PhD THESIS

Research on the dynamics and impact of *Acer negundo* on poplar and willow galleries in the Mures Middle Plain

(SUMMARY OF PhD THESIS)

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Introduction

Acer negundo, traditionally known as the American maple, is a fast-growing invasive tree species ranging in height from 8 to 25 m in native habitats and up to 15 m outside. Although native to the USA, Acer negundo is now found in many other areas of the world, including our own country. In Romania, this species was intentionally introduced îin 1887, both for ornamental purposes and for forest plantations and anti-erosion forest belts (DUMITRAȘCU MONICA et al., 2014). It is found especially in lowland and hilly areas, being observed in riverbeds, near streams and on the edge of lakes or ponds, but also in parks, gardens and urban areas, where it is cultivated as an ornamental tree due to its decorative leaves (BRÎNZAN et al., 2020).

Acer negundo is highly adaptable and widespread, characteristics that can give it a competitive advantage in different environments. Recent studies have shown that this invasive tree can compete with native species for resources such as light, water and nutrients (DUMAS, 2019; RAFIKOVA et al., 2020; SIKORSKA et al., 2019). This can lead to reduced local biodiversity and changes in the structure and functionality of invaded ecosystems.

Given the high capacity of this species to spread into new habitats in our country (by dredging and budding), the aggressiveness of this species in competition with native species and its lack of economic value (PATTERSON, 1985), studies that can contribute to the understanding of the invasion mechanism, as well as the trajectory followed by this species in colonizing new habitats, are essential for the conservation of poplar and willow forests (both inside and outside Natura 2000 sites).

1. Structure of the doctoral thesis

The doctoral thesis comprises a total of 150 pages and is structured into two parts, totalling 9 chapters, 82 figures and 22 tables.

The first part is devoted to the bibliographical study and represents 29.33% (44 pages) of the thesis, while the second part comprises the personal contribution, representing 70.67% of the thesis (106 pages).

Part I of the thesis consists of 3 chapters. **Chapter 1** summarises information on the current state of knowledge on invasive species and presents issues related to: the definition of invasive species, discussion of factors influencing their spread, pathways of introduction into the natural environment, mechanisms of invasion, European Union strategy on the control of invasive species, and finally how the Natura 2000 Network is managing this issue. **Chapter 2** covers issues related to the impact of invasive tree species on stands specific to riparian ecosystems, with particular

attention to *Salix alba* and *Populus alba* galleries. **Chapter 3** presents a synthesis of the literature on invasion concerns of *Acer negundo*, highlighting aspects of the biological and ecological characteristics of the species as well as the most relevant research on its spread, intensity and invasiveness.

Part II of the thesis is the personal contribution and comprises 6 chapters. In this part are presented:

- The aim and objectives of the research (**Chapter 4**);
- The particularities of the natural environment in which the research was carried out (**Chapter 5**);
- Materials and methods used (**Chapter 6**);
- Results and discussion (**Chapter 7**);
- Conclusions and recommendations (**Chapter 8**);
- Originality and innovative contributions of the thesis (**Chapter 9**);
- **Bibliography**, which contains 147 bibliographic titles.

2. Purpose and objectives of the research

The aim of the research was to identify and map the invasive species *Acer negundo* in the Middle Mures Plain and to assess the risks and impacts it has/may have on native stands, both inside and outside Natura 2000 sites.

In order to achieve the proposed goal, the following specific objectives were pursued:

(1) General characterization of aspen and willow forests in the Middle Mures Plain.

(2) Assessment of native tree species distribution and invasiveness of invasive tree and shrub species.

(3) To investigate the impact of invasive tree and shrub species on poplar and willow stands.

(4) Evaluate the effectiveness of the Natura 2000 Ecological Network in conserving aspen and willow galleries from invasive *Acer negundo*.

(5) Distribution dynamics of *Acer negundo* in the Middle Mures Plain.

3. The particularities of the natural environment in which the research was carried out

The research carried out to achieve the aim of this PhD thesis was carried out in the Middle Mures Plain, a region along the Mures and Cugir rivers. Geographically speaking, the area investigated is located in the Transylvanian Plateau, a geographical unit in the inner area of the Carpathian arc characterized by a temperate continental climate. Two Natura 2000 sites (ROSCI0419 Mureşul Mijlociu-Cugir and ROSCI0313 Confluence Mureş with Arieş), important for the conservation of habitat 92A0 - *Salix alba* and *Populus alba* galleries, were also included in the perimeter of the research area. The dominant vegetation in the surveyed region is represented by moisture-loving species, the edifying stands being represented by *Populus alba* (aspen) and Salix alba (willow), together with species such as *Alnus glutinosa* (black maple), *Salix fragilis* (willow) and, according to the studies carried out so far, invasive species such as *Acer negundo.*

4. Material and method

Observations and determinations were carried out from March 2020 to May 2023, both at the beginning of the growing season (spring) and at the end of the growing season (autumn).

The experimental protocol involved, in the first phase, the delimitation of the research stations, which were selected on the basis of the data obtained using the drone. Seven representative research stations were delimited: (1) Gligoreşti (located within the Natura 2000 site ROSCI0313 Confluence Mureş with Arieş), (2) Hădăreni, (3) Ciumbrud, (4) Rădeşti, (5) Zărieş, (6) Căpud and (7) Balomiru de Câmp (located within the Natura 2000 site ROSCI0419 Mureşul Mijlociu-Cugir). The 7 Research Stations have been named after the names of the localities on whose radius they are located and are presented in Figure 1.

The research methodology included both field methods specific to such studies, namely the transect and polygon methods, and innovative methods such as the use of unmanned aerial vehicles - drones (for preliminary studies and delimitation of representative research stations) and satellite imagery (for mapping the invasive species *Acer negundo* along the surveyed area). In some research stations it was decided to use both field methods, while in others only transects could be made, as the stationary conditions in the field did not make it possible to make polygons.

Fig. 1. Location of the research stations

The study polygons were organized according to the method proposed by MANNING et al. (2011), each with a radius of 70 m, with three sample areas per polygon (0.6-0.8 ha/each). The transects were made so that as large a distance as possible could be covered within the polygon analysed (approximately 10-20 km), depending on the area and the number of invasive species identified.

Specifically, the following indicators were determined:

a) Indicators of stand structure: composition of poplar and willow stands (expressed as the proportion of each tree species per surveyed area), consistency, density (expressed as the number of trees per surveyed area) and degree of invasion.
b) Dendrometric indices of inventoried tree and shrub species (diameter, height, age) and diameter growth of American maple.
c) Indices of stand diversity: richness (expressed by the number of tree species per surveyed area) and tree species diversity (expressed by the Shannon-Weiner (H′) and Evenness (J) diversity indices).
d) The establishment of stands forming the poplar and willow galleries.

Data recorded during field surveys were processed using Statistics vs 10, t-test and Hutcheson/diversity t-test (for the two diversity indices calculated, using Pastsoftware).

5. Results and discussions

The results and discussion are presented in **Chapter 7** and represent the largest part of the PhD thesis. This chapter is structured in 5 sub-chapters, in line with the 5 specific objectives stated above.

In sub-chapter 7.1. research was carried out on the general characterisation of poplar and willow forests in the Middle Mures Plain. It was noted that the meadow forests in the researched area are characterized by a compact, well-developed stand of mainly poplar and willow. Along with these two native species, other species characteristic of riparian forests, such as *Alnus glutinosa* and *Sambucus nigra* (black elder), were also observed in the stand structure in different proportions from one region to another. Preliminary analysis of the structure of aspen and willow stands by drone revealed the presence of invasive tree and shrub species in some areas of the study area, of which *Acer negundo* (American maple) and *Amorpfa fructicosa* (dwarf acacia) were the most important.

In sub-chapter 7.2. research was carried out on the assessment of native tree species distribution and the degree of invasiveness of invasive tree and shrub species in the Middle Mures Plain. The obtained results revealed that the stand structure of the Gligoreşti Research Station (located within the Natura 2000 site ROSCI0313 Confluence Mureş with Arieş) consisted of young aspen and willow stands (specimens with a maximum age of 9 years for *Salix alba* and 7 years for *Populus alba*), with a full consistency (cover of about 90-100%), composed of mixed stands. The tree layer was predominantly composed of *Salix alba* (up to 77%) and *Populus alba* (up to 43%). The characteristic species Alnus glutinosa and the invasive species *Acer negundo* and *Amorpha fructicosa* (isolated specimens) were inventoried in small proportions. The *Acer negundo* specimens inventoried were young (age category 4-6 years), with height and diameter values lower than the average height/diameter of the inventoried tree species/polygon. This observation suggests that native species have a competitive advantage over American maple for food and/or light resources in this Research Station. A similar stand structure was also observed in Hădăreni, where white willow occupied the largest share (about 71-85%), followed by white poplar with 20%. Black maple was present in the stand composition in 5% and American maple in 3-8%.

The results obtained for Ciumbrud and Căpud revealed a different structure of the poplar and willow galleries than that observed for the first two research stations analysed. More mature aspen and willow stands were found (with a maximum age of 22 years for *Salix alba* and 14 years for *Populus alba*). The stands had a higher density and 90% cover. The tree layer was predominantly composed of *Salix alba* (between 27-69%) and *Populus alba* (7-14%), with *Alnus glutinosa* (between 6-12%). The *Acer negundo* specimens inventoried were more mature (maximum age of 12 years), with a higher share in the stand structure than described for the first two research stations. Also in these two research stations, an incipient invasion of *Acer negundo* was assessed.

In Rădeşti and Zărieş, mature aspen and willow stands (up to 35 years old for willow and 22 years old for aspen) were inventoried. The percentage of participation in the stand structure of white willow was up to 35% and of white poplar up to 12%. Most of the stand structure was composed of adult (age category 14-16 years), vigorous *Acer negundo* (in some sample areas even more than 70%), a species that in these research areas appeared arranged in compact groups. The stand structure in the Balomiru de Câmp Research Station (located within the Natura 2000 site ROSCI0419 Muresul Mijlociu-Cugir) was relatively similar to that described for Rădeşti and Zărieş, being predominantly composed of mature, vigorous specimens of American maple (age category 8-24 years), which had the largest share in the forest phytocenoses analysed. The inventoried stands were mature, up to 35 years old for willow and 90 years old for poplar. In general, the American maple specimens inventoried had greater height/diameter than the average height/diameter/pole, which may indicate intensified competition for resources in the poplar and willow stands analyzed. Further, this competitive advantage of invasive species over native species may indicate significant changes in stand structure, with potential consequences for ecosystem composition and functioning, if effective management practices are not adopted. Alongside the American maple, the dwarf acacia has also been inventoried, and its share in the stand structure has increased in proportion to that of the American maple. In areas where there are no more aspen and willow stands, *Acer negundo* has occupied a very large share in the stand structure, taking full possession of the banks of the Mures River.

In sub-chapter 7.3. research was carried out on the impact of invasive tree and shrub species on poplar and willow stands. The results showed that in Gligoreşti (research station located within the Natura 2000 site ROSCI0313 Confluence Mures with Arieş) the lowest tree species richness (average of 3 tree species) and the highest values of the Shannon - Weiner diversity index (between 0.54-1.47), i.e. the most uniform species population distribution, were obtained. In Rădeşti, Ciumbrud, Zărieş and Balomiru de Câmp a greater richness in tree species was inventoried (4-5 species), but the value of the Shannon - Weiner diversity index (H') was lower than that obtained for Gligoreşti (maximum 1.27), and a less uniform distribution of tree and shrub species than in Gligoreşti (Pielou index value of maximum 0.85).

The species of trees and shrubs in the research stations analysed were generally arranged in two vegetation stages:

- the lower ceiling, located on the river bank (Mures or Cugir), mostly composed of tree and shrub species characteristic for the type of habitat, such as *Salix alba* and *Alnus glutinosa*;

- the upper ceiling, mainly composed of *Populus alba*.

In the research stations where a high degree of invasion was observed (Zărieș, Rădești and Balomiru de Câmp), the upper canopy was mostly composed of American maple, along with a small number of *Populus alba*. In the research stations where an incipient level of American maple invasion was observed (Gligorești, Hădăreni, Ciumbrud and Căpud), a higher percentage of grassy vegetation species characteristic for the habitat type (A*egopodium podagraria, Brachypodium sylvaticum, Glechoma hederaceae, Urtica dioica*) was observed in the lower canopy. These results explain the higher diversity (by H' index value) found in research stations with incipient invasion compared to those strongly invaded by this invasive species.

In sub-chapter 7.4. research was carried out on the effectiveness of the Natura 2000 ecological network in the conservation of poplar and willow galleries from invasion by *Acer negundo*. The results showed that the inclusion of *Salix alba* and *Populus alba* galleries in ROSCI0419 Mureșul Mijlociu - Cugir in N2000 failed to ensure their conservation from biological invasion. As the research carried out in the framework of the thesis shows, the research area within the N2000 network was highly threatened by two invasive species, namely the American maple (poses particular problems) and the dwarf acacia.

In sub-chapter 7.5. research was carried out on the distribution dynamics of *Acer negundo* in the Middle Mures Plain. The results obtained from the processing of satellite images taken with the Sentinel-2 instrument (and trained/tested with field data collected with polygons and transects), allowed the identification and differentiation of *Acer negundo* species from the other species present in the structure of poplar and willow forests in the Middle Mures Plain. The most relevant data for training the algorithms (which were the basis for the recognition of this invasive species) were those from the research stations Zărieș, Rădești and Balomiru de Câmp, sample areas where the American maple showed a higher degree of invasion. The accuracy achieved in the identification of American maple along the Middle Mures Plain using satellite images was 49% maximum.

6. Conclusions and recommendations

(1) **As a general conclusion, it was observed that the invasion of the *Acer negundo* species is getting stronger and older (the inventoried specimens being more and more mature), as we go downstream on the Mures river, suggesting the hypothesis that the invasion started from downstream to upstream.** The fact that the invasion comes from downstream to upstream suggests that water is not the factor propagating the invasion. One of the most likely factors that could have contributed to the invasion of American maple is wind, which could facilitate the

spread of seeds of this species (due to their shape and light weight they are extremely easy to transport by air currents), but we recommend further study of this aspect, given that this invasive species, which is particularly prolific, propagates by seeds, shoots and drakes.

(2) The highest degree of invasion was observed in the Balomiru de Câmp Research Station, located within the Natura 2000 Site ROSCI0419 Muresul Mijlociu-Cugir. At the opposite pole, the lowest degree of invasion was observed in the Gligoreşti Research Station, located inside the Natura 2000 Site ROSCI0313 Confluence Mureş with Arieş (where isolated, young specimens of American maple were inventoried, demonstrating the existence of an incipient invasion). These results demonstrate that the inclusion of Salix alba and Populus alba galleries in the N2000 network failed to ensure their conservation against the biological invasion of American maple. We therefore recommend that both land managers and managers of Natura 2000 sites intervene with silvicultural measures to eliminate invasive species and protect native species.

(3) In sample areas heavily invaded by *Acer negundo*, intensified competition for resources was observed, where this invasive species seems to have a competitive advantage (higher density, cover and dendrometric index values).

(4) Diameter growth in *Acer negundo* specimens was about 1.5 cm/year. It was found that in male specimens the increase in diameter was several mm higher. This phenomenon can be explained by the fact that female specimens fruit abundantly and consume part of the nutrients with the seeds which are extremely vigorous.

(5) In the sample areas with heavy invasion of American maple, a tendency to change the stand structure has already been observed, with native and edible species being largely replaced by this invasive species. This observation might suggest that American maple already has an influence on the ecological succession of vegetation in the surveyed ditches.

7. Originality and innovative contributions of the thesis

Although the presence of *Acer negundo* has already been observed in many habitats in Romania (including some sites under the protection regime of the Natura 2000 ecological network), the studies carried out so far on this topic are extremely few and have been limited, for the most part, only to mention the presence of the American maple, without research on the degree of invasion and impact of this species. Given the particular importance of aspen and willow forests for the biodiversity and ecological functioning of riparian ecosystems, studies on the identification and management of invasive species in these regions are essential for their conservation.

The research carried out in this PhD thesis has completed the scientific information base in the field of invasive species by:

(1) Conducting pioneering research in our country, which revealed the extent of invasion and the impact of American maple on poplar and willow forests in the Middle Mures Plain.

(2) To highlight, for the first time in our country, the trajectory followed by *Acer negundo* in the colonization of poplar and willow forests in the Middle Mures Plain (from downstream to upstream).

(3) Description of the aggressiveness of American maple in competition with native species in aspen and willow stand structure and their involvement in ecological succession.

(4) To carry out pioneering studies on the evaluation of the effectiveness of the Natura 2000 ecological network in the conservation of poplar and willow galleries in our country.

(5) Demonstrate the effectiveness and utility of innovative methods for studying stand structure and mapping American maple.

Selective references

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