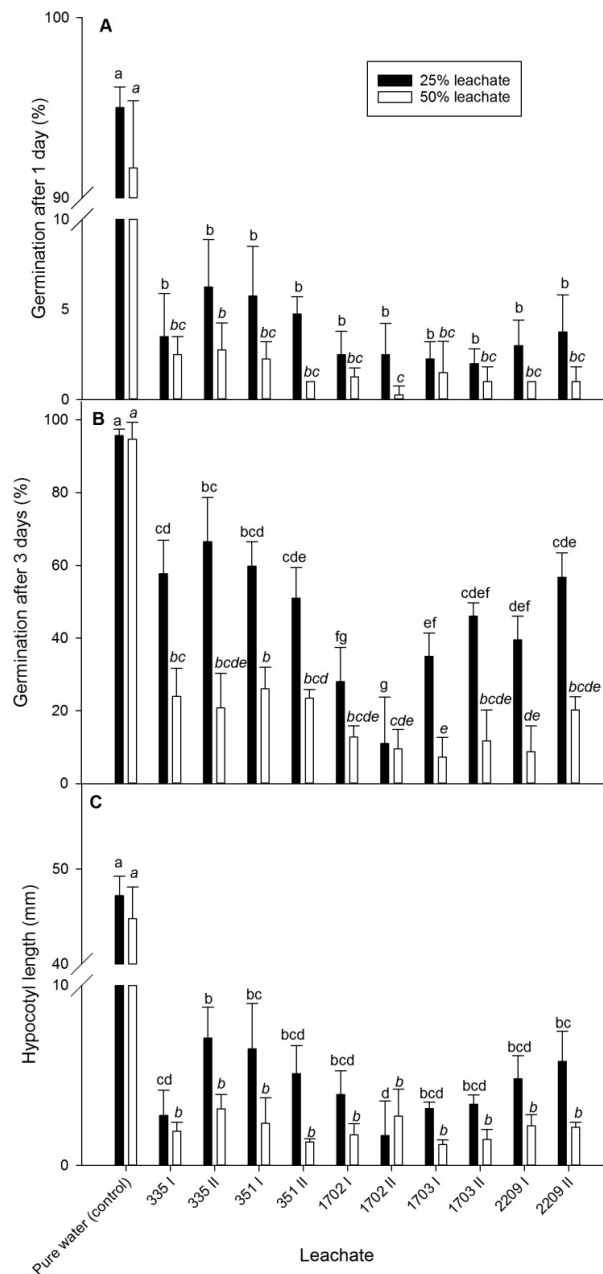
**Experiment 1**

All leachates significantly affected the germination and hypocotyl length in comparison to the control. There were significant differences between allelopathic effects of leachates from different clones in experiment 1 (Table 2). The greatest differences were observed for two parameters, i.e. germination after 3 days and hypocotyl length. These measures were the best to identify the dissimilarities between effects. The 25% concentration of leachates was optimal for estimating the allelopathy effect, differences were greater in the samples with addi-

**Table 2.** Results of one-way ANOVA with the leachate effect as the factor. Variables are the following: germination of seeds of test plant (%) after one day under the influence of leachate; germination of seeds of test plant (%) after three days under the influence of leachate; medium length of hypocotyl (mm) of test plants after three days of growth under the influence of leachate. Percentage data were transformed with Bliss function before the analysis. Effects were statistically significant ( $p < .000$  for all)

Effect	DF	Germination after 1 day		Germination after 3 days		Hypocotyl length	
		F	p	F	p	F	p
<b>Experiment 1</b>							
25% leachates	10	728.17	$p < .000$	25.12	$p < .000$	214.57	$p < .000$
50% leachates	10	1519.05	$p < .000$	55.93	$p < .000$	445.72	$p < .000$
<b>Experiment 2</b>							
25% leachates	10	432.128	$p < .000$	32.728	$p < .000$	308.1	$p < .000$
50% leachates	10	126.39	$p < .000$	40.38	$p < .000$	232.17	$p < .000$
<b>Experiment 3</b>							
	30	53.10	$p < .000$	50.87	$p < .000$	12.38	$p < .000$

tion of the 25% leachates than in the samples with 50% leachate addition (Figure 1). The concentration of 50% was too strong for testing because it affected the growth



**Figure 1.** Results of experiment 1. Mean ( $\pm$  SE) percentage of germinated seeds of the test plant after one day (A) or 3 days after imbibition (B) and mean ( $\pm$  SE) hypocotyl length of the test plant (C) after 3 days of growth. The test plant grew under the influence of two concentrations (25 and 50%) of leachates, which were obtained from needles harvested from different Scots pine clones (e.g. 335) planted in two blocks (e.g. II) in one seed orchard. Different lowercase characters within one concentration indicate statistical differences at a 0.05 alpha level in *post-hoc* Bonferroni test

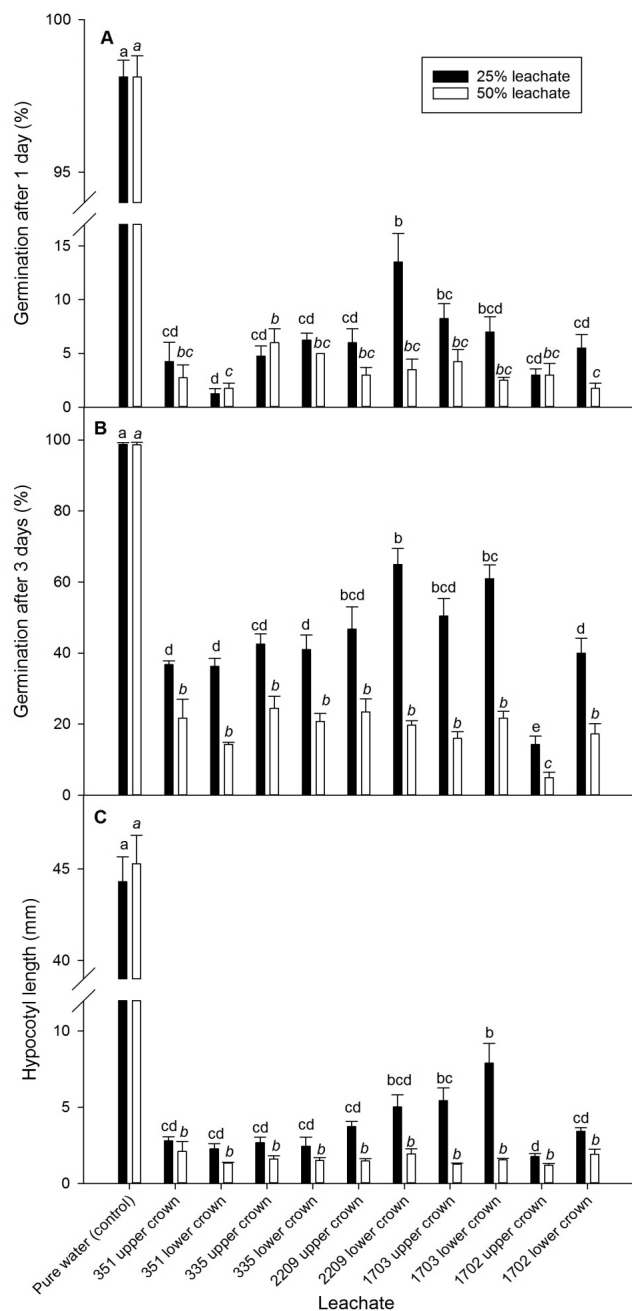
and germination too dramatically. No differences were observed between blocks (Figure 1), but the sample was relatively small (two blocks per clone). In experiment 3, five blocks were taken, therefore this experiment would be better for estimating the localization (blocks) effect than experiment 1 (see *experiment 3* in this chapter). Leachates of clone 1702 reveal the strongest allelopathic potential. Also, a similar situation was observed in both blocks, clone 1702 also had the strongest allelopathic potential in block I and block II. Leachates from clone 355 from block II had the slightest allelopathic effect on germination, but leachates from block I had a moderate effect on hypocotyl length for this clone. The moderate allelopathic effect was also observed in the case of leachates prepared from 351, 1702 and 2209 clones from both blocks.

**Experiment 2**

Both concentrations (25 and 50%) of leachate from needles of Scots pine had a strong allelopathic influence on the analysed measures in comparison to the control in experiment 2 (Figure 2A, B, C). Germination of the test plant after one day and after three days, and hypocotyl length were significantly inhibited by leachates (Table 2). There were no significant differences between effects of leachates derived from different positions in the crown for each clone in all three measured parameters (*post-hoc* comparisons in Figure 2). Especially, the leachates from clone number 2209 and 1703 slightly inhibited germination of the test plant in comparison to the leachates from other clones (Figure 2). Leachates derived from needles collected from both parts of the crown of clone 1702 had a stronger allelopathic potential than the others. A comparable situation was observed in experiment 1, which suggests that these clones had the strongest allelopathic potential as well. Leachates derived from needles collected from the upper part of the crown from clone 1702 exerted a significantly stronger influence on the germination of the test plant after 3 days of growth than the leachates derived from the lower part of the crown (Figure 2B).

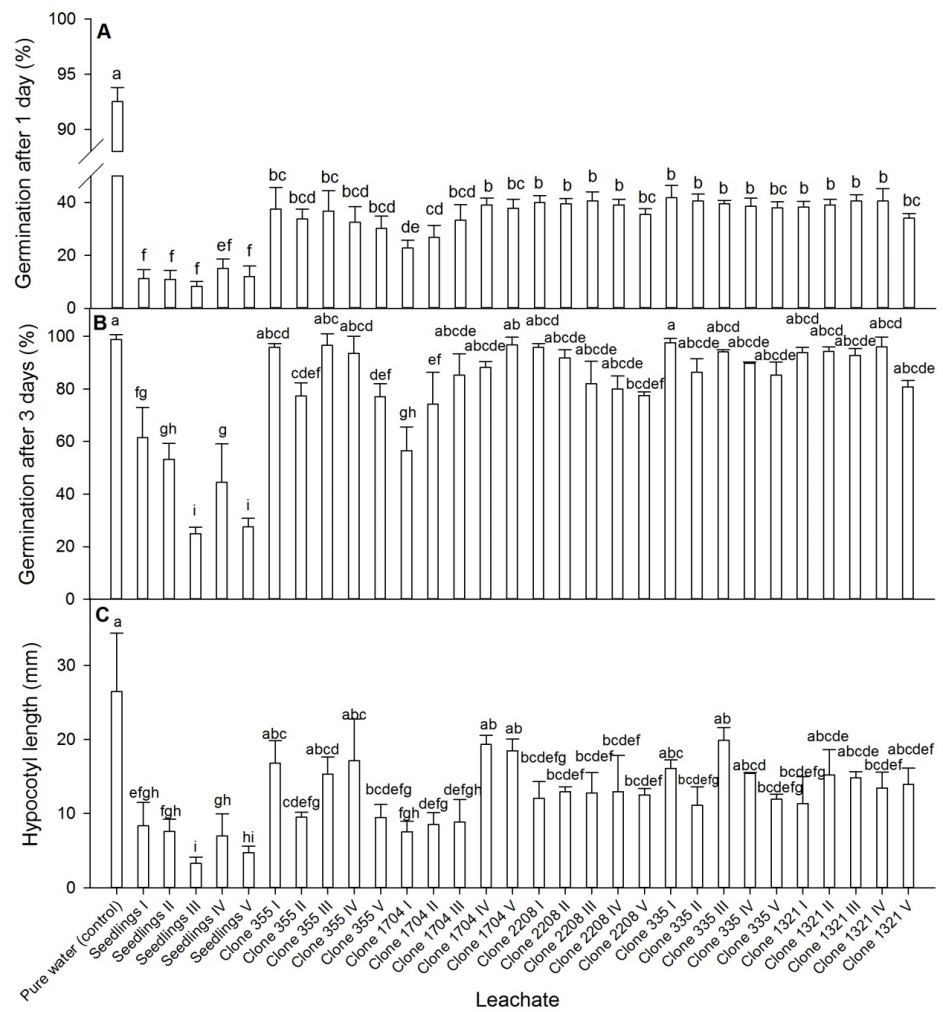
**Experiment 3**

The one-way analysis of variance conducted in experiment 3 (Table 2) showed that the leachates significantly influenced germination and hypocotyl length of the test plant in comparison to the control. After 3 days, the test plant growing under the influence of 25% leachates was significantly different from the control (Table 2). The Bonferroni test used in *post-hoc* analysis showed that the leachates from young seedlings had a significantly higher allelopathic potential than the leachates from mature trees from all clones (Figure 3). Also, most of the leachates from mature trees signifi-



**Figure 2.** Results of experiment 2. Mean ( $\pm$  SE) percentage of germinated seeds of the test plant after one day (A) or 3 days after imbibition (B) and mean ( $\pm$  SE) hypocotyl length of the test plant (C) after 3 days of growth. The test plant grew under the influence of two concentrations (25 and 50%) of leachates, which were obtained from needles harvested from two part of the crown, lower and upper, and from different Scots pine clones (e.g. 335) planted in one seed orchard. Different lowercase characters within one concentration indicate statistical differences at a 0.05 alpha level in *post-hoc* Bonferroni test





**Figure 3.** Results of experiment 3. Mean ( $\pm$  SE) percentage of germinated seeds of the test plant after one day (A) or 3 days after imbibition (B) and mean ( $\pm$  SE) hypocotyl length of the test plant (C) after 3 days of growth. The test plant grew under the influence of one concentration (25%) of leachates, which were obtained from needles harvested from five clones (e.g. 355) or seedlings (Seedlings) planted in five blocks (e.g. II) in a seed orchard at Sulechów. Different lower-case characters within one concentration indicate statistical differences at 0.05 alpha level in *post-hoc* Bonferroni test

cantly decreased the percentage of test plant germination, especially regarding germination after one day, and also decreased the hypocotyl length (Figure 3C). Leachates from clones with number 355 (block I, II, IV), 1704 (block IV and V, 335 block III) had the highest allelopathic effect on hypocotyl length. Germination after 1 day and hypocotyl length were more sensitive indicators of allelopathic potential than germination after 3 days. The differences between blocks were shown in the case of leachates obtained from seedling and from different clones.

**Discussion and Conclusions**

It is a well-known fact that Scots pine is, on the one hand, a species with a considerable allelopathic potential and, on the other hand, a species sensitive to allelopathy. It was treated both as an acceptor (Šežienė et al. 2012) and as a donor in the allelopathy research (Bulut and Demir 2007). In the current research, the most interesting was how strong allelopathic potential of Scots

pine is, and which clones originating from maternal trees growing in one location have a higher allelopathic potential. All analyzed leachates significantly inhibited the germination and the growth of the test plant in comparison to the control (Table 2), which confirms that Scots pine has indeed the allelopathic potential. However, the main aims of the research were to investigate differences in the allelopathic potential of various clones. Only half of this scientific objective was achieved, because sometimes local conditions modified the allelopathic potential of clones (Figure 3). It means that the phenotypic plasticity of this feature is high, thus the same clone in different localities can express different properties. High variability of the allelopathic potential of clones, grown in different blocks but inside the same plantation, can raise doubts regarding the possibility of high allelopathic clone selection in the case of the Scots pine. On the other hand, clone 1702 showed consequently a higher allelopathic potential in two experiments (in two different localities), which may hold promise for further positive selection.

The results obtained in these studies do not exclude the possibility of selecting Scots pine clones with a high allelopathic potential and, thus, with a higher resistance to biotic stress. The concept of this “super-pine” is not a new idea, previously other authors proposed the selection of Scots pine clones to obtain trees more resistant to pathogens (Sierota et al. 1998). But the concept that a plant with a high allelopathic potential can have an advantage in plant-plant competition is relatively new (Hofmann 2015).

It is worth noticing that seedlings have a stronger allelopathic potential than mature trees. According to Wójcik-Wojtkowiak et al. (1998), the higher allelopathic potential of young plants was decreasing as they achieved maturity. Also, in allelopathic tests conducted by Pereira (2015) younger leaves collected from *Serania lethalis* A.St.-Hil. had a higher allelopathic potential than older leaves did. It is consistent with our results (Figure 3), the leachates obtained from needles collected from seedlings had stronger allelopathic potential than the leachates collected from mature trees. Two possible hypotheses could be checked to explain these results. The first explanation: it is a common situation when a young plant has a higher allelopathic potential than the mature plant, because possibly the lower biomass of the young plant produces a small amount of allelopathic substances, thus the plant has to compensate for this relatively small biomass by a higher production of allelochemicals in the struggle for existence. It is likely that also seedlings of Scots pine have a stronger allelopathic potential than the mature trees, which is the element of the living strategy of this species. A similar situation was observed in the case of other plants (Weston 1996). The second explanation: it is possible that the higher allelopathic potential might be the expression of cross-generational adaptations in the second generation (Gilbert and Parker 2010). This hypothesis is worth considering because Scots pines used in experiment 3 were planted in a seed orchard beyond their habitat of the natural population. In experiment 3, the first-generation clones probably responded to the new environment by producing a lower amount of allelopathic compounds (low adaptation to the environment) in comparison to experiment 1 and 2 (good adaptation to the environment). In turn, the second-generation Scots pine’s half-clones (seedlings), growing beyond their natural range, might adapted to new environment, which might have resulted in their higher allelopathic potential. This ambiguity needs further research and explanations.

Leachates from Scots pine needles collected from two heights of the crown in experiment 2 (from the upper and from the lower part of the crown) differed in the allelopathic potential. In the case of clone 1702, needles collected from the upper part of the crown had a higher

allelopathic potential than these from the lower part. A clear explanation of this fact is problematic. In other research, the lower part of the crown was related with lower intensity of solar radiation, and with a higher concentration of allelochemicals (Robakowski et al. 2016), but an opposite tendency was observed in our experiment. It is possible that Scots pine, as a pioneer, light-preferable species, has an opposite strategy.

The results obtained allow concluding that some clones of Scots pine could be subjected to the genetic selection, of course if the object of the genetic selection are seedlings or mature trees with a high allelopathic potential, both in agriculture (Duke et al. 2001) and in forestry (Oguchi et al. 2014), because the seedlings or trees with a higher allelopathic potential might be more resistant to the influence of competitors, pathogens or herbivores (Xuan et al. 2005). Summing up, the clones cultivated from maternal trees 1702 and 1704 showed a notably higher allelopathic potential. Planting material originating from these two clones might be used in forest regeneration practice to produce more competitive seedlings (Uesugi and Kessler 2013).

Noteworthy is that the allelopathic tests conducted in this research might not correspond with a real situation in the forest. Many ecological, natural processes modify allelochemicals in the air and in the soil (Fritz and Schneider 2015), but leachates from needles collected from the crown have probably a stable profile of chemicals at the moment of interaction. This scientific work is based on the presumption that trees with the examined, higher allelopathic potential (like clones 1702 and 1703) might have a higher allelopathic potential in the forest and be more resistant to competitors and insects. That hypothesis is worth being examined in future experiments.

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