

## Allelopathic Effects of Invasive Woody Plant Species in Hungary

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**Abstract** – Allelopathy may play an important role in the invasion success of adventive plant species. The aim of this study was to determine the allelopathic potential of invasive woody plant species occurring in Hungary. Juglone index of fourteen invasive woody plant species in Hungary was determined by the method of Szabó (1997), comparing the effects of juglone and substance extracted of plant species with unknown allelopathic potential on the germination rate, shoot length and root length of white mustard (*Sinapis alba* L.) used as receiver species. Results have proven a more or less expressed allelopathic potential in case of all species. The juglone index at higher concentration extracts (5 g dry plant material extracted with 100 ml distilled water) of almost every studied species approaches to 1 or is above 1, this means the effect of the extracts is similar to juglone or surpasses it. In terms of juglone index, the allelopathic potential of false indigo (*Amorpha fruticosa* L.), tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle) and hackberry (*Celtis occidentalis* L.) were the highest. Besides these species the treatment with the extracts of black walnut (*Juglans nigra* L.), black cherry (*Prunus serotina* Ehrh.) and green ash (*Fraxinus pennsylvanica* MARSH. var. *subintegerrima* (Vahl) Fern.) reduced extremely significantly the germination rate, shoot and root length, compared to the control.

**species / allelopathy / juglone index / germination inhibition / growth inhibition**

**Kivonat – Magyarországon előforduló fás szárú özönfajok allelopatikus hatásának vizsgálata.** Az allelopátia egyes adventív növényfajok inváziós sikerében jelentős szerepet tölthet be, ezért jelen vizsgálat a Magyarországon előforduló, fás szárú özönfajok allelopatikus potenciáljának megállapítását tűzte ki céljává. A vizsgálat során tízennégy, hazánkban inváziós fás szárú növényfaj juglon-indexe került meghatározásra Szabó (1997) által leírt módszer szerint, amely az ismeretlen allelopatikus potenciálú növényfajból készített kivonat hatását a juglonéval hasonlítja össze a fehér mustár (*Sinapis alba* L.) tesztnövény csírázási százaléka, gyökér- és hajtáshosszúságára nézve. A vizsgálat eredményeként bebizonyosodott, hogy a Magyarországon előforduló, fás szárú inváziós növényfajok mindegyike rendelkezik kifejezett vagy kevésbé kifejezett allelopatikus potenciállal, a magasabb koncentrációjú kivonatok (5 g szárított növényi anyag 100 ml desztillált vízben kivonva) esetén csaknem mindegyik faj juglon-indexe közelít az 1-hez vagy meghaladja azt, vagyis hatása a juglonéhoz közelít, vagy azét felülmúlja. A juglon-indexet tekintve kiemelkedő a gyalogakác (*Amorpha fruticosa* L.), a bálványfa (*Ailanthus altissima* (Mill.) Swingle) és a nyugati ostorfa (*Celtis occidentalis* L.) allelopatikus potenciálja. E fajokon kívül a fekete dió (*Juglans nigra* L.), a kései meggy (*Prunus serotina* Ehrh.) és a zöld kőris (*Fraxinus pennsylvanica* Marsh. var. *subintegerrima* (Vahl) Fern.) kivonatával történt kezelés mind a csírázási százalék, mind a hajtáshosszúság, mind pedig a gyökérhosszúság esetén rendkívül szignifikáns eltérést mutatott a kontrollhoz képest.

**fás szárú özönfajok / allelopátia / juglon-index / csírázásgátlás / növekedésgátlás**

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## 1 INTRODUCTION

Records about allelopathy are known since ancient times, however the inhibitory effect was not attributed to the secondary metabolites (Plinius Secundus 1<sup>st</sup> century AD cit. Rice 1984). After introducing the term allelopathy by Molish (1937), the definition has undergone several changing and widening of meaning (Csontos 1997, Brückner – Szabó 2001), nowadays it refers to the positive or negative, direct or indirect effect by one plant, fungus or microorganisms to the other through the production of chemical compounds that escape into the environment (RICE 1984). Most observations of allelopathy connected with agriculture, several weeds are known which are allelopathic to cultivated plants (Terpó – Kotori 1974, Mikulás 1981, Kazinczi et al. 1991), plant species allelopathic to weeds can offer a selective and an environmentally friendlier way of weed biocontrol.

Because of the complexity of allelopathy and problems of its practical applications the beneficial effects of allelopathy have not been exploited in the forestry yet, although the competition and allelopathic effects are more explicit in the forested plant communities because of huge biomass of canopy and root layers (Pellissier – Gallet – Souto 2002). It is also notable that almost one third of allelopathic plant species are woody plants in Hungary (Szabó 1997). Allelopathy can influence the succession, determine the pattern of cut area, inhibit the forestation and forest regeneration; understory species can inhibit the germination of woody plants therefore the secondary metabolites of woody plant species can greatly influence the pattern of understory (Lodhi 1975, 1976, 1978, Kuiters – Denneman 1987, Csontos 1991). The effect of understory species can be expressed indirectly through the microbiological activity of the soil (Szabó et al 1987), however the effect can show different rate in laboratory experiments and under field conditions (Fekete 1974). The role of allelopathy in the forest ecosystem has been proven by Čaboun (2004) in the seed production, forest regeneration and protection, forest structure and production of biomass. According to Carroll (1994): „Within the forest ecosystem allelochemical interaction plays a much larger role than previously thought, affecting growth, germination, plant succession, and vegetative patterning of forest ecosystem.”

It is not surprising that the percentage of alien species among the allelopathic plants is very high; since the species living close to each other were able to adapt to metabolites produced by the other species during the evolution, and an alien species is more likely to emit chemicals that native plants are less resistant to (Callaway – Aschehoug 2000). Many studies suggest that allelopathy may contribute the ability of an alien species to become dominant in invaded plant communities (Ridenour – Callaway 2001, Hierro – Callaway 2003). Among native plant communities the forests are especially endangered by the invasion of alien plant species in Hungary (Bartha 2002), the number of alien woody plant species can exceed the native woody species locally. Among invasive woody plant species occurring in Hungary the allelopathic effect of Manitoba maple (*Acer negundo* L.), false indigo (*Amorpha fruticosa* L.), black locust (*Robinia pseudoacacia* L.), black walnut (*Juglans nigra* L.), white mulberry (*Morus alba* L.), tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle), hackberry (*Celtis occidentalis* L.), black cherry (*Prunus serotina* EHRH.) and Russian-olive (*Elaeagnus angustifolia* L.) has been proven (Čaboun 1994, Nandal et al. 1994, Elakovich – Wooten 1995, 1996, Heisey 1996, Szabó 1999). This study aimed at investigating the allelopathic effects of invasive woody plant species occurring in Hungary under the same conditions providing comparison of allelopathic potential of species and results which can contribute to understanding of complex function of forest ecosystems and hereby the protection and conservation of our forests.

## 2 MATERIALS AND METHODS

Juglone index (SZABÓ 1999) was chosen to study the allelopathic potential of invasive woody plant species in Hungary. Juglone is a strong allelopathic naftoquinone has been isolated from Persian walnut (*Juglans regia* L.), black walnut (*Juglans nigra* L.) and many species in the *Juglandaceae* family; it is washed into the soil by the rain from leaves (DAGLISH 1950, PRATAVIERA et al. 1983). Juglone has an inhibitory effect on germination and seedling growth of several plant species (Kocacaliskan – Terzi 2001), this inhibition is expressed by reduced intensity of photosynthesis and respiration (Hejl et al. 1993, Jose – Gillespie 1998), and increased oxidative stress (Segura-Aguilar et al. 1992). ERCISLI et al. (2005) treated day-neutral strawberry (*Fragaria x ananassa* L.) by juglone and found that the fruit yield per plant, the number of fruit per plant, average fruit weight, crowns per plant, number of leaves, leaf area, fresh root weight, total soluble solid (TSS), vitamin C, acidity and uptake of some nutrient element decreased. Method of juglone index was introduced by SZABÓ (1999), it is based on comparing the effects of treatment with 1 mM juglone and substance extracted of plant species with unknown allelopathic potential. Juglone index is a quotient created by the germination rate, shoot length and root length of white mustard (*Sinapis alba* L.) treated with juglone and substance with unknown allelopathic potential. If this quotient is above 1, the allelopathic potential is more expressed than the juglone's and the inhibitory effect is stronger; if it is below 1, the allelopathic potential is less expressed than the juglone's and the inhibitory effect is weaker. If the juglone index is smaller than 0,5, we can not practically speak about an allelopathic potential (Szabó 1999). The juglone index ( $I_j / x$ ) of a substance with unknown allelopathic potential is:

$$I_j / x = (H_j + R_j + G_j) / (H_x + R_x + G_x)$$

Abbreviations:  $H_j$ : average of shoot lengths of 3 times 10 white mustard seeds treated with 1 mM juglone (mm),  $R_j$ : average of root lengths of 3 times 10 white mustard seeds treated with 1 mM juglone (mm),  $G_j$ : average of germination rates of 3 times 100 white mustard seeds treated with 1 mM juglone (number),  $H_x$ : average of shoot lengths of 3 times 10 white mustard seeds treated with substance from species "x" with unknown allelopathic potential (mm),  $R_x$ : average of root lengths of 3 times 10 white mustard seeds treated with substance from species "x" with unknown allelopathic potential (mm),  $G_x$ : average of germination rates of 3 times 100 white mustard seeds treated with substance from species "x" with unknown allelopathic potential (number).

For the examination shoots of invasive woody plants were collected in May 2008 and dried at room temperature. The studied species were the following: Manitoba maple (*Acer negundo* L.), false indigo (*Amorpha fruticosa* L.), black locust (*Robinia pseudoacacia* L.), black walnut (*Juglans nigra* L.), white mulberry (*Morus alba* L.), tree-of-heaven (*Ailanthus altissima* (Mill.) SWINGLE), hackberry (*Celtis occidentalis* L.), black cherry (*Prunus serotina* EHRH.), Russian-olive (*Elaeagnus angustifolia* L.), thicket creeper (*Parthenocissus inserta* (A. Kern) Fritsch), golden currant (*Ribes aureum* PURSH), riverbank grape (*Vitis riparia* MICHX.), common hoptree (*Ptelea trifoliata* L.), red ash (*Fraxinus pennsylvanica* Marsh. var. *austini* Fern.) and green ash (*Fraxinus pennsylvanica* Marsh. var. *subintegerrima* (Vahl) Fern.).

Aqueous extracts were prepared at two different concentrations by soaking 1 g and 5 g dried plant material (leaves and stems) of the donor species in 100 ml distilled water at 20 °C for an hour, shaken in every 10 minutes and filtered through filter paper. White mustard seeds were germinated between two filter papers wetted with 5 ml extract, in darkness, placed in a biological thermostat at 20 °C. 100 white mustard seeds were placed in each petri dishes, I repeated the experiment with each concentration and species three times. Germination rate, shoot and root length were registered on the 6th day counted from the beginning of imbibition. The effects of the different plant species extracts on the germination rate, shoot and root length of white mustard were compared to the treatment with using distilled water (control). The statistical analysis of the results was made by using Chi-square test in case of

germination rate, with Mann-Whitney test in case of shoot and root length using the InStat statistical program (InStat 1997). During the statistical analysis the categories of InStat statistical program [extremely significant ( $P < 0.001$ ), very significant ( $P < 0.01$ ), significant ( $P < 0.05$ ), not quite significant ( $P \leq 0.1$ ), not significant ( $P > 0.1$ )] were used.

### 3 RESULTS

Regarding juglone index of invasive woody plant species in Hungary it is conspicuous, that the juglone index at higher concentration extracts of almost every studied plant species approaches to 1 or is above 1, this means the effect of the extracts is similar to juglone or exceeds it (Table 1). *Amorpha fruticosa* is preeminent among the species, its juglone index is the highest at both concentrations, and it is the only one which juglone index is more than 1 at lower concentration extract. In case of higher concentration extracts *Amorpha fruticosa* was followed by *Ailanthus altissima* and *Celtis occidentalis* with juglone index 1.49 and 1.36. *Juglans nigra*, *Prunus serotina*, *Ptelea trifoliata*, *Fraxinus pennsylvanica* var. *subintegerrima* and *Robinia pseudoacacia* have a juglone index higher than 1. The juglone index of *Acer negundo*, *Parthenocissus inserta*, *Vitis riparia*, *Elaeagnus angustifolia* and *Ribes aureum* are only with some hundredth lower than 1. *Morus alba* and *Fraxinus pennsylvanica* var. *austini* can be characterized by the lowest juglone index in case of higher concentration extracts. Lower juglone index belonged to lower concentration extracts than to higher concentration extract in case of every species, although this difference was more considerable in case of species having a higher juglone index.

Table 1. Juglone index of invasive woody plant species occurring in Hungary

Species	Juglone index	
	at lower concentration extract (1 g plant material extracted with 100 ml distilled water)	at higher concentration extract (5 g plant material extracted with 100 ml distilled water)
<i>Acer negundo</i> L.	0.93	0.99
<i>Ailanthus altissima</i> (MILL.) SWINGLE	0.80	1.49
<i>Amorpha fruticosa</i> L.	1.11	2.00
<i>Celtis occidentalis</i> L.	0.86	1.36
<i>Elaeagnus angustifolia</i> L.	0.74	0.93
<i>Fraxinus pennsylvanica</i> Marsh. var. <i>austini</i> Fern.	0.79	0.85
<i>Fraxinus pennsylvanica</i> Marsh. var. <i>subintegerrima</i> (Vahl) Fern.	0.76	1.01
<i>Juglans nigra</i> L.	0.80	1.08
<i>Morus alba</i> L.	0.80	0.86
<i>Parthenocissus inserta</i> (A. Kern) Fritsch	0.76	0.96
<i>Prunus serotina</i> Ehrh.	0.77	1.04
<i>Ptelea trifoliata</i> L.	0.73	1.03
<i>Ribes aureum</i> Pursh	0.77	0.91
<i>Robinia pseudoacacia</i> L.	0.84	1.01
<i>Vitis riparia</i> Michx.	0.81	0.94

Species can be ranked based on expression of their allelopathic potential using the juglone index at different concentrations of extracts, but it does not give any information about how the species inhibit the growth and development of the test plant. To find this out I compared the germination rate, shoot and root length data in case of each plant species and concentration to the data of control (treatment with distilled water) (Table 2).

Table 2. Inhibitory effects of extracts of invasive woody plant species in Hungary on the germination of *Sinapis alba* L. according to the analysis by Chi-square test and Mann-Whitney test

Donor species	Weight of plant material (g)	Germination rate	Shoot length	Root length
<i>Acer negundo</i> L.	5	*	***	***
	1	-	-	***
<i>Ailanthus altissima</i> (Mill.) Swingle	5	***	***	***
	1	-	-	***
<i>Amorpha fruticosa</i> L.	5	***	***	***
	1	**	***	***
<i>Celtis occidentalis</i> L.	5	***	***	***
	1	-	-	***
<i>Elaeagnus angustifolia</i> L.	5	-	-	***
	1	-	-	***
<i>Fraxinus pennsylvanica</i> Marsh. var. <i>austini</i> Fern.	5	-	-	***
	1	-	o	***
<i>Fraxinus pennsylvanica</i> Marsh. var. <i>subintegerrima</i> (Vahl) Fern.	5	***	***	***
	1	-	-	***
<i>Juglans nigra</i> L.	5	***	***	***
	1	-	-	***
<i>Morus alba</i> L.	5	-	o	***
	1	-	-	***
<i>Parthenocissus inserta</i> (A. Kern) Fritsch	5	*	*	***
	1	-	*	***
<i>Prunus serotina</i> Ehrh.	5	***	***	***
	1	-	-	***
<i>Ptelea trifoliata</i> L.	5	***	**	***
	1	-	*	**
<i>Ribes aureum</i> Pursh	5	-	-	***
	1	-	-	***
<i>Robinia pseudoacacia</i> L.	5	**	o	***
	1	-	-	***
<i>Vitis riparia</i> Michx.	5	***	*	***
	1	-	*	***

Abbreviations: The inhibitory effect: \*\*\*: extremely significant, \*\*: very significant, \*: significant, o: not quite significant, -: not significant

Examining the germination rate the treatment with higher concentration extracts of *Ailanthus altissima*, *Amorpha fruticosa*, *Celtis occidentalis*, *Fraxinus pennsylvanica* var. *subintegerrima*, *Juglans nigra*, *Prunus serotina*, *Ptelea trifoliata* and *Vitis riparia* considered extremely significantly different compared to the control. The germination rates were very significantly lower than the control following the treatment with higher concentration extract

of *Robinia pseudoacacia* and the treatment with lower concentration extract of *Amorpha fruticosa*. There were significant difference between the control and the treatment with higher concentration extracts of *Acer negundo* and *Parthenocissus inserta*. Treatment with other extracts did not show significant difference to the control.

Analyzing the shoot length data the effects of various species and concentrations divided more considerably: eight extracts caused extremely significant, one very significant, five significant, three not quite significant and thirteen not significant differences. Higher concentration extracts of *Acer negundo*, *Ailanthus altissima*, *Amorpha fruticosa*, *Celtis occidentalis*, *Fraxinus pennsylvanica* var. *subintegerrima*, *Juglans nigra* and *Prunus serotina* and lower concentration extract of *Amorpha fruticosa* resulted extremely significant difference to the data of control. Higher concentration extract of *Ptelea trifoliata* reduced very significantly the shoot length. After the treatment with lower concentration extract of *Ptelea trifoliata* and both concentration extracts of *Vitis riparia* and *Parthenocissus inserta* the shoot lengths were significantly shorter.

The root lengths influenced with treatment using different species and concentrations brought more unified results. With the exception of lower concentration extract of *Ptelea trifoliata* – which showed very significant difference compared to the control – extracts at both concentration in case of every species reduced extremely significantly the root length.

#### 4 CONCLUSION

The results of study have proven that every invasive woody plant species occurring in Hungary has a more or less expressed allelopathic potential. These allelopathic effects are known from the literature in case of some studied species, but there are not any publications or there are some ones about the allelopathy of other studied species such as *Fraxinus pennsylvanica* var. *austini*, *Vitis riparia*, *Parthenocissus inserta*, *Ptelea trifoliata* and *Ribes aureum*. In term of juglone index the allelopathic potential of *Amorpha fruticosa*, *Ailanthus altissima* and *Celtis occidentalis* were the most preeminent. Besides these species the treatment with *Juglans nigra*, *Prunus serotina* and *Fraxinus pennsylvanica* var. *subintegerrima* extracts reduced extremely significantly the germination rate, shoot and root length, compared to the control.

The comparison of the juglone index of investigated adventive woody species with the values of adventive herbaceous species reported by Szabó (1999), revealed that for higher concentrations extracts, juglone indices of woody species were in general similar, around 1. No extreme values were found comparable to Eastern daisy fleabane (*Erigeron annuus* (L.) Pers.) with a juglone index of 8.77 (Szabó 1999). In case of *Elaeagnus angustifolia*, I found at lower extract concentration an index lower by 0.14, at higher concentration higher by 0.18, respectively, than reported by Szabó (1999). This difference is not considerable and does not reduce the authenticity of methods; on the other hand it highlights the fact, that the concentration of allelopathica is influenced by several biotic and abiotic factors and may have seasonal variability.

It is known, that Petri dish bioassays could greatly overestimate the allelopathic effect compared to realistic field conditions (Keeley 1988, Wardle et al. 1998). The explanation of different allelopathic effects can be the transformation of allelochemicals by organic and anorganic compounds of soil, adsorption by colloids and dilution by the precipitation (Brückner – Szabó 2001). During these processes the allelochemicals can lose their allelopathic effects although it can be preserved too. To sum up in vitro researches are suitable for determining the allelopathic potential, but allelopathic effect should be proven under field conditions too.

Nowadays several uses of allelochemicals are known. Undesirable deciduous plants were controlled successfully in Canada sowing allelopathic plant species to the understory or incorporating barley, oat and wheat residues in soil (Jobidon et al. 1989). As the results of HEISEY's (1997) studies the ailanthone, the allelochemicals of tree-of-heaven was considered a broad-spectrum, pre- and postemergent herbicide. Most herbicides derived from plants are biodegradable and usually considered to be safer from human health and environmental point of view (Rizvi – Rizvi 1992). The invasive alien species cause considerable environmental, economical and human health harm worldwide, therefore we can agree with findings of Hierro and Callaway (2003 p. 29): „We do not recommend allelopathy as a 'unifying theory' for plant interactions, nor do we espouse the view that allelopathy is the dominant way that plants interact, but we argue that non-resource mechanisms should be returned to the discussion table as a potential mechanism for explaining the remarkable success of some invasive species.”

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