THE EVALUATION OF ALLELOPATHIC COMPOUNDS ACTION OF *AILANTHUS ALTISSIMA* ON SOME OF THE BROAD-LEAVED SPECIES

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Abstract. *Our primay focus was to assess the tolerance of local broad-leaved species such as Acer platanoides and Quercus cerris to the effect of aqueous extracts of Ailanthus altissima. This type of assessment has been chosen because of the increasinngly developing species that visibly colonize a wide range of habitats, often with prolific populations in forest ecosystems and protected areas, affecting through dynamics and influence the local species it shares the habitat with. The rapid growth and prolific reproduction through both sexual and vegetative contribute to its success as invasive species, but the large amount of biochemical substances present in all plant tissues suggest that allelopathy is another mechanism that gives the species Ailanthus altissima invasiveness. The study was founded in greenhouses, under the study of "Plant Physiology" at the Faculty of Agriculture Timisoara (Romania) under controlled conditions of humidity, temperature and light. The plant material used in bioassays consisted in roots of A. altissima and seedlings of Acer platanoides and Quercus cerris, in the 2-4 leaf stage. The obtained solutions were considered the reference and were prepared in three different doses: version 1 (V1) - 5 ml / seedling; Version 2 (V2) - 10 ml / seedling; Variant 3 (V3) - 20 ml / seedlings. The experience was maintained for 25 days, during which time the plants were treated with the extracts obtained, the following determinations were performed: plant height; number of leaves / plant; leaf area; chlorophyll content. By applying the extracts, the following have occurred: proportionally to the amount of extract applied: reducing the number of leaves, decreasing the content of chlorophyll, the beginning of chlorosis, the lack of their development overt the time, reduced leaf area and the appearance of necrosis, partial or total drying plant. Based on these results, we can preliminary conclude that A. altissima, based on the allelopathic compounds that it provides, may replace local species in natural ecosystems, representing a problem for the present and future of biodiversity.*

Key words: *Ailanthus altissima, broad-leaved species, allelopathic compounds, aqueous extracts*

INTRODUCTION

The study perspectives proposed in this study include determining the effect of aqueous extracts from *Ailanthus altissima* on some broad-leaved species such as *Acer platanoides* and Quercus cerris. For this purpose, a study has been set-up under controlled conditions, combining established methods containing measurements and determinations on some morphological and physiological characteristics, which led to a set of results and useful information on the topic addressed.

Broad-leaved species were chosen because of the increasing expansion (invasion) of the species *A. altissima* in forest ecosystems and protected areas, affecting the dynamics and influences on native species with whom it shares the habitat.

Studies carried out by BOSTAN C. ET AL. (2014) on the invasiveness, distribution, inventory and bio measurements on *Ailanthus altissima* species in ecosystems and protected areas in Moldova Noua - Berzasca, revealed the presence of both species in clumps, alignments and isolated trees on the embankment and on the slopes; the study area is included in the protected area Porţile de Fier Natural Park.

The forest ecosystem is dependent on the interaction established between the biological community consisting of different species of trees, with other bodies contained in the system, and their interrelationship with the environment (SPURR AND BARNES, 1980).

Within an ecosystem, the effects of biotic stress factors are far more complex compared to those produced by abiotic factors. The interactions between the biotic and abiotic factors stand as the basic premise that bodies or plants have to cope with, and knowledge about the proportion of interconnection is essential for understanding the area of distribution of plant species and their association at a certain moment (AUSTIN, 1990; AND TRAVIS DUNSON, 1991).

At the core of the consequences of interactions established between the plants there are the competitors to their access to light, nutrients and water from the soil, as well as the reaction of vegetable organisms in relation to a series of chemical substances secreted by various plants, such as various secondary metabolism products, emanating from them in the natural or artificially created environment (CORBU AND COSMA, 2010).

Secondary metabolites with alelochimic potential have a large chemical diversity and are involved in many metabolic and environmental processes (AIRES ET AL., 2005). These substances belong to different types of secondary metabolites: phenols, terpenes and alkaloids, which may be present in different organs: leaves, flowers, fruits and roots of some plant species (MARASCHIN - SILVA AND AQUILA, 2006). Secondary metabolic compounds help plants to better and easier adaptation to the environment, to establish more complex interrelationships between plants and animals (MOTA ET AL., 2011).

Recent studies show that interactions between invasive species and native species, based on allelopathy might be one of the mechanisms underlying the remarkable success of some of the most aggressive invasive plants (BAIS ET AL. 2003; CALLAWAY AND RIDENOUR 2004 PRATI AND BOẞDORF 2004; STINSON ET AL., 2006, LORENA GÓMEZ APARICIO AND CHARLES D. CANHAM, 2008).

Ailanthus altissima is classified as harmful, a major invader in many regions (HUNTER, 2000; WEBER, 2003; BRUNEL, 2005; WITTENBERG, 2005). The consequences for species in danger of extinction are largely unknown, but obviously some types of endangered habitats are affected, especially in warmer parts of Europe. *Ailanthus* is part of the first 20 trees identified as targets of classical biological control in Europe (SHEPPARD ET AL., 2006).

In the case of *Ailanthus altissima* species, at least 10 quassinoides, together with alkaloids and other products were isolated from different plant parts (HEISEY&HEISEY, 2003). A quassinoid (ailanthona) has been identified as a component responsible for phytotoxic effects (LIN ET AL., 1995; HEISEY 1996).

ROD M. HEISEY AND KISH TERESA HEISEY (2003) investigated through a series of experiments in the field of Ailanthus activity and selectivity of the extract on some species of weeds and crops, showing strong herbicidal effects of the extracts.

In a laboratory experiment conducted by C. BOSTAN ET AL., we have studied the effect of the allelopathic compounds present in *Ailanthus altissima* on legume species *Sinapis alba* and *Brassica napus*, demonstrating the strong inhibitory effects of the extracts.

Although the rapid growth and prolific reproduction (both sexual and vegetative) undoubtedly contribute to its success as invasive species, big arsenal of biochemical substances present in all tissues of the species suggest that allelopathy is another mechanism that gives the species *Ailanthus altissima* invasiveness.

Detailed studies based on the assessment of positive or negative impact of *Ailanthus altissima* species are rare, although *Ailanthus* visibly colonizes a wide range of habitats, often with prolific populations.

PELLISSIER ET AL. (2002), following investigations carried out in forest ecosystems, have demonstrated that allelopathy studies require completion of three operational steps. The first step is to observe symptoms of allelopathy in the field and can be represented by the lack of the natural regeneration of species, brood rotting, and changes in the small species that fall under the canopy of trees. The second step involves identifying potential chemical interactions involved in alelocompounds, to be set in the substrate, derived from roots, leaves or fruit plants of the alleged donor. The third step serves to prove the existence of the phenomenon of allelopathy, it consists in performing experiments in vitro, by administering the suspected compounds into the receiving plants.

MATERIAL AND METHODS

The plant material used in biotests consisted of roots of *A. altissima* collected from the didactic park USAMVB Timişoara, Romania as well as from seedlings of *Acer platanoides* and *Quercus cerris*, in the 2-4 leaf stage, obtained from IARAC nursery within the forest reservation Iuliu Moldovan.

Preparing the material

Biologic dosing is an integral part of allelopathy in all studies. These dosages are needed to assess the allelopathy potential of species as a result of activity during the extraction, purification and identification of active biocompounds. In its simplest form, these biotests, as well as isolation and identification alelochemicals, are techniques for providing initial information (OLIVEIRA, 2006).

Biotests techniques vary greatly and no researcher follows the same procedure. The biggest problem is the lack of standardized biotests. Incomplete information about the source of alelochimical substances, method of extraction, concentration, fractions and the absence of known compounds with demonstrated activity in bioassays prevent their validity; frequently it is difficult to establish the relationships of correspondence between bioassay results and distinct patterns of vegetation in the field (AKADÉMIAI KIADÓ, 2007).

Within our experience, the aqueous extracts have been obtained from the mixture of 250 g of plant material (roots), previously dried and then milled until a fine powder was obtained, and 2 liters of distilled water.

After mixing, the mixture thus obtained was allowed to stand to soak for 72 hours after which it was filtered. The extracts were kept in the dark at a temperature of 2-4 ° C.

The obtained solutions were considered the reference and were prepared in three different doses as follows: version 1 (V1) - 5 ml / seedling; Version 2 (V2) - 10 ml / seedling; Variant 3 (V3) - 20 ml / seedlings.

Setting up the experiment

The experiment was founded in the greenhouses of the discipline "Plant Physiology" within the Faculty of Agriculture Timisoara (Romania) under controlled conditions of humidity, temperature and light.

Seedlings were transplanted into vegetation pots (500 ml), which were filled with topsoil.

The experience was maintained for 25 days, during which time the plants were treated with the extracts obtained according to the three formulas presented. In the first 14 days, at an interval of three days, the following determinations were made: plant height; number of leaves / plant; leaf area; chlorophyll content.

The total chlorophyll in leaves (SADP) was determined by the chlorophyllmeter.

Leaf area was determined with a planimeter.

Plant height was measured to the terminal bud by a graduated tape measure.

Plants treated with the extract have also been tested for determining the chlorotic and necrotic areas and the degree of dryness in the lamellae or of the whole plant.

RESULTS AND DISCUSION

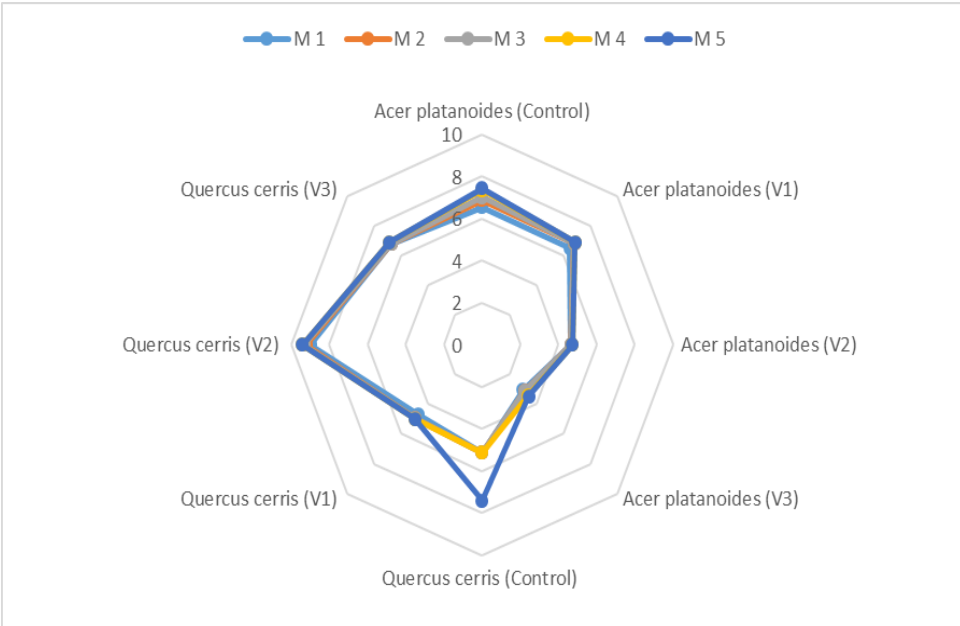
Testing the influences given by extracts obtained can shape in the future the possibility of obtaining a natural herbicide that can be used successfully in emphasizing the idea of sustainability in agriculture, but also to determine to what extent it can be influenced by the diversity of habitats influx of *A. altissima*.

An important objective of our study was to determine the ability of the extracts of *A. altissima* to influence the quality of broad-leaved species studied.

Analysis of processed data on plant height regardless of the applied dose proves that there is a growing trend in mm with the plant height, but that extracts of *A. altissima* don't have a significant influence on this feature during the period the experiement took place.

Growth is manifested by a measured interval to another. For example, with *Acer platanoides* for V1, in the day I (when the first biometric measurements were made) the average height of the plants measured was 6.5 cm, three days after the first measurement there was a slight increase in plant height (6.8 cm), exactly the same size and keeping the plant at the time of three determinations carried out by following the M4 and M5 plant height values to be 6.86 (fig.1). In the case of Quercus cerris in V2 for example, the tendency is the same, the slight increase between the measurements as follows: M1 = 9; M2 = 9.23; M3 = 9.33; M4 = 9.43; M5 9.43.

The height difference between witnesses and variants are mainly due to the waist plant from the beginning, i.e. from the moment of transplanting them from their nursery to vegetation pots, and not necessarily because of the influence of extracts with potential allelopathic effect.



Legend: V1-5 ml; V2-10 ml; V3-20 ml; M1-measurement I; The measurements M2-II; M3 measurement III; M4-IV measurement; M5-V measurement; measurement unit: cm.

Fig.1. Height (cm) in the control and treated plants

The number of leaves in the control variant increases in the measured period, from 4 leaves in *Acer platanoides* for M1 to M5 6.33, and for the *Quercus cerris* from 7 to 7.66 leaves.

In the case of plants treated with root extract, allelopathic effects are manifested by a slight decrease in vaiants treated with 5 ml of extract (V1), except for *Q. cerris* where there is an unsignificant increase, and in those treated with 10 ml and 20 ml (V2, V3) having a significant decrease reaching values of 0.66 for *Acer platanoides* and 1.66for *Quercus cerris* (fig. 2).

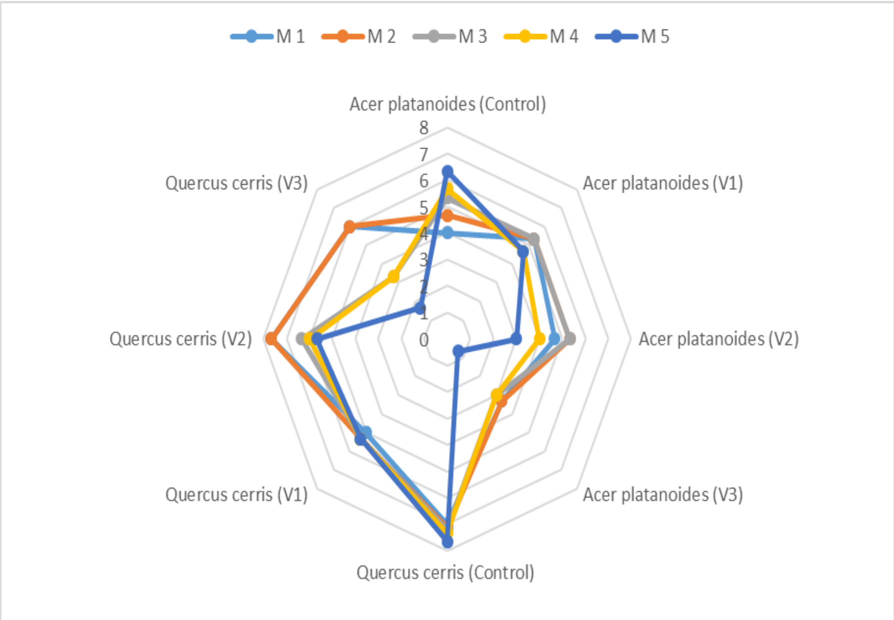


Figure 2. The number of leaves in the control and treated plants

In the case of leaf area of the control plants during measurement, values are rising from 22.23 to 41.02 for the Norway maple, while in the case of studied variants there are significant decreases reaching values of 8.05 and even 7.45 (fig. 3).

Q. cerris stands as an exception again, where the V2 shows an increase of leaf area compared to M1, from 21.97 to 49.85 and from 37.84 to M2 to M3.

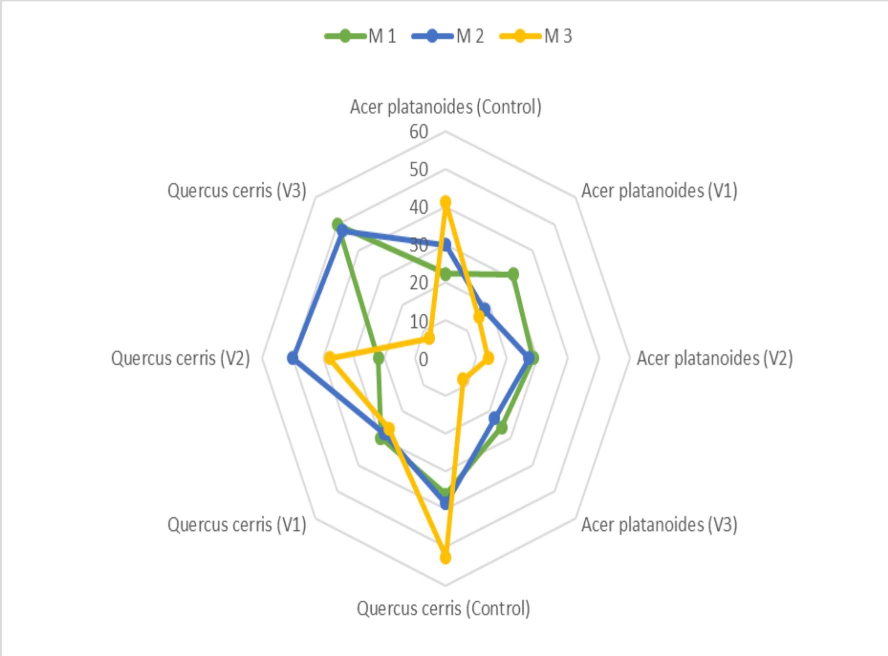


Figure 3. Leaf area in the control and treated plants

Determination of chlorophyll at control plants and at those treated with different doses of extracts of *A. altissima* was performed according to the method described above. The measurement results indicate different values both between witnesses and three variants, as well as between variants studied.

A first observation is that that with longer time application of aqueous extracts containing alkaloids, chlorophyll is inhibited registering a decrease proportional to the dose applied.

Data analysis demonstrates that chlorophyll content varies in the case of the same variant with increases and decreases at intervals of time the measurements were made. For example, in the last measurement carried out, it was found that by applying a dose of 20 ml extract (V3), the amount of chlorophyll decreased significantly, in the case of ther *Acer platanoides* value is 2.6, and at the *Q. cerris* 2.43 which may mean that root extracts potently inhibits the activity of the treated plants chlorophyll.

At *Q. cerris* as the dose of extract is higher, the inhibiting effect is more concentrated, decreasing in chlorophyll content, for example at M1, from 28.73 to 28.16 at V1 and V2 24.93 at V3, and at the M5 from 21.16 to 16.5 V1 2.43 V2 and V3 (Fig. 4).

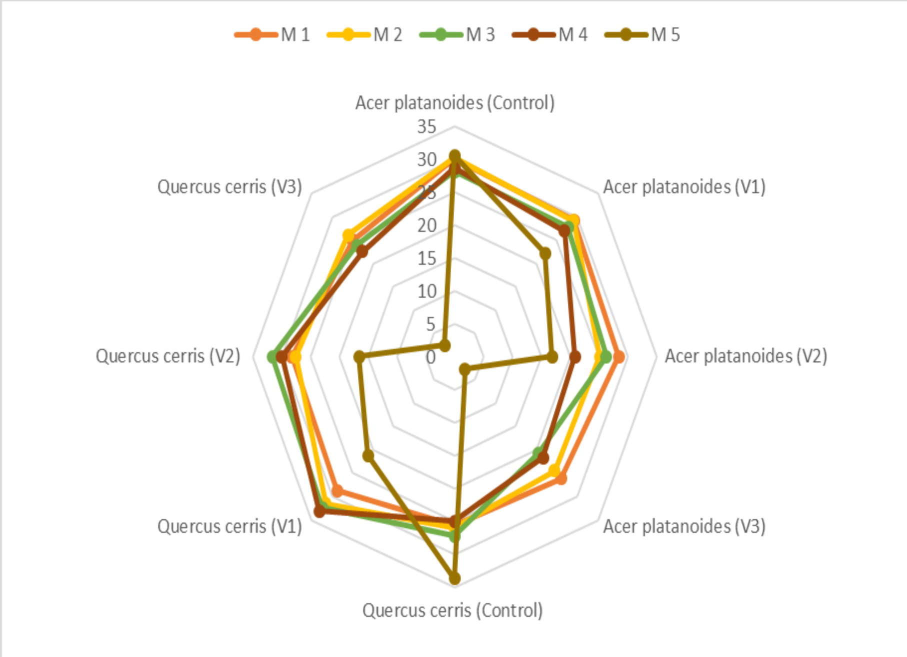


Figure 4. The content of chlorophyll in the control and treated plants

Chlorosis is a plant disease that is manifested by yellowing of leaves due to loss of chlorophyll.

In the case of our study, determining plant chlorosis was done visually, although this can be seen from the data present in table and graph made for the concentration of chlorophyll. Leaf chlorosis was gradually installed on their surface, becoming more and more obvious with the increasing the amount of extract applied.

As a result of disturbances caused by *Ailanthus altissima* root extracts on *Acer plantanoides* plants and after chlorosis of leaves, necrosis followed, namely tissue death, followed by drying of leaves or the whole plant.

CONCLUSIONS

A. altissima is a tree species originating in Asia (China), introduced into Europe approximately 200 years ago, and in Romania it is found in plain and hill.

The invasiveness of the species is manifested both in open areas (pastures, meadows) as well as in stands where it replaces the native species. Competing with native species, *A. altissima* manages to dominate due to the outstanding features that this species has.

Basically, when *A. altissima* occupies a space, it can be removed mechanically or chemically with effort and high costs. Also, it exhibits an important allelopathy effect on neighboring species and we believe this is the most important competitive advantage for *A. altissima.*

Based on the study carried out, it can be preliminary concluded that invasive species such as *A. altissima*, can replace native species in natural ecosystems due to the allelopathic compounds that it eliminates. This entire process represents for now and especially for the future a problem for biodiversity.

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