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Allelopathic Effect of Black Cherry (*Prunus serotina* Ehrh.) on Early Growth of White Mustard (*Sinapis alba* L.) and Common Buckwheat (*Fagopyrum esculentum* Moench): Is the Invader a Threat to Restoration of Fallow Lands?

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Abstract: Abandoned agricultural land present in most European countries constitutes a resource of land that can be restored to agricultural production. Fallow colonization by invasive plant species contributes to changes in the course of natural secondary succession. This may modify the possibilities of returning fallow lands to agriculture, which constitutes an issue that needs to be investigated. In this study, the phytotoxic effect of invasive black cherry (*Prunus serotina* Ehrh.) on two crop plants commonly used for fallow land utilization, white mustard (*Sinapis alba* L.) and common buckwheat (*Fagopyrum esculentum* Moench), was assessed. The influence of water extracts from *P. serotina* litter and the soil collected under the individuals of this species was investigated. *Sinapis alba* was found to be more sensitive to allelochemicals released by *P. serotina* than *F. esculentum*. Litter extracts and soil with residues of *P. serotina* significantly inhibited both germination and growth of *S. alba* seedlings, estimated with length and mass of the above-ground and underground parts. In the case of *F. esculentum*, a negative effect of the tested extracts and soil on root mass reduction was observed. Preliminary results of our laboratory tests suggest that cultivation of white mustard should be avoided in the reclamation of fallows with black cherry.

Keywords: fallow land restoration; invasive plant; *Prunus serotina*; allelopathy; germination; seedling growth; *Sinapis alba*; *Fagopyrum esculentum*

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1. Introduction

Abandoned farmlands are a permanent element in the agricultural landscape of Europe [1]. Along with the prolonged abandonment of cultivation, vegetation regrowth and changes in the soil environment, occurring as a result of natural secondary succession, are observed [2]. The latest research works indicate that long-term fallowing does not lead to such deterioration of soil chemical properties that would make it difficult to restore plant production [3,4].

Unfortunately, the encroachment of invasive plant species on uncultivated fields causes negative changes, displacing native plant species from their growing area and modifying soil conditions [5–8]. This impedes the possibility of reclaiming fallow land for food or biomass production [4].

The most common invaders on abandoned farmland in Poland are goldenrods (*Solidago canadensis* L., *S. gigantea* Aiton), which enter after the segetal phase of natural succession and create one-species dominating communities [9,10]. The colonization of early fallows by the invasive black cherry (*Prunus serotina* Ehrh.) is also being observed more and more frequently [11,12] with a tendency to clustered distribution of individuals [13].

Black cherry, a forest tree of North American origin, was introduced to central Europe in the 17th century as an ornamental plant and as a timber species. Later, it was repeatedly

planted in poor forest habitats [14,15], which facilitated its uncontrolled spread and expansion of the range of invasion [16]. The documented influence of *P. serotina* invasion concerns forest photocoenoses where it forms a close shrub layer and outcompetes photophilous species [17,18] and contributes to encroachment of species with high nutrient demand at the forest floor [19,20].

There are many ecological traits that facilitate *P. serotina* invasion. This alien species has high tolerance for environmental conditions in the introduced range, has a high ability of generative reproduction and vegetative sprouting, produces numerous seeds dispersed by birds and mammals, and it establishes a long-living bank of seedlings [16,21,22].

The interaction of invasive plants and native plants in communities is a complex combination of competition for resources (water, nutrients, light) and reaction to plant secondary metabolites, called allelochemicals, and belonging to a wide range of chemical classes [23] that are involved in the process of allelopathy. Allelopathy is defined by Rice as "any direct or indirect harmful or beneficial effect by one plant on another through production of chemical compounds that escape into the environment" [24]. Allelochemicals are released by rainfall leachates, volatilization from living plant parts, root exudation, or decomposition of plant residues, which are decomposed and/or modified by soil microorganisms [25–27]. The work of Zhang et al. [28] confirms that plant residues exert the most negative effect on plant communities.

Previous research work of *P. serotina* allelopathy has focused on the influence of this species on the natural regeneration of Scots pine (*Pinus sylvestris*). The negative effects of *P. serotina* fresh leaf extract [29], litter extract [30], and volatile organic compounds [31] on germination and growth of *P. sylvestris* seedlings were confirmed. Allelopathic interactions of black cherry on fallows, in the context of the potential to restore the agricultural function to these lands, remain unknown.

The most reliable and common method for the evaluation of allelopathic interactions is by examining the inhibitory effects of different plant parts' extracts against growth of the tested plant species in vitro and in pots [32]. We conducted two separate experiments within the study using decomposed *P. serotina* leaves and the soil collected from individuals of this species. The aim of this study was to evaluate the phytotoxic effects of black cherry on two species of great economic importance and commonly used for fallow land utilization: white mustard (*Sinapis alba* L.) and common buckwheat (*Fagopyrum esculentum* Moench). We tested the following hypotheses: (1) *P. serotina* litter extract affects germination and seedling growth of *S. alba* and *F. esculentum*, and (2) soil with *P. serotina* exudates modifies germination and early growth of the tested plant species.

2. Materials and Methods

2.1. Seed Selection

We chose white mustard *S. alba* L. (cultivar Nakielska) and common buckwheat *F. esculentum* Moench (cultivar Kora) as the tested plants. Both species are annual, short vegetation plants, which have low habitat requirements. They are grown primarily for seeds, and in Europe, they are also used as a green manure/cover crop and a fodder for livestock. Cultivation of *S. alba* or *F. esculentum* has a positive effect on the agrophysical, chemical, and biological properties of the soil. These species release allelochemicals that suppress weed development [33–35]. In addition, *S. alba* is a model species in allelopathy studies due to its fast germination, sensitivity to allelochemicals, and the possibility of comparing the obtained results based on numerous research works by other authors [36,37]. The seeds of the tested species were purchased in a package at a garden store.

2.2. Preparation of Decayed Leaf Litter Extracts

The decayed leaf litter of *P serotina* was collected in the first half of December 2018 in an area of approximately 500 m^2, under dense *P. serotina* bushes, on a 10-year fallow land. About 10 g of plant material was sampled from each of 10 randomly selected individuals and combined in one sample. The sampling site was located on Podzolic soil in the southern

suburbs of the city of Wrocław (SW Poland: 51°2′52.86″ N, 17°19′21.61″ N and 118 m a.s.l.). This area is dominated by traditional agricultural landscapes characterized by a great variety of ecosystems. The seed source of *P. serotina* on the selected fallow consisted of mature trees located about 300 m away in a backyard garden.

Black cherry litter was cleaned by removing bark, twigs, and leaves of other plants, then 30 g of plant material was immersed in 100 mL of deionized water [30]. After 24 h maceration at 25 °C in the dark, the extract was filtered through filter paper (density 65 g/m^2). The resulting filtrate was considered as a 100% solution, and then dilutions were made to various concentrations (75%, 50%, and 25%). Extracts were kept in refrigerator.

2.3. Preparation of Pot Experiment

On the selected fallow in April 2018, two soil samples were collected up to a depth of 20 cm (each about 50 L), one directly under a *P. serotina* individual and the other at a 10 m distance away. The selected shrub was located at the intersection of the diagonals of the decayed leaf sampling area. After being transported to the laboratory, the soil was dried at room temperature and passed through a sieve with a mesh diameter of 2 mm. Then, four variants of the substrate were prepared: (1) soil without *P. serotina*, (2) soil without *P. serotina* + activated carbon (AC), (3) soil under *P. serotina*, and (4) soil under *P. serotina* + AC. Activated carbon, used in allelopathic tests to neutralize the effects of allelochemicals [25,38,39], was added to the soil in a ratio of 1:3 (100 mL AC + 300 mL soil). The substrate was placed in 500 mL pots, perforated on the underside. For each of the variants of the substrate, 10 replications were prepared.

2.4. Germination and Growth Assay

To determine the influence of extracts from black cherry litter on the target species, the tests of germination were conducted in sterile, transparent plastic cups (diameter 9.5 cm, height 8 cm) with filter paper (diameter 9 cm, density 50 g/m^2) inside. In each cup, ten seeds of specific plant species (*S. alba* or *F. esculentum*) were placed on filter paper. Then, 5 mL quantities of water extracts of each concentration (including 0% as a control and 25%, 50%, 75%, 100%) were added directly to the filter paper and the cups were closed with a lid. Treatments were replicated five times for each species and each concentration. The experiment was carried out in controlled conditions with a daily light/dark cycle, day 16 h = 10 klx, night 8 h, 24 °C/14 °C, 60% relative humidity. After 14 days germination capacity (defined as percentage of seeds which had germinated during the test), mean root length, mean stem height, mean dry weight of the roots, and mean dry weight of the shoots were determined. Biomass was dried to constant mass at 60 °C.

In the experiments with soil in each pot with a specific substrate, five seeds of the tested plant (white mustard or common buckwheat) were placed. Mustard seeds were sown in the soil to a depth of about 1 cm, and buckwheat to a depth of about 5 cm. Immediately after setting up the experiment, each container was watered with 400 mL of tap water. Then, each container was watered every 4 days with 100 mL of water. Pots were stored under the conditions described above. Plants were harvested 28 days after sowing. Allelopathic activity of soil was estimated on the basis of seed germination and three seedling growth parameters (analyzed in the previous experiment). Measurement of length of roots was abandoned because the roots washed in a strainer under running water were broken.

2.5. Statistical Analyses

The data for germination and growth parameters were calculated as the mean value (Tables 1–4) and the percentage of inhibition/stimulation over the control (Figure 1). The compatibility 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. In order to determine the significance of differences between the groups, in the case of variables with a normal distribution and homogeneity of variance in the group, parametric tests were used: Student's t-test (pot experiment) or one-way analysis of variance (ANOVA), followed by

Tukey's HSD post hoc test (litter extracts experiment). In the case of variables that did not have a normal distribution or homogeneity of variance, non-parametric methods were used: the Mann–Whitney U test (pot experiment) or the Kruskal–Wallis test followed by a post hoc test (litter extracts experiment). Significance was evaluated at $p \leq 0.05$. The above analyses were performed using STATISTICA software v. 13 (TIBCO Software Inc., Palo Alto, CA, USA).

Table 1. The effect of water extracts from *Prunus serotina* litter with different concentrations on germination and seedling growth of *Sinapis alba*. Data are mean ± SE, n = 5. Different letters following values in rows, indicating significant differences for the parameter between control and extract concentrations, were estimated by Tukey HSD test or Kruskal–Wallis test with $p \leq 0.05$.

Parameter	Concentration of *P. serotina* Litter Extract				
	Control (0%)	**25%**	**50%**	**75%**	**100%**
Germination capacity (%)	94 ± 0.24 a	68 ± 0.58 ab	48 ± 0.58 b	56 ± 0.75 ab	22 ± 1.1 b
Stem height (mm)	33 ± 8.53 a	30 ± 2.47 a	22 ± 4.51 ab	17 ± 2.5 b	5 ± 3.49 b
Shoot dry matter (mg)	106 ± 5.72 a	66 ± 10.77 ab	33 ± 6.65 ab	23 ± 6.95 b	10 ± 8.3 b
Root length (mm)	41 ± 9.81 ab	87 ± 12.59 a	46 ± 14.35 ab	25 ± 7.54 ab	9 ± 7.97 b
Root dry matter (mg)	26 ± 3.42 a	16 ± 3.08 a	9 ± 2.04 ab	8 ± 2.94 ab	1 ± 1.42 b

Table 2. The effect of water extracts from *Prunus serotina* litter with different concentrations on germination and seedling growth of *Fagopyrum esculentum*. Data are mean ± SE, n = 5. Different letters following values in rows, indicating significant differences for the parameter between control and extract concentrations, were estimated by Tukey HSD test or Kruskal–Wallis test with $p \leq 0.05$.

Parameter	Concentration of *P. serotina* Litter Extract				
	Control (0%)	**25%**	**50%**	**75%**	**100%**
Germination capacity (%)	84 ± 0.5 a	86 ± 0.24 a	76 ± 0.51 a	74 ± 0.51 a	72 ± 0.58 a
Stem height (mm)	33 ± 1.77 a	33 ± 2.01 a	33 ± 2.18 a	31 ± 1.24 a	35 ± 1.86 a
Shoot dry matter (mg)	46 ± 3.1 a	48 ± 3.78 a	46 ± 2.77 a	43 ± 3.2 a	47 ± 3.08 a
Root length (mm)	45 ± 3.4 a	37 ± 0.93 ab	26 ± 2.57 abc	16 ± 2.35 bc	10 ± 1.39 c
Root dry matter (mg)	20 ± 2.06 a	13 ± 0.71 b	8 ± 0.86 c	5 ± 0.97 cd	3 ± 0.37 d

Table 3. Effect of different variants of soil substrate on germination and seedling growth of *Sinapis alba* in pots. Data are mean ± SE, n = 10.

Parameter	Variant of Soil Substrate			
	without *P. serotina* Exudates	**without *P. serotina* Exudates + AC**	**with *P. serotina* Exudates**	**with *P. serotina* Exudates + AC**
Germination capacity (%)	68 ± 6.80	64 ± 10.7	44 ± 5.80	70 ± 9.10
Stem height (mm)	109.71 ± 4.75	51.25 ± 6.6	102.2 ± 10.30	46.77 ± 5.17
Shoot dry matter (mg)	130.1 ± 0.01	38.4 ± 0.01	59.2 ± 0.01	31.9 ± 0.01
Root dry matter (mg)	98.1 ± 0.02	11.3 ± 0.00	5.9 ± 0.001	9.6 ± 0.00

Table 4. Effect of different variants of soil substrate on germination and seedling growth of *Fagopyrum esculentum* in pots. Data are mean ± SE, n = 10.

Parameter	Variant of Soil Substrate			
	Without *P. serotina* Exudates	**without *P. serotina* Exudates + AC**	**without *P. serotina* Exudates**	**with *P. serotina* Exudates + AC**
Germination capacity (%)	60 ± 11.16	80 ± 4.22	76 ± 9.78	62 ± 9.16
Stem height (mm)	83.33 ± 13.27	97.08 ± 6.09	112.8 ± 7.82	114.84 ± 6.52
Shoot dry matter (mg)	130 ± 0.01	66 ± 0.00	94 ± 0.02	84 ± 0.01
Root dry matter (mg)	98 ± 0.02	22.7 ± 0.00	32.3 ± 0.01	23.1 ± 0.01

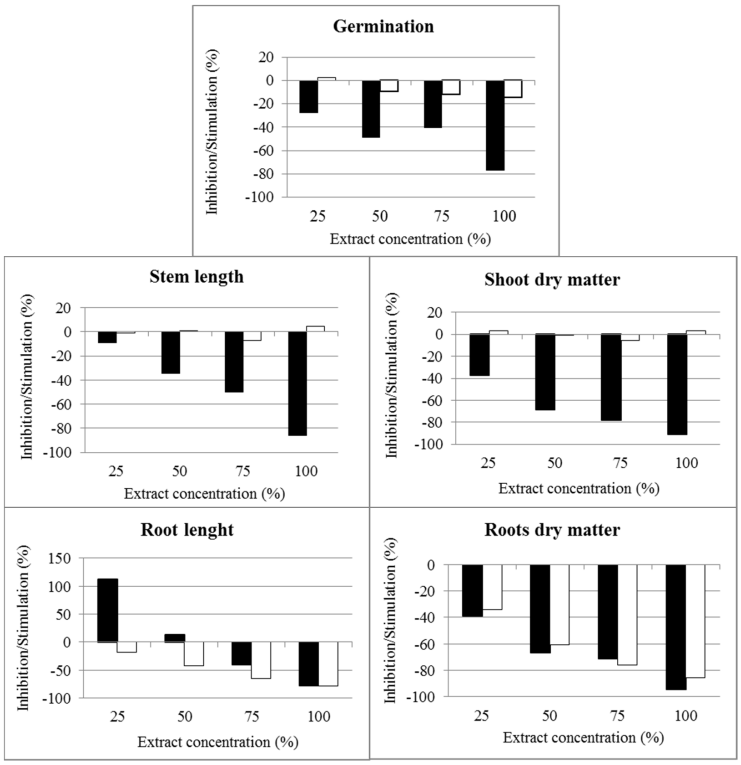


Figure 1. Inhibitory (−) or stimulatory (+) effects (over control) of leaf litter extracts of *Prunus serotina* on *Sinapis alba* (black bars) and *Fagopyrum esculentum* (white bars).

3. Results

3.1. Influence of P. serotina Litter Extracts on Germination and Growth of Tested Species

The phytotoxic potential of *P. serotina* litter extracts on *S. alba* and *F. esculentum* was examined based on changes in germination and growth of seedlings. The extracts showed variation in allelopathic activity toward the two target species.

The results showed a significant effect of the water extract from *P. serotina* litter in concentrations of 50% and 100% on the germination capacity of *S. alba* in comparison to the seeds treated only with distilled water (H = 16.19, p = 0.0028) (Table 1). At the highest concentration of extracts, a decrease in the value of the germination parameter by 77% in relation to the control was observed (Figure 1). The stem height decreased compared with the control by approximately 50% after treatment with extract of concentration 75%, and by 86% at concentration 100% (H = 25, p = 0.0036). Extracts of concentration of 75% and 100% also significantly influenced shoot dry matter, which resulted in a weight loss of 78% and 91%, respectively, compared with the control (H = 18.15, p = 0.0012). No statistically significant differences were found in the length of seedling roots treated with

tested extracts and grown under control conditions. Only the extract of concentration 100% had a negative effect on root dry matter (H = 17.3, p = 0.0017); the weight loss was 94% relative to the control.

Regarding *F. esculentum*, a significant allelopathic influence of water extracts from *Prunus serotina* litter was observed only on roots (Table 2, Figure 1). Length of roots of target species decreased by 78% with extract at 100% concentration, and 65% with extract of concentration 75%, compared with the control (H = 21.64, p = 0.0002). All analyzed *P. serotina* extracts showed an inhibitory effect on the weight of seedling roots, which decreased with increasing concentration of extract (F = 2.75, p = 0.05). The differences in the value of this parameter were from 34% to 86% in comparison to control untreated seedlings.

3.2. Evaluation of the Suitability of the Soil Inhabited by P. serotina for Cultivation of the Tested Plants

In the pot experiment with four variants of soil substrate, *S. alba* seeds showed the lowest germination rate in soil collected under the *P. serotina* bushes. The numerical value of this parameter differed significantly from the germination capacity in the soil collected on the site without this invasive species (F = 1.37, p = 0.05) (Table 3). The addition of activated carbon (AC) to the soil with *P. serotina* exudates significantly increased the germination capacity of *S. alba* (F = 2.43, p = 0.02). Stem height of young mustard plants in pots with soil with and without black cherry exudates was comparable. The presence of AC significantly inhibited stem growth as compared with the first (U = 0, p = 0.0002) and the second soil variant quoted above (U = 0, p = 0.0002). The dry matter of both above-ground parts and roots of *S. alba* was significantly lower in the case of plants germinating in the soil with *P. serotina* exudates compared with those grown in the soil free of this species (F = 1.70, p = 0.044 and F = 192.94, p = 0). Activated carbon added to the soil without *P. serotina* exudates contributed to the inhibition of the mass growth of both the above-ground and underground parts of seedlings (F = 2.78, p = 0.014; F = 116.72, p = 0). The presence of AC in the soil from the *P. serotina* site reduced the weight of *S. alba* shoots (F = 2.03, p = 0.031).

No influence of the applied soil variants on the germination capacity of *F. esculentum* seeds was found (Table 4). The tallest plants of *F. esculentum* were recorded in pots filled with soil taken from the habitat of *P. serotina* with the addition of activated carbon. The stems of buckwheat growing in the soil with black cherry exudates were significantly taller compared with the stems of the specimens growing in the soil not inhabited by this analyzed invasive species (U = 16, p = 0.011). The weight of *F. esculentum* stems and leaves in the soil collected under *P. serotina* did not differ significantly from the weight of the above-ground part of the seedlings growing in soil free from this species. Activated carbon added to the soil without black cherry exudates significantly inhibited the weight gain of the above-ground part of common buckwheat compared with the soil free from this species (F = 6.21, p = 0.012). The above-ground parts of *F. esculentum* seedlings in the soil from the *P. serotina* site with the addition of activated carbon weighed significantly less than the seedlings growing in the soil without *P. serotina* exudates (F = 1.097, p = 0.0089). The dry matter of the roots of *F. esculentum* was significantly lower for the seedlings germinating from the seeds sown into the soil collected under the *P. serotina* bushes, compared with the dry weight of the roots of the seedlings growing in the soil free of this species (F = 6.38, p = 0.01).

4. Discussion

Most of the fallowed land in Poland retains its potential to be restored for food or biomass production. Even on long-term fallows, inhabited by native shrubs and trees, changes in the physicochemical properties of the soil (an increase in organic matter content with an increase in soil acidity) allow agricultural production to be resumed [4]. Allelochemicals released into the environment by invasive plants additionally affect the soil by influencing the composition and diversity of the soil microorganism community [40,41], which may limit the possibility of returning fallow land to agriculture [7].

The results of the current study indicated the negative effect of *P. serotina* litter extracts with the highest concentration on tested crop species. In the case of *S. alba*, it concerned both germination and growth of whole seedlings, while for *F. esculentum*, only the reaction of roots of young plants was observed. Previous studies showed the strong influence of extracts from decomposed black cherry leaves on the length of *Pinus sylvestris* roots with a slightly weaker effect on stem growth [30]. The greater sensitivity of the roots compared with above-ground parts of the tested plant species has also been demonstrated by other authors in allelopathic tests [42,43]. Sarkar et al. [44] suggest that constant contact of young roots with filter paper, treated with the analyzed solution, and direct uptake by root hairs, may cause greater sensitivity of this part of the seedling to biologically active compounds.

Our results of the soil experiment suggest the inhibitory effect of the soil collected from under *P. serotina* on the germination capacity of seeds and the weight of both roots and shoots of *S. alba* seedlings. In the case of *F. esculentum* cultivated in soil collected from the *P. serotina* site, a stimulating effect on stem growth while limiting the root mass, compared with seedlings growing on soil not inhabited by the invader, was found. Some authors point out that the positive allelopathic effect is short-lived, and the chemical compounds that cause it, undergoing natural processes of decomposition in the soil, cause the long-term fertilization effect [45]. The positive effect of soil under *P. serotina* on *F. esculentum* demonstrated in our research, even after adding activated carbon, may suggest stimulation induced by nutrients. In the research works of other authors, the addition of activated carbon to the soil obtained from the sites of invasive species, such as *Reynoutria* × *bohemica* or *Solidago canadensis*, usually reduces the negative impact of such soil on the growth of target species [46,47]. Moreover, it cannot be ruled out that activated carbon may have changed the availability of nutrients in the soil, even in the absence of allelochemicals, which is confirmed by the study of Lau et al. [48].

It is difficult to predict the durability of the found phytotoxic or fertilizing effect of *P. serotina*, because plant growth is a process modified by the type of soil [49], soil physical properties [50], the content of nutrients [51], activity of soil microorganisms [52,53], and the interactions among the above-mentioned factors. Complex biochemical interactions occurring in the soil environment play a very important but still poorly understood role in the course of plant invasion [54,55].

The methods used to assess the allelopathic potential of black cherry, despite the small amount of plant material used, confirmed the possibility of the impact of this invasive plant species on selected crop species. The authors are aware of limitations resulting from tests carried out in laboratory conditions. It is likely that the applied concentrations of extracts from decaying leaves of *P. serotina* were higher than those in the natural environment. It should also be taken into account that allelopathic interactions between species in the same plant community occur simultaneously with the competition phenomenon and are subject to certain seasonal dynamics, and the phytotoxic effects observed in laboratory conditions may not be significant in nature [56]. Additionally, laboratory tests with filter paper do not take into account the processes taking place in the natural soil environment, where the activity of allelopathic compounds undergoes very dynamic changes [57]. They are influenced by physical (texture, structure, organic matter content, moisture, and aeration), chemical (reaction, ion exchange capacity, nutrient dynamics, and O_2 and CO_2 concentrations), and biological (soil microorganisms) soil properties. The set of all closely related interactions exerts a multidirectional effect on the retention, transport, and transformation of allelochemicals, as well as it determines their bioavailability and the level of phytotoxicity [58].

5. Conclusions

The results obtained in the current study indicated that litter extracts of *Prunus serotina* and soil with residues of *P. serotina* significantly inhibit growth of the cultivated crops white mustard and common buckwheat. Both seeds and whole seedlings of *Sinapis alba* were found to be more sensitive to allelochemicals released by *P. serotina* than *Fagopyrum*

esculentum. In the case of common buckwheat, a negative effect of the tested extracts and soil on root mass reduction was observed. Preliminary results of our laboratory tests suggest that cultivation of white mustard should be avoided in the reclamation of fallows with black cherry. Further research is recommended, in particular in field conditions, which will make it possible to assess the potential to restore fallow lands with black cherry to their agricultural function.

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