IMMEDIATE ALLELOPATHIC EFFECT OF TWO INVASIVE HERACLEUM SPECIES ON ACCEPTOR-GERMINATION

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Successful spread of introduced alien plant species in new territories might occur due to following features: rapid growth and reproduction, ability to colonize disturbed habitats, short life cycle, early flowering and seeding, production of large quantities of seeds and vegetative propagules, different phenology from native species, disease- and pest-resistance. Moreover, recently ecological significance of secondary metabolites in ecosystem interactions is approved. Thus, this study is aimed on research of allelochemicals phytotoxicity which is likely to be involved in the invasion success of the invasive species. Assessment of the total phenolics content (TPC) of both Heracleum spp. and its germination suppression of perennial ryegrass (monocots) and winter rapeseed (dicots) seed was done ex situ.

The results suggested that invasive plant species may acquire spreading advantage in new territories due to their ability to inhibit germination of other species. The complete inhibition (0%) was observed in 0.2% leaf extracts of both tested Heracleum species due to highest TPC.

The TPC varied depending on the H. mantegazianum parts and leachate concentration. The highest content of phenolic compounds (87.98 and 92.06 mg mL\(^{-1}\)) accumulated in leaf 0.2% leachates of H. sosnovskyi and H. mantegazzianum respectively, and impacted the lowest acceptor germination. Strong negative correlations were found between TPC in tested Heracleum spp. and germination of acceptor rapeseed (r= -0.8) and ryegrass (r= -0.7). Consequently, the germination response of neighbouring species to invaders allelochemicals might be addressed to regeneration capacity of native plant community. These findings are important to explain variation in the response of native to invasive species at habitat range.

Key words: invasion, allelopathy, Heracleum spp., phenolics, phytotoxicity.

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INTRODUCTION

Though ecosystem biochemical interaction was long-stated, nonetheless just by Fraenkel (1959) approved ecological significance of secondary metabolites (Iason et al. 2012). These compounds are involved in different plant allelochemical interactions which are a key for a complete understanding for plant responses in ecosystem (Rice 1984). Plants presented passive organisms, capable only of responding to the ‘raw materials’ that they encounter, or they can transmit, receive and respond to non-resource signals that allow them to interact with other...
works has focused on aboveground, more and more researches have connected plant invasion to their biochemical impact in ecosystem (Stinson et al. 2006). Numerous studies that investigate the impacts of invasive species on neighbouring plant species suggest that plant species richness is generally reduced (Baležentienė & Bartkevičius 2013, Hejda & Pyšek 2006, Mallik 2003).

Sensu Jones & Gutiérrez (2007), the direct and indirect impacts of invasive species on ecosystem processes rates could be realized by means of two techniques: throughout biochemical assimilation-dissimilation or/and changing ecosystems structure (Fig. 1).

Some invasive species, such as a tall forb giant hogweed *Heracleum mantegazzianum* Somm. et Lev. and cow parsnip *Heracleum sosnowskyi* Mandel. (*Apiaceae, Magnoliophyta*) originated from the Western Greater Caucasus (Russia, Georgia) have become widely naturalized and are recently most dangerous invasive species throughout Europe, North America, Australia, New Zealand, etc. with a continuing increase in its distribution (Perglova et al. 2007, Kowarik 2003, Weber 2003). Both these species belong to *Heracleum* section Pubescentia (*H. pubescens, H. mantegazzianum, H. sosnowskyi* and *H. sommieri*) and they cover most Europe (except arctic, Mediterranean regions and temperate zone of Asia and North America). The *Heracleum mantegazzianum* and *Heralceum sosnovskyi* are two of the three *Heracleum* spp. found in most Central Europe countries with phototoxic effects.
Among the secondary metabolites, phenolic compounds influenced the interactions in communities thus playing an important role in plant-plant interference (Callaway et al. 2002). These compounds also play important role in seed germination, plant development, growth, xylogenesis and flowering (Callaway & Ridenour 2004). Considering all above, we attempted to investigate into the allelopathic activity of *H. mantegazzianum*. This study aimed to assess the secondary metabolites namely total phenols content (TPC) and phytotoxic effects of *H. sosnovskyi* and *H. mantegazzianum* on perennial ryegrass (*Ryegrass perenne*; Monocot) and winter rapeseed (*Brassica napus*; Dicot) seed germination in Petri dishes and to record their response *ex situ*.

**MATERIAL AND METHODS**

**Experimental setup**

*H. sosnovskyi* and *H. mantegazzianum* belonged to *Heracleum* sect. *Pubescentia* (*H. pubescens, H. sosnovskyi* and *H. sommieri*). Their recentanthropogenic areal covers near all Europe excepting arctic, Mediterranean regions and temperate zone of Asia and North America. Lithuania lies in the very Centre of Europe on Baltic Sea and houses a temperate climate with 660 mm precipitation, 17°C and 4°C summer and winter mean temperature respectively (Oleni 2002).

Allelopathic impact (total phenolics content, total concentration and dynamic, influence on seed germination as well) of *Heracleum* spp. was examined during 2012-2013 at Aleksandras Stulginskis University. The plants were sampled in spring (May, rosette), summer (June, flowering) and autumn (September, seed maturity) for preparing the aqueous extracts for bio-screening. The biochemical (allelopathic) characteristics of aqueous extracts were examined at different plant growth stages: rosette (39 BBCH; end of May), flowering (65 BBCH; end of June) and milky maturity stage (76 BBCH; end July). Principal due to photoactive furanocoumarins injurious to human and animal health, as well as suppresses the local biodiversity. *H. mantegazzianum* is dangerous invader in about two-thirds of German districts (Reinhardt et al. 2003). In Czech Republic, the front of populations of *H. mantegazzianum* are advancing at 10 m per year (Perglova et al. 2007). *H. mantegazzianum* has also invaded the Slovakia from Ukraine by seed. *Heracleum sosnovskyi* is among the 18 dangerous invader species successfully spread along the roads and naturalized in Lithuanian and other Europe countries’ habitats and plant communities.

Individual plants of *H. mantegazzianum* over 12 years old have been reported from extremely dry localities. Though both *Heraleum* species mature plants are strictly monocarpic and dies after flowering, but there are some reports of *H. mantegazzianum* polycarpy. At maturity stage the *Heracleum* spp. have accumulated enough resources for reproduction. *H. sosnovskyi* and *H. mantegazzianum* develop generative stem up to 3 or 5 m in height and 10-12 cm in diameter at the base and each plant annually produces 10,000-20,000 fruits respectively. The species forms pure stands, resulting in change in ecosystems diversity and landscape (Starfinger & Kowarik 2003). The both species have phototoxic effects by means of photoactive furanocoumarins, which could be injurious for human and animal health (Jaspersen-Schib et al. 1996). These species are enlisted as invasive alien plants and are strongly recommended to take measures to prevent its introduction and spread to new areas, or to manage unwanted populations.

One of the mechanisms of invasive species success is the production and release of allelopathic compounds by invader that are harmful to plant neighbours in the introduced range (Blumenthal, 2005). Moreover, plant biochemistry may also influence the invasions either directly (i.e. production of phytotoxins) or indirectly (i.e. by making a plant less payable to insects). Consequently, allelopathy is likely to play an important role in shaping the community structure of successive invaders after the successful establishing on the restored sites (Kowarik & Starfinger 2002).
The germination was recorded when the seed germination >50% ($G_{50}$) in distilled water (control). Thereafter the $G_{50}$ rate was equated to 100%. This method enables to assess not only inhibitory, but also stimulatory effect of extracts. Fast germinating and high germination energy oil rapeseed (Brassica napus L., Dicot) cv. Kasimir (NPZ / Saaten-Uninio, Germany) and perennial ryegrass (Lolium perenne L., Monocot) cv. Sodrē were chosen as receptor plants. One hundred seeds were placed on filter paper in each Petri dish (6-cm dia). Five mL aqueous plant extracts (0, 0.02, 0.05, 0.1, and 0.2 % w/v) concentrations was added per Petri dish as per treatment. Treatments were replicated four times. Petri dishes were kept at 26°C for 16 h. Seeds sown in distilled water served as control. Germination was considered when radicle emerges from seed coat. Seed germination rate was used to calculate the allelopathic potential of aqueous extracts in conventional coumarine units (CCU). In this mode an universal index of allelochemicals activity- CCU, is evaluated by nomogram based on the coumarine activity and germination.

Germination bioassay

Allelopathic activity of Heracleum spp. and their parts was estimated on the basis of seed germination bio-screening and recalculating to conventional coumarine units (CCU). The germination responses of biologically different seeds belonging to different taxon group (Monocot and Dicot) were tested.

![Graph showing TPC accumulation in different plant parts of H. sosnovskyi and H. mantegazzianum.](image)

**Fig. 2.** TPC accumulation in different plant parts of *H. sosnovskyi* and *H. mantegazzianum.*
direct measurement of photo absorption in the ultraviolet. In determining the TPC, standard curve with chlorogenic acid ($C_{16}H_{18}O_9$, C3878, Sigma, Aldrich, Germany) was used. Equivalent value was calculated by multiplication of the absorbance of each sample by a single value of equivalent chemical weight per absorbance unit determined under the same condition. In crude extracts and each fraction TPC of Heracleum spp. was expressed on fresh weight basis as mg per g chlorogenic acid equivalent (CAE).

Statistical analysis: The confidence limits of the data were based on Student theoretical criterion. Standard deviation (SD) were calculated \( p<0.05 \). The results of allelopathic effects were statistically evaluated by using the statistical package STATISTICA of Stat Soft for Windows standards. Results of germination, phenols concentration and CCU are presented as mean \( \pm \) SD of 4 independent analyses at the \( P<0.05 \) probability level.

RESULTS

Phenolics content

Phenolics compounds play major role in ecosystems functionality and are involved in many plants interactions with their biotic and abiotic environment (Gents et al. 2005). They can accumulate in different plant parts, tissues and cells during ontogenesis and under the influence of various environmental stimuli (Inderjit 1996, Inderjit & Duke 2003, Padda & Picha 2008). TPC in hogweed parts was significantly variable (1.99 and 92.06 in \( H. \) sosnovskyi and 8.86 and 87.98 mg mL\(^{-1} \) in \( H. \) mantegazzianum), and thus exhibited different effects on germination of test species (Fig. 2, 3-4).

The variability in phenolics content could substantiate different phytotoxicity of tested species and their parts. As aboveground parts is more stressed due to aerial conditions than underground parts (Nielsen et al. 2005), it was found, that aboveground parts of both species were richer in TPC than that of root.

It was found the highest accumulation of phenolic compounds in leaf (92.61 and 87.98 mg mL\(^{-1} \)) possibly due to most intensive conversion of synthesized materials in \( H. \) sosnovskyi and \( H. \) mantegazzianum respectively (Fig. 2). These findings correlate with Grodzinsky (1990), that the phenolic compounds are produced naturally by most plant species and usually synthesized in leaves.

Fig. 3. Inhibition level of acceptor-rapeseed germination in leachates of \( H. \) sosnovskyi and \( H. \) mantegazzianum (\( p<0.05 \); mean\( \pm \)SE).
The lowest TPC was observed in roots of *H. sosnovskyi* (1.99 mg ml\(^{-1}\)) and *H. mantegazzianum* (10.04 mg ml\(^{-1}\)). However the content of phenolics compounds is similar in two invasive *Heracleum* species, namely *H. mantegazzianum* and *H. sosnovskyi*.

**Germination and conventional coumarin units (CCU)**

The response of germination varied significantly (p < 0.05) with plant parts, leachate concentration, *Heracleum* and acceptor species. Rapeseed (0–82% and 2–62%) and ryegrass germination values (1-87% and 4-76 %) ranged in aboveground parts and root extracts respectively (Fig. 3–4). These germination differences corresponded with findings for other species (Baležentienė, Sampietro 2009, Inderjit et al. 2009, Meng et al. 2009). Moreover, acceptor germination responded to the content of phytotoxic phenolics in stem, leaf and seed of both tested *Heracleum* species. Strong negative correlations were found between TPC in tested *Heracleum* spp. and germination of acceptor species, namely rapeseed (r= -0.8) and ryegrass (r= -0.7).

The common trend was observed, that acceptor-species responded differently on leachates phytotoxicity. This outcome was testified by strong correlations between TPC and germination of acceptor species, namely rapeseed (r= -0.8) and ryegrass (r= -0.7) due to impact of TPC on germination.

According to former findings (Hanley & Whiting, 2005), the different germination response may be due to acceptor-species, seed size, seed structure and differences in permeability of seed coat. Thus we found out that the rapeseed was more responsive than ryegrass possible due to higher permeability of seed coat and the faster germination. Also the germination of both acceptor species depended on tested *H. sosnovskyi* and *H. mantegazzianum* plant parts and their leachates concentration. The complete inhibition of rapeseed germination (0%) occurred in 0.2% leaf extracts of both tested *Heracleum* species. Accordingly, the highest germination was observed at the lowest 0.02% concentration extracts.

Due to higher TPC values in shoot parts extracts, these were more harmful and phytotoxic on acceptor germination than root extracts. These variations in TPC agree with Liu et al. (2010) results that metabolic balance correlates between the biosynthesis and further catabolism of plants.

![Inhibition of acceptor-ryegrass germination in leachates of *H. sosnovskyi* and *H. mantegazzianum* (p<0.05; mean±SE).](image)

Fig. 4. Inhibition of acceptor-ryegrass germination in leachates of *H. sosnovskyi* and *H. mantegazzianum* (p<0.05; mean±SE).
Fig. 5. CCU content accordingly rapeseed germination in leachates of *H. sosnovskyi* and *H. mantegazzianum* (p<0.05; mean±SE).

Fig. 6. CCU content accordingly ryegrass germination in leachates of *H. sosnovskyi* and *H. mantegazzianum* (p<0.05; mean±SE).

The last three decades brought evidence that phenolic compounds represent a striking example of metabolic flexibility enabling plants to adapt to changing biotic and abiotic environments. The phenolic compounds can play an important role in the control of many processes including seed development and germination and thus may protect plants against different stresses (Boudet 2007, Heidarabadi et al. 2011). Therefore universal expression of TPC, namely...
conventional coumarine units (CCU), might be used (Chick 2008) to compare the biochemical activity of various plant species and induced by different phenolic compounds. CCU content of *H. mantegazzianum* and *H. sosnovskyi* increased from minimal values 42.9 and 100.3 in 0.02% root extract (acceptor-rapeseed) up to maximal values 1364 in 0.02% root and seed extract (acceptor-ryegrass) (Fig. 5, 6).

The similar trend of CCU variation was observed in ryegrass leachates (6). Nonetheless, CCU content was lower of *H. mantegazzianum* than that of *H. sosnovskyi* possibly due to different biological peculiarities of acceptor seed and their permeability. Such explanations correspond with findings generalized for other plant species (Moise et al. 2005, Kabouw et al. 2010). Lower CCU content in *H. mantegazzianum* leachates resulted in higher germination rates than that in *H. sosnovskyi* leachates.

Consequently, the highest CCU values occurred in leachates which exhibited the strongest germination of acceptor-rapeseed and ryegrass. The strong negative correlation between CCU and rapeseed (r=-0.7) and ryegrass (r=-0.6 and r=-0.8) germination values of tested *H. sosnovskyi* and *H. mantegazzianum* leachates.

**CONCLUSION**

In generally, this assessment confirms that *H. mantegazzianum* and *H. sosnovskyi* contain phenolics that are phytotoxic for seed germination of different systematical groups, e.g. Monocots (ryegrass) and Dicots (rapeseed). The both tested *Heracleum* species leachates phytotoxic exhibited inhibition effect on acceptor species germination. Strong negative correlation confirmed leachates phytotoxicity on the acceptors-species germination. Thereof, the phytotoxicity of tested *Heracleum* species may significantly impact germination and consequently recruitment or regeneration of neighbouring species. Thus invasive *H. mantegazzianum* and *H. sosnovskyi* may substantially contribute to the reduction the species diversity or locally out-compete of native plants in habitats. The determined allelochemical phytotoxicity of *H. mantegazzianum* and *H. sosnovskyi* should be addressed to the partially explanation of highly aggressiveness of species. These findings are important to explain variation in the response of native species to invasive species biochemical mediators at a habitat range. The hypothesis that allelopathy is expected to be an important mechanism in the plant invasion may encourage development of general research models of invasive susceptibility in ecosystems.

**REFERENCES**


