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Herbicidal effect of *Ailanthus altissima* leaves water extracts on *Medicago sativa* seeds germination

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Abstract

Ailanthus altissima (Mill.) Swingle is a deciduous tree native to Southeast Asia, and one of the worst invasive plant species in Europe and North America. A feature probably contributing to its invasiveness is a production of a secondary metabolites, one of which ailanthone, is shown in several studies to have, amongst other, a herbicidal effect on many plant species. In this study we have tested the herbicidal effect of *A. altissima* leaves water extracts on *Medicago sativa*L. seed germination. *M. sativa* has been shown in previous studies to be sensitive to *A. altissima* extracts. The main phytotoxic compound in *A. altissima* was previously shown to be ailanthone, although probably it is not the only one. Water extracts of leaves have been prepared and diluted to multiple concentrations in order to assess the relation between the extract concentration and the intensity of herbicidal activity. The obtained data showed that there was a significant difference between the emergence of treated and untreated seeds, precisely, the emergence of seeds treated with the highest concentration was on average 30% lower than control. These results were expected and consistent with the previous observations of ailanthone being phytotoxic to a wide variety of plants, causing germination inhibition and injuries in older plants. The data available so far show great promise for the possible future applications of ailanthone as a natural product herbicide.

Key words: *Ailanthus altissima*, invasive species, herbicide effect, germination, ailanthone

1 Introduction

Ailanthus altissima (Mill.) Swingle is a deciduous tree native to Southeast Asia (Hu, 1979), and today one of the most widespread invasive plant species in Europe and North America. It was introduced into Europe in 1740s and North America in 1780s (Hu, 1979) primarily as an ornamental tree in cities, due to its high esthetic value and a resistance to pollution and herbivory. Later its uses have been extended to other areas, such as reforestation, erosion control and a food source for silk worms and honey bees (Kowarik and Säumel, 2007). Wide usage in the past has resulted in diffusion of *A.*

altissima in natural environments where it became established, widespread and uncontrollable. Today it is present on all continents except Antarctica, being the most widespread in the meridional part of the Northern temperate zone (Kowarik and Säumel, 2007). The areas it occupies have similar climatic conditions as its native areal, of which most important being moderate winter cold. As it is limited by cold, it's highly widespread in southern areas, such as the Mediterranean, where it's present in a wide variety of habitat types (Vilà *et al.*, 2008). On a south to north gradient its distribution is getting more confined to urban areas which provide milder winter temperatures. Due to its tolerance to pollution (Gatti, 2008; Gravano *et al.*, 2003), *A. altissima* is widespread in urban environments and other disturbed sites such as transportation corridors, abandoned lots and agricultural fields.
High invasive potential is caused by many properties, such as tolerance of a wide span of ecological conditions and pollution, high reproduction, growth and regeneration rate and a production of secondary metabolites with herbicidal and insecticidal activities. The main component responsible for a herbicidal effect in *A. altissima* is shown to be ailanthone, a chemical in the group of quassinoids (Heisey, 1996), mainly present in the *Simaroubaceae* family. Ailanthone is in several studies shown to be toxic for many plant species, including weeds, crops and trees (Mergen, 1959; Heisey, 1990a; Lawrence *et al.*, 1991; Heisey, 1996; Heisey and Heisey, 2003). It's believed that, by producing and releasing ailanthone trough it's tissues, in largest part by roots, *A. altissima* has an allelopathic effect on nearby plant species, slowing their growth and in such way outcompeting them (Heisey, 1990b). In addition, insecticidal activities of ailanthone probably serve as a deterrent of herbivores (Caboni *et al.*, 2012; Lü and Shi, 2012), limiting the number of species able to control its growth.
A. altissima had a great importance in Chinese folk medicine, which induced the research of the active properties of quassinoids. Today, they are shown to have antiviral (Chang and Woo, 2003; Tamura *et al.*, 2003), antitubercular (Rahman *et al.*, 1997), antimalarial (Okunade *et al.*, 2003), antifungal, antibacterial (Rahman *et al.*, 1997; Zhao *et al.*, 2005; Huo *et al.*, 2012) and anticancer properties (Tamura *et al.*, 2003; DeFeo *et al.*, 2005). Considering this, *A. altissima* extracts could pose an important source of compounds with a wide variety of possible applications in medicine and agriculture.
The purpose of this study was to test the herbicidal effects of *A. altissima* leaves water extracts on emergence of *Medicago sativa* seeds. Water extracts of dry leaves have been diluted to multiple concentrations in order to determine the relation between herbicidal activity and concentration of extracts. *M. sativa* has previously been shown to be susceptible to ailanthone (Tsao *et al.*, 2002), so the goal here was to repeat and confirm the toxicity, and possibly describe its correlation to the extract concentration.

2 Materials and methods

2.1 Extract preparation

Leaves of *A. altissima* were collected around the city of Poreč and were left to dry in a warehouse. Water content was determined by weighing the air-dried leaves for several days, until their weight remained constant and gravimetrically by sample drying on 105°C. A 100 g of dry leaves were soaked in 1L of water and after 48 hours the mixture was filtered through Whatman filter paper (Grade 5). The approximate ratio of tissue weight and solvent volume was taken from Lawrence *et al.* (1991). The initial solution was used as a 100% treatment and dilutions of 80% and 60 % were prepared by adding water. A volume of 100 ml of each concentration was prepared for treatments.

2.2 Treatment

M. sativa seeds have been planted in 12 planting pots on depth of approximately 0.5 cm. In each pot 20 seeds have been planted. The seeds were watered with tap water and left to dry for 2 hours before the application of extracts. Pots have been divided in 4 groups and treated with following treatments: 33 ml of 100%, 80% and 60% initial extract concentration for test groups (1-3) and 33 ml of tap water for control group (C). They were incubated in an indoor laboratory on room temperature. The results were observed 2 and 6 days after the treatment by counting emerged seedlings.

2.3 Statistical analysis

The obtained data were analyzed by chi-square (χ^2), $p \leq 0{,}05$ test to determine if there was any significant difference between data groups.

3 Results

The number and percentage of germinated seeds are presented in Table 1. Two days after the treatment only seeds from the control group and one seed from the 60% group have germinated. Six days after the treatment all of the pots contained emerged seeds although in different quantities. The quantities of emerged seeds were being reduced going from the control group towards the 100% group (Figure 1, Table 1). On average, 68% of seeds in the control group (C) have germinated 6 days after the treatment, in comparison to 42% in groups 2 and 3 and 20% in group 1. The significant difference is shown to exist between all data groups except between groups 2 and 3 which had the same average percentage of emerged seedlings.

Table 1: Number of germinated seeds and a corresponding percentage of initial seed number in 3 treated groups and control group, 0, 2 and 6 days after the treatment.

		0 days			**2 days after**			**6 days after**		
Containers		1	2	3	1	2	3	1	2	3
Treatments	1. 100%	0	0	0	0	0	0	4 (20%)	7 (35%)	1 (5%)
	2. 80 %	0	0	0	0	0	0	13 (65%)	5 (25%)	7 (35%)
	3. 60 %	0	0	0	0	0	1 (5%)	5 (25%)	17 (85%)	3 (15%)
	C	0	0	0	3 (15%)	1 (5%)	7 (35%)	10 (50%)	11 (55%)	20 (100%)

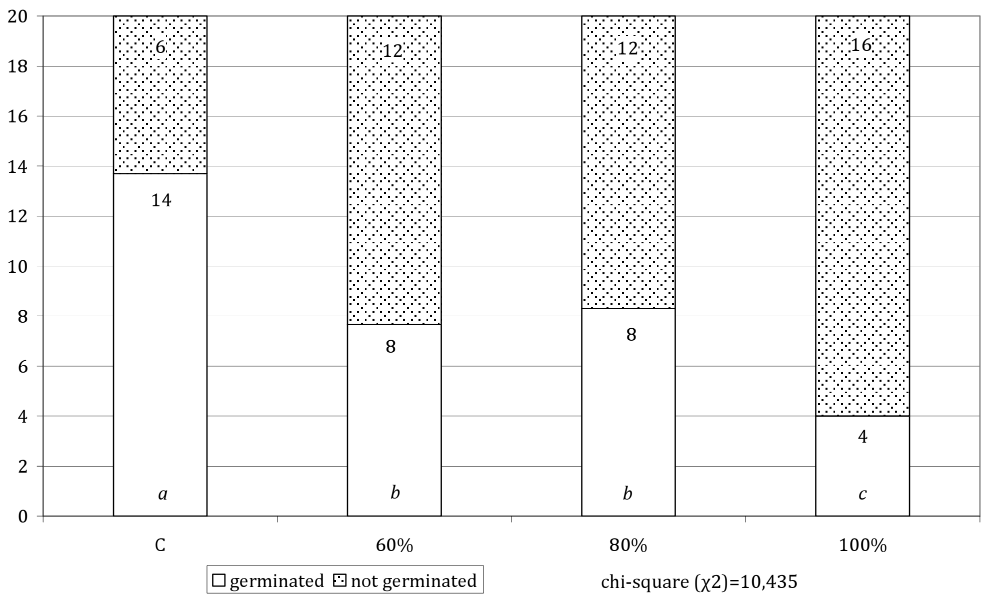

Figure 1: Emergence of *M. sativa* seeds in relation to the extract concentration, 6 days after the treatment.

4 Discussion

The obtained results clearly showed the reduction in plant germination rate by the increased concentration of extracts. Although between groups 2 and 3 there was no significant difference in seedling emergence, the difference between other groups, especially group 1 and control group showed the strong inhibitory effect *A. altissima* extracts had on germination of *M. sativa* seeds. This effect on *M. sativa* was previously observed in the work of Tsao *et al.* (2002) where it was tested by measuring the radicle elongation of treated and untreated seeds. The results showed a strong inhibitory effect, especially on seeds incubated under the presence of light, in which case the activity of the extracts was shown to be 2 to 3 fold higher than those of seeds incubated in the dark. This enhanced phytotoxic activity of ailanthone in the presence of light was previously observed by Lin *et al.* (1995).
Although ailanthone may not be the only phytotoxic compound in *A. altissima* tissues it is the most active as shown by Heisey (1993) who identified it as a component responsible for the phytotoxic effect, and Heisey (1996) who compared the toxicity of pure ailanthone to those of root bark extracts. One of the obtained fractions of solutions extracted with dichloromethane and etyl-acetate had nearly identical level of toxicity as pure ailanthone (0,7 mgL^{-1} causes 50% inhibition of radicle elongation of *Lepidium sativum* L. seeds). Ailanthone is a polar compound easily extracted by polar solvents like water, although the extraction is more efficient with the increase of the solvent polarity as shown in mentioned study. In this study water was used as a solvent, so the solution contained lower levels of ailanthone although sufficient to cause inhibition of germination. This effect of aqueous extracts of *A. altissima* tissues was first observed by Mergen (1959) who tested leaves extracts on 46 tree species. The extracts caused different levels of damage in all species except *Fraxinus americana* L.
The difference between the quantities of extracts needed for inducing a phytotoxic effect can be caused by the different tissues used in the extraction. Heisey (1990a) showed that the highest concentration of active compounds is present in the root bark (inner), followed by stem bark, leaves, wood and flowers as tissue containing the lowest concentration. In this study leaves were used as they are the most accessible and easily collected and the toxicity of their extracts is sufficient.
To date several studies have been made concerning the toxicity of *A. altissima*, with different tissues, extraction methods and testing species used. In Heisey and Heisey (2003) phytotoxic activity was tested on 17 plant species in field condition, using methanol extracts of inner bark. The lowest application rate, which was equivalent to 0,3 kg ha^{-1} of pure ailanthone caused mortality and damage of more than 50% in 9 species and a significant reduction in shoot biomass in 13 species. In the second trial extracts were applied on 4 crops in field to determine the effect on natural growing weeds. The treatments provided partial weed control (highest reduction of biomass was 40%) but also caused significant crop injuries. Altogether all of the tested species except cotton (*Gossipium hirsutum* L.) and yellow nutsedge (*Cyperus esculentus* L.) were affected by the treatment in different intensities. Lawrence *et al.* (1991) tested the effect on *A. altissima* leaves and stem water extracts on *Lactuca sativa* L. seeds germination rate and radicle elongation and showed by the obtained results that both processes were significantly inhibited by applied extracts.
Although it is highly phytotoxic, the effects of ailanthone are short-lasting, as it is easily degradable by soil microorganisms. This was shown by Heisey (1996) who observed the elongation of cress radicle in sterile and non-sterile soil. In non-sterile soil the toxicity was lost after 3 - 5 days, depending on the application rate, in contrast to the sterile soil where the toxicity persisted for 21 days of the experiment duration.
Considering its high phytotoxicity, ailanthone shows potential as a possible future natural product herbicide, although its nonselectivity, observed in multiple studies, would present an obstacle if not resolved in some way. In addition, its rapid biodegradability could be a positive feature from the conservational aspect as it has a short lasting effect in the environment, but a negative one if possible applications as a herbicidal compound would be taken into account. Its usage in such way would require repeated applications every few days for the toxicity to persist.

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