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Examination of the spread and control possibilities of black cherry (*Prunus serotina* Ehrh.)

Theses of doctoral (PhD) dissertation

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Sopron
2022

Doctoral School:

Roth Gyula Forestry and Wildlife Management Doctoral School

Program:

Biological Basis of Forest Management (E2)

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1. Introduction and Objectives

Black cherry (*Prunus serotina* Ehrhardt, 1784) is recognized Europe-wide as a rapidly expanding invasive species, it is identified as one of the most dangerous invasive plants in several international lists. It is also recognized as a fast-expanding invasive species in Hungary. Its impact on the natural vegetation is very diverse, beyond its allelopathic potential and competitive behaviour it transforms the soil by accelerating the leaf litter and humus formation, gradually changing the shrub-layer of the forest, after that the lower canopy layer, and finally due to its strong shading, the herb layer as well.

In addition to the problems it causes in nature conservation, black cherry is the source of significant problems in forest management both in terms of intensive sylviculture for timber production and inhibiting the natural regeneration of our native forests. With its good competitive capacity, it can suppress the main species, forming dense regeneration at the herb layer, inhibiting the natural regeneration of native tree species by its high cover and allelopathy.

Because of this, problems caused in nature conservation and sylviculture, factors associated with the spread of the species and possibilities of its control were the main points of this research.

Objectives of the research:

- Exploring various opinions on the sylvicultural problems caused by black cherry, its sylvicultural importance, and to learn about the control methods and their effectiveness via an online questionnaire.
- Examining the effect of black cherry on the understorey of different forests via nationwide surveying of the layers of five types of forests, and also by surveying the vegetation of the Nagylózs 5F experiment.
- Investigating of the factors shaping the spread and invasion success of the black cherry, such as seed bank, allelopathic interactions, and sprouting capacity.
- Investigation of chemical control options by studying the efficiency of different herbicides, combinations, doses, application methods at different phenological phases.

2. Materials and Methods

2.1. Questionnaire about the sylvicultural significance of black cherry

The questionnaire, which was aimed to provide information about the sylvicultural significance of black cherry, was based on the method of MOLNÁR (2014). All the operational units of the 22 state forestries were contacted. The questionnaire enquired about the damage caused by black cherry in various forests, the type of damage, the control methods used and their cost implications.

2.2. Coenological surveys

In the spring and summer of 2017, coenological surveys were carried out in the 12 different forest types of the Nagylózs 5F forest subcompartment. During the surveys, three quadrats of 20 x 20 m were delineated in each plot, in which species cover data were recorded by layers. In the spring and summer of 2019, the same survey method was used to study forests' vegetation in 5 different parts of Hungary (Bács-Kiskun, Győr-Moson-Sopron, Pest and Szabolcs-Szatmár-Bereg Counties). The investigations were carried out in 41 different forest subcompartments. In every subcompartment, three 20 m x 20 m quadrats were surveyed, in total 123 quadrats. The majority of the surveyed areas were black locust (*Robinia pseudoacacia* Linnaeus, 1753) plots, but scots pine (*Pinus sylvestris* Linnaeus, 1753) turkey oak (*Quercus cerris* Linnaeus, 1753), pedunculate oak (*Quercus robur* Linnaeus, 1753) and Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco, 1950) plots were also studied.

Sample areas were analysed according to the social behaviour categories of the species explored there (BORHIDI, 1993). To examine the similarities of the studied plots, hierarchical cluster-analysis was carried out (Jaccard index, Bray-Curtis index). To study the relationship between the observed flora and closure of black cherry in the canopy and shrub layer, canonical correspondence analysis (CCA) was carried out. Statistical evaluation of the results was performed by non-parametric analysis of variance (Friedman test, Dunn test) and Spearman's rank-order correlation ($p<0,05$).

2.3. Examination of the seed bank of black cherry

The evaluation of the seed bank of black cherry was made with the following question in mind: Can the species regenerate from the seed bank, and can this regeneration be the source of a potential invasion?

The soil samples for this research were collected during the nationwide vegetation survey carried out in spring of 2019. This way the sampling was done after the germination of black cherry, but before seed dispersal – as this would allow detection of a persistent seed bank. In each forest subcompartment, 3 samples were collected from a selected specimen. The samples were collected at nearly equal distances to a depth of 30 cm.

2.4. Examination of the allelopathic potential of black cherry

The experiment on the allelopathic effect of black cherry was carried out in 2019 using the method described by SZABÓ (1999). In the experiment, observation of the allelopathic effect of black cherry was made on 10 different test plants. As test plants, fast germinating commercially available species were selected, several of which are used as test plants for allelopathy studies.

Test plants included wheat (*Triticum aestivum* Linnaeus, 1753), broccoli *(Brassica oleracea* convar. *botrytis* Linnaeus, 1753), sugar beet (*Beta vulgaris* ssp. *vulgaris* var. *altissima* Döll, 1843), alfalfa (*Medicago sativa* Linnaeus, 1753), white mustard (*Sinapis alba* Linnaeus, 1753), sunflower (*Helianthus annuus* Linnaeus, 1753), lettuce (*Lactuca sativa* Linnaeus, 1753), carrot (*Daucus carota* subsp. *sativus* (Hoffmann) Arcang., 1882), red onion (*Allium cepa* Linneus, 1753) and garden cress *(Lepidium sativum* Linnaeus, 1753).

Two different concentrations of aqueous extracts (1 g/100 ml and 5 g/ 100 ml) and a control with distilled water were prepared for each plant species. Percentage of germination, shoot and root length were recorded on the 6th day from the beginning of germination. The control treatments with distilled water were used for comparison. Germination percentage was evaluated by χ^2 test, while shoot and root length were evaluated by Mann-Whitney test.

2.5. Examination of the sprouting capacity of black cherry

Two experiments were carried out to study the sprouting capacity of black cherry, the first one in 2017, the second one in 2018-2019. Investigations were conducted in two forest subcompartments, one of which was a clear-cut black locust regeneration (open), the other was a middle-aged black locust plot (closed).

In 2017, 25-25 sample trees were felled in both areas, with a diameter of 20-25 cm. In the spring of 2018, another 10-10 trees were felled. The emerging sprouts were cut back in 2019, then the re-emerging sprouts were also examined. The surveyed data were compared using paired-sample t-test using the InStat software.

2.6. Exploring herbicide control options

The aim of the experiments was to determine the effectiveness of two methods of control: trunk injection and bark treatment, as well as finding the lowest effective dose if their effectiveness is proven.

The trunk injection experiments were carried out in the Gödöllő 84/E subcompartment, the bark treatment experiments were carried out in the Gödöllő 84/C subcompartment in 2016 and after that in 2018, in middle-aged black locust plots. Evaluation was based on the condition of the crown (weighted on a ten-point scale) and the possible appearance of sprouts. In order to explore the difference between the treatments, results were statistically evaluated by non-parametric analysis of variance (Friendman test, Dunn test) ($p<0.05$).

Names and doses of applied herbicides are given in section 3.6.

2.6.1. Trunk Injection

In 2016, chosen trees were injected with aqueous solutions of 8 different formulations. In 2018, the effects of 11 different herbicides, some at reduced doses from the previous experiment, were tested. Following preliminary field trips, 10 trees per each treatment were injected. On 8th of August 2016, 80 trees were injected and on 25th of July 2018 and on 15th of September 2018, 110-100 individuals were treated.

2.6.2. Bark treatment

In 2016, specimens were treated with a linseed oil solution of 7 formulations. In 2018, three treatments from the previous experiment were applied, but the solvent was water. Following preliminary field trips, 15 trees per each treatment were treated. On the 8th of August 2016, 105 trees were treated, on the 25th of July of 2018 and on 15th September 2018, 45-45 specimens were treated.

3. Results and conclusions

3.1. Results of the questionnaire

The questionnaire received 54 responses, that is 56% of the respondents who completed the questionnaire.

On a scale of 1 to 5, the sylvicultural significance of black cherry was rated 2.19 overall. In areas with larger populations, it is considered a major threat, such as: Nyírség, Gödöllői-dombság, Szigetköz-Rábaköz. According to the answers, in these areas black cherry requires a targeted intervention in the majority of forest regenerations (value of 4).

The methods of control were detailed by 39 experts, thanks to which 2831 hectares of forest regeneration were carried out and their financial implications became known.

Over two-thirds (69%) of all control methods were chemical, 1/3rd of which was trunk injection, and just over 1/5th was spraying. Regarding mechanical forms of control, girdling was the most effective method.

All chemical methods were considered more effective than mechanical interventions. Bark treatment (with causing wound) was considered the most expedient (3.67) by the respondents, followed by trunk injection (3.63).

According to the respondents, chemical control methods were specifically more expensive than mechanical ones. The most cost-effective method of control was trunk injection.

The total area where respondents applied the various control methods were 2831 hectares. A total of 146.9 million HUF was allocated to combat the invasion of black cherry.

Based on the responses, black cherry caused the most problems in black locust (3.52), pedunculate oak (3.52) and scotch pine forests. This is not surprising given that the centre of its distribution area is concentrated in sandy areas. However, it also requires targeted control in noble poplar (3.2) and European turkey oak (2.9) forests. Based on the responses, it is not a problem in floodplain forests, and beech forests are also not endangered.

Overall, the present research is the first questionnaire survey that specifically studies the sylvicultural importance of black cherry. Respondents attributed low importance of the species at the national level. However, locally, where there are larger populations, the results of the questionnaire suggest that a greater emphasis could be placed on controlling the species to prevent its further spread.

3.2. Results of the coenological survey

As a result of the coenological survey of the Nagylózs 5F tree species comparison experiment, it was found that there was a negative correlation between the presence of black cherry in the shrub level and the closure of the canopy level. The plots of different species were conquered to varying degrees by the species. In plots with a less closed canopy layer (such as sweet chestnut (*Castanea sativa* Miller, 1768) and scots pine), black cherry colonized more successfully than in those with a more closed canopy layer (such as small-leaved linden (*Tilia cordata* Miller, 1768) and large-leaved linden (*Tilia platyphyllos* Scopoli, 1771)). This suggests that the presence of a second canopy layer may play an important role in black cherry control, due to the second canopy layer's additional shading (NEMES *et al.*, 2018).

When analysing the data of the nationwide coenological survey, a slight negative correlation was found between the cover of black cherry in the canopy layer and the number of species indicating naturalness at the herb layer. There was a significant positive correlation between the cover of black cherry in the shrub layer and the number of ruderal species, presumably due to the fact that in an environment which was already changed by the invasion of black cherry, ruderal species were able to utilize natural resources more efficiently and were better adapted to the new environment compared to other species (CHABRERIE *et al.*, 2010).

When comparing surveyed areas of the same forest type, there was a difference between the number of species in the flora of different black locust and scots pine target areas.

Hierarchical cluster analysis based on Jaccard index and Bray-Curtis index also showed the greatest variance between areas of the black locust and scots pine target areas. Based on the results of the canonical correspondence analysis, no clear correlation could be found between the cover of black cherry and the location of the ruderal and species that indicate naturalness.

Overall, this research confirms the existing scientific literature, but to establish an accurate picture, long-term experiments are necessary.

3.3. Results of the seed bank examination

None of the samples taken from 28 soils in the sample areas of 5 forest types contained viable, germination-capable black cherry seeds. Some soil samples (Gödöllő 83/A, Hegykő 2/E, Hegykő 2/F) contained seeds which were dispersed after applying light pressure.

Overall, according to the results of the experiment, black cherry does not form a persistent seed bank in Hungary, confirming the findings of existing foreign literature (THOMPSON *et al.*, 1997, PHARTYAL *et al*., 2009). However, confirmative information can also be important, because thorough exploration of all the factors influencing the spread of black cherry may play a role in predicting the invasion and long-term consequences of the species, as well in its control.

3.4. Results of the allelopathical experiments

The negative effect of black cherry on the germination parameters of test plants could be observed for the 1 g/100 ml solution, and for the 5 g/100 ml solution it significantly reduced the root and shoot length compared to the controls. In the case of several test plants (alfalfa, white mustard, carrot, garden cress) the germination process did not even start. The extracts significantly inhibited the development of the most commonly used experimental plants, so beside white mustard (CSISZÁR, 2009), the allelopathic effects of black cherry was also proved in other agriculturally important plants.

Seeds of all species included in the study showed nearly 100 percent germination vigour in the control Petri dishes, thus the plants included in this experiment were suitable test plants for allelopathy testing.

In conclusion, the phytotoxic compounds found in the shoots of black cherry can potentially inhibit the germination of several plant species found in nature, but further experiments would be important to investigate the effect of black cherry on the germination of sylviculturally important species (like pedunculate oak and scots pine), as black cherry interacts with these species in nature way more often than agricultural test plants.

3.5. Results of the sprout formation experiment

In the study conducted in 2017, stumps under the shade of the canopy of black locust subcompartment produced more spouts (~ 49 pieces/stump) than those exposed to full sun in the forest regeneration (~ 31 pieces/stump). The average length of the sprouts developing under shade was shorter throughout the whole experiment, with a later growth start and a sharp slowdown during the summer. At the end of the study, sprouts developing in full sun were more than twice as long (143.2 cm) compared to sprouts developing under shade (63.9 cm), but had significantly smaller leaves.

In the 2018 and 2019 studies, the stumps under the shade of the canopy of the black locust subcompartment produced far fewer shoots in the year after cutting back than those developing in full sun, and the shoots were also shorter under the closed canopy.

A difference was also found in the number of sprouts formed in the two experiments conducted in different years. Trunks in full sun ($p=0.005$, $r=0.7455$) and under shade ($p=0.0010$, $r=-0.3071$) produced more sprouts in 2018 than in 2019 following the cutback. Subsequently, sprouts were much shorter in 2019 compared to 2018 in the closed canopy area, while trunks in full sun produced sprouts in 2019 that were closer to length to those formed in 2018. In terms of length, there was a significant difference in the sprouts developing in full sun ($p=0.0015$, $r=1,000$) and under shadow ($p=0.0098$, $r=0.04863$) in favour of the sprouts that developed in 2018. Sprouts developing in full sun in 2018 were nearly twice, and in 2019 were almost three times longer than their counterparts developing in shade.

Therefore, out of all mechanical control methods simply felling the tree is not economical, as shown by the large number and length of sprouts formed. Although they were not as vigorous as those developed in 2018, sprouts that developed in 2019 were absolutely viable and showed a faster growth than the rest of the vegetation, thus they were capable of becoming dominant. It would presumably require several years of continuous cutback, which is not a cost-effective enough method. However, in sensitive ecosystems, it may be a worthwhile solution despite the high labour and cost implications.

3.6. Results of the study of herbicide control options

The results of trunk injection experiment are summarized in *Table 1*.

Table 1: Summary table of the trunk injection experiments

Herbicide	Concentration	Mean Dbh (cm)	Date of injection	Efficiency	Remarks
Medallon Premium	70%	14.5	2016.08.08.	effective	
	55%	13.6	2018.07.25.	effective	
		13.4	2018.09.15.	ineffective	strong sprouting
Medallon Premium Mecomorn 750 SL	60% 10%	13.6	2016.08.08.	effective	
	50% 5%	12.9	2018.07.25.	effective	
		19.4	2018.09.15.	ineffective	strong sprouting
Medallon Premium DMA-6	60% 10%	14.5	2016.08.08.	effective	
Medallon Premium Banvel 480 S	60% 10%	17.8	2016.08.08.	effective	
	50% 5%	18.3	2018.07.25.	effective	
		15.5	2018.09.15.	ineffective	strong sprouting
Medallon Premium Tomigan 250 EC	60% 10%	16.5	2016.08.08.	effective	
	50% 5%	12.1	2018.07.25.	effective	
		18.6	2018.09.15.	ineffective	strong sprouting
Medallon Premium Chikara 25 WG	60% 10%	14.4	2016.08.08.	effective	
Kyleo Mezzo 20 WG	40% 1%	15.2	2016.08.08.	effective	
	40% 1%	18.3	2018.07.25.	ineffective	strong sprouting
		15.9	2018.09.15.	effective	
Medallon Premium Lontrel 300	60% 10%	14.7	2016.08.08.	effective	
	50% 5%	15.5	2018.07.25.	effective	
		12.8	2018.09.15.	effective	
Chikara Duo	55%	15.5	2018.07.25.	ineffective	strong sprouting
		16.8	2018.09.15.	ineffective	strong sprouting
Kyleo	55%	17.4	2018.07.25.	ineffective	strong sprouting
		16.6	2018.09.15.	effective	
Mecomorn 750 SL	55%	18.5	2018.07.25.	ineffective	strong sprouting
		14.3	2018.09.15.	ineffective	strong sprouting
Banvel 480 S	55%	16.5	2018.07.25.	ineffective	strong sprouting
		14.5	2018.09.15.	effective	
Lontrel 300	55%	19.8	2018.07.25.	ineffective	strong sprouting
		15.3	2018.09.15.	ineffective	strong sprouting

The results of bark treatment experiments are summarized in *Table 2.*

Table 2: Summary table of the bark treatment experiments

Herbicide	Concen-tration	Mean Dbh (cm)	Date of the treatment	Efficiency	Remarks
Medallon Premium	33%	4.7	2016.08.08.	ineffective	minimal yellowing, fully healthy crown in the following year
Medallon Premium Mecomorn 750 SL	30% 3%	4.6	2016.08.08.	effective	
		4.3	2018.07.25.	effective	
		4.4	2018.09.15.	effective	
Medallon Premium DMA-6	30% 3%	4.4	2016.08.08.	ineffective	minimal yellowing, fully healthy crown in the following year
Medallon Premium Banvel 480 S	30% 3%	4.4	2016.08.08.	ineffective	minimal yellowing, fully healthy crown in the following year
		4.6	2018.07.25.	effective	
		4.5	2018.09.15.	effective	
Medallon Premium Tomigan 250 EC	30% 3%	4.9	2016 08.08.	effective	
Medallon Premium Chikara 25 WG	30% 3%	4.8	2016.08.08.	effective	
Medallon Premium Lontrel 300	30% 3%	4.5	2016.08.08.	effective	
		4.2	2018.07.25.	effective	
		4.8	2018.09.15.	effective	

Tested formulations were not always effective in the tested doses, but the formulations of the effective treatments can be used in practice and provide a basis for further series of dose-reducing experiments. Formulations that resulted in significant sprouting in addition to the destruction of the crown should not be used.

In conclusion, trunk injection was found to be an effective chemical control method which can be used reliably after a detailed risk assessment. Bark treatment can also be an effective control method but should only be used in younger individuals with small breast-height diameter due to the risk of drift of the herbicides.

4. Theses

The most important scientific results of the studies are the following:

1. At the national level, the impact of black cherry was surveyed in 123 quadrats of 41 forest subcompartments of 5 forest types. Based on the results, a significant negative correlation can be demonstrated between the canopy cover of black cherry and the number of species indicating naturalness, and a positive correlation can be seen between the cover of black cherry in the shrub layer and the number of ruderal species.

2. As a result of the soil seed bank study on 28 sample areas in 5 forest types that had black cherry, it was confirmed that black cherry does not form a persistent seed bank under Hungarian conditions. These results are new for the Hungarian conditions and confirm the findings of previous experiments documented in European literature on black cherry seed banks.

3. The allelopathic effect of the extract of the foliage of black cherry was confirmed on nine test plants. The study confirmed the literature knowledge concerning white mustard and demonstrated the germination and growth inhibitory effects of black cherry on wheat, broccoli, sugar beet, alfalfa, lettuce, carrot, red onion and garden cress.

4. Trunk sprouting response of black cherry differs between open and closed forest subcompartments, with differences in the number and length of shoots, the rate of growth and the size of the leaves. Despite the differences, sprouting capacity of black cherry is significant even in the closed forest subcompartments.

5. Felling of black cherry, by itself is an ineffective control method. Cutting back the emerging sprouts significantly reduces the number and length of sprouts appearing in the following year; however, the reduction is not sufficient to control the species.

6. Experiments show that trunk injection, and in case of trees with a diameter less than 5 cm breast-height, damage-free bark treatment are effective methods for controlling black cherry. Trunk injection when executed in the summer is more effective, causing the permanent destruction of the trees. Trunk injection carried out at the end of the vegetation period can cause the root system to overcome and it can respond with vigorous sprouting.

7. The eight herbicide mixtures applied in the dose-reduction trunk injection experiment proved to be equally effective in terms of defoliation, but there were differences in terms of efficacy, as some mixtures elicited a sprouting response. Of the mixtures that did not induce sprouting, the formulation that could be more recommended is the combination of Medallon Premium (glyphosate) and Lontrel 300 (clopyralid) in a 50 + 5% aqueous solution, which was effective in both summer and autumn applications and did not result in sprouting response.

8. For young trees with thin trunks, bark treatment is an effective control method in both summer and autumn applications. Medallon Premium (glyphosate) at 33% dose was not effective by itself, but it was effective in 30 + 3% linseed oil solutions of the following herbicides: Mecomorn 750 SL (MCPA), Lontrel 300 (clopyralid), Tomigan 250 EC (fluroxypyr) and Chikara 25 WG (flazasulfuron). In an aqueous mixture, Medallon Premium 30 + 3% mixing ratio with the following herbicides caused complete plant death: Mecomorn 750 SL (MCPA), Banvel 480 S (dicamba) and Lontrel 300 (clopyralid).

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