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Accumulation and phytotoxicity of secondary metabolites in invasive *Heracleum sosnovskyi* Manden

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Increasing human activity has resulted in a concomitant increase of alien *Heracleum sosnovskyi* Manden migration and spread into new habitats suppressing native species due to giant size, and biochemical activity. The preliminary bioassay *ex situ* showed different phytotoxicity of 1 and 2-year old plant extracts due to variation in accumulated phenolics content. The extracts inhibited seed germination and the level of inhibition was concentration- dependent. Phenolics contents vary throughout the plant age (0.22–81.03 mg ml^{-1}), growth stage (0.17–81.03 mg ml^{-1}) and across different plant parts (2.97–92.61 mg ml^{-1}) inhibiting the acceptors plants germination. The results suggested that invasive *H. sosnovskyi* possesses allelopathic activity therefore may acquire spreading advantage in new territories throughout use of allelochemicals. Nonetheless, species evidence for allelopathic effects should also include research *in situ*.

Key words: allelopathy, phytotoxicity, environment, germination, *H. sosnovskyi* Manden, phenolics

INTRODUCTION

Dispersion of invasive alien species is a major environmental problem which changes the ecosystem functioning and threat the ecosystem biodiversity (Hulme et al., 2009; Pyšek, Hulme, 2011). *Heracleum sosnovskyi* Manden (giant hogweed, giant cow parsnip, or cartwheel flower) is among 18 dangerous invader species successfully spread along the roads and naturalized in Lithuanian and other Europe countries' habitats and plant communities (List…, 2012). *H. sosnovskyi* is endemic to Caucasus where it occurs in the upper forest belt of the southern slope, mainly in meadows, clearings and forest edges (Nielsen et al., 2005). In 1947, it was promoted as crop in northwest Russia and introduced as forage crop in Latvia, Estonia, Lithuania, Belarus, Ukraine and the former German Democratic Republic (Nielsen et al., 2005). This species is harmful not only due to its threat to native species, biodiversity and ecosystems, but also due to its bioactivity, induced by accumulated allergic furanocumarins in all plant parts (Vanaga et al., 2006).

In Lithuania, it was introduced as fodder in the 1960's at Research Station, Aleksandras Stulginskis University, hence, spread in this area. The naturalized monocarpic *H. sosnovskyi* is comprised of pure stands and changes the ecosystems diversity by outcompeting autochthonic species in native habitats thus causing extensive damage to native flora and landscape. Plants which produce higher biomass also cause negative impacts due to more intense shading of its neighbours leading to competition for light (Finnoff, Tschirhart, 2009). *H. sosnovskyi* establishes itself in different habitats (pastures, river banks, road and rail network sides and wastelands) out-competing the native species (Pyšek, Hulme, 2011). *H. sosnovskyi* and other allied species belong to *Heracleum* section *Pubescentia* (*H. pubescens, H. mantegazzianum, H. sosnovskyi and H. sommieri*) and they cover most of Europe (except Arctic, Mediterranean regions and temperate zone of Asia and North America). Lithuania lies in Central

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Europe at the Baltic Sea with temperate climate (660 mm precipitation, 17 °C and 4 °C summer and winter mean temperature, respectively). As per EU goals to prevent degradation of biodiversity till 2010, great efforts have been made to stop the spread of *H. sosnovskyi* in other territories of Lithuania. Thus, since 2009 this species is enlisted in EPPO A2 (A2/355) and in the National list (2009) of most invasive plants.

Researchers have found plant chemicals (allelochemicals) that prevent seed germination and inhibit tree growth independently of competitive factors (Goodrich-Mahoney et al., 2008). These bioactive materials involve both inhibitory and stimulatory biochemical interactions between plants (Pyšek, Hulme, 2011). The chemical influence of one plant on another is referred to as allelopathy and is a facet of the broader field of chemical ecology. Allelopathy as a science has flourished in agriculture but has not been applied to manage the alien and invasive plants (Chick, 2008; Goodrich-Mahoney et al., 2008). The traits enabling the alien species to successfully establish in natural ecosystems around continents and dominate the new ecosystems remain unclear (Foxcrofta et al., 2011). One mechanism explaining the success of invasive species is the production and release of allelopathic compounds by the invader that are harmful to plant neighbours in the introduced range (Hulme, 2007). Moreover, plant biochemistry may also influence the invasions either directly (i. e. production of phytotoxins) or indirectly (i. e. by making a plant less attractive to insects). Consequently, allelopathy is likely to play an important role in shaping the community structure of successive invaders after seeding on the restoration sites (Hulme, 2007). Furthermore, community neighbours (insects, other plants or possibly soil microbes) can induce these changes. Horvath et al. (2003) suggested how different invaders can succeed through different strategies and circumstances and stop this growing global phenomenon.

The *Centaurea diffusa*, *Centaurea maculosa*, etc. produce bioactive compounds which are more active against native plant species in the introduced range than against co-evolved species in the native range (Blair et al., 2005; Inderjit et al., 2009). However, there is a gap in the knowledge about allelopathic activity of *H. sosnovskyi* and other biochemically related processes for the success of invasive species. Understanding of allelopathic effects, including allelochemicals produced by roots and shoots, enables a far-reaching analysis of the defensive and aggressive abilities of an invasive plant. Therefore integrated microbiological, biogeographical and plant biochemical studies are needed to expand the allelopathy hypothesis and to evaluate its relative importance. Among the secondary metabolites phenolic compounds are constituents of all parts of plants, many have antioxidant activity and influence the interactions in communities thus playing an important role in plant-plant interference (Li et al., 2010). These compounds also play an important role in seed germination, plant development, growth, xylogenesis and flowering. Considering all above, we attempted to investigate the allelopathic activity of *H. sosnovskyi*.

This study aimed to assess the total phenols content and biochemical impact of *H. sosnovskyi* on perennial ryegrass (monocots) and winter rapeseed (dicots) seed germination in Petri dishes and to record their response *ex situ*.

MATERIALS AND METHODS

Monocarpic herbaceous *H. sosnovskyi* and other allied species belonged to *Heracleum* sect. *Pubescentia (H. pubescens, H. mantegazzianum, H. sosnovskyi and H. sommieri).* Their recent anthropogenic range cover near all Europe except Arctic, Mediterranean regions and temperate zone of Asia and North America. Lithuania lies in the very centre of Europe at the Baltic Sea and has a temperate climate with 660 mm precipitation, 17 °C and 4 °C summer and winter mean temperature respectively (Lietuvos klimatas, 2007).

Local samples of *H. sosnovskyi* at an individual block scale were collected in a 6 km transect along highway *Via Baltica* near Kaunas, the centre of Lithuania. Plant samples were analyzed for biochemical peculiarities of *H. sosnovskyi*. The plants were sampled in spring (May, rosette), summer (June, flowering) and autumn (September, ripening) for preparing the aqueous extracts for bio-screening. The biochemical (allelopathic) characteristics of *H. sosnovskyi* aqueous extracts of root and shoot were examined at different plant growth stages: rosette (39 Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH); end of May), flowering (65 BBCH; end of June) and seed milky maturity stage (76 BBCH;

end July). Additionally, aqueous extracts were produced from plant parts at flowering stage and from seed at maturity stage. The amount of phenols and their impact on seed germination were assessed on plants of different ages, 1-year old (rosette growth stage) and 2-year old (full maturity). Principal (0–9) and secondary (0–9) growth stages as per universal BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) scale description and coded using uniform two-digit code of phenologically similar growth stages of all mono- and dicotyledonous plant species (Meier, 2001). The plant samplings were taken when 50% of plants had reached the same developmental stage. Plant leaves, stems, blossoms, seeds and roots were separated and chopped into 0.5 cm long pieces before extraction which was immersed in a 15 × 20 × 5 cm plastic tray containing distilled water. Containers were closed with glass plates and kept at 5 °C in an incubator. After 12 h, the aqueous extracts were filtered through Whatman No 1 filter paper and diluted to 0.02, 0.05, 0.1 and 0.2% (w/v) concentrations and used for germination assays. Leaves, stems, blossoms at flowering stage and seeds at dough stage were used to prepare 0.1 and 0.2% (w/v) extracts.

Allelopathic activity of *H. sosnovskyi* and plant parts was estimated on the basis of seed germination bio-screening and recalculated to conventional coumarine units (CCU) as per Grodzinsky's method (1990). The prepared extracts were tested on different acceptor species specific with different biological features. Fast germinating and high germination energy monocot oil rapeseed (*Brassica napus* L.) cv. *Kasimir* (NPZ / Saaten-Uninio, Germany, 98% germination) and dicot perennial ryegrass (*Lolium perenne* L.) cv. *Sodrė* (82% germination) were chosen as receptor plants. One hundred seeds were placed on filter paper in each Petri dish (6-cm). Five ml of aqueous plant extract concentrations (0, 0.02, 0.05, 0.1, and 0.2% w/v) were added per Petri dish as pretreatment in four replications. 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.

Total phenolics content (TPC) in extract samples was determined by Singleton and Rossi's method (1965) which relied on a colorimetric reaction and 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. One ml of extract was mixed with 45 ml distilled water. One ml of Folin-Ciocalteu reagent (Merck, Darmstadt, Germany) was added and mixed thoroughly. After 3 min 3 ml of Na_2CO_3 were added then the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm. Samples were analyzed in two replicates. Identification and quantification of individual target polyphenolic compounds were done by UV-Vis spectrophotometry (Bechman DU-40, Germany). To evaluate the effects of selected chemicals as a standard equivalent on total phenolics in *H. sosnovskyi*, the content was calculated based on the standard curve of chlorogenic acid. 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 *H. sosnovskyi* was expressed as mg of chlorogenic acid equivalent per g of fresh weight of plant (CAE).

Statistical analysis. The confidence limits of the data were based on Stjudent theoretical criterion. Standard errors (SE) were calculated at $p < 0.05$. 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 ± SE of 4 independent analyses at ≤ 0.05 probability level.

RESULTS AND DISCUSSION

Germination response on *H. sosnovskyi* extracts

The response of acceptor plants varied significantly ($p < 0.05$) with plant parts (shoot or root). Germination rate G_{50} of rapeseed and ryegrass was 0–40% and 4–42% in shoot and 2–47% and 2–47% in root extracts, respectively.

1-year old *H. sosnovskyi* plants at rosette stage deistically inhibited the seed germination of both acceptors (rapeseed and perennial ryegrass) (Fig. 1). Therefore, a positive significant correlation ($r = 0.89$, $p < 0.001$) was found between the amount of fresh material added to the water extract and the resulting inhibitory effects on germination.

The same ryegrass germination trends were determined across 2-year *H. sosnovskyi* extract concentration gradient (Fig. 2). The lowest ryegrass

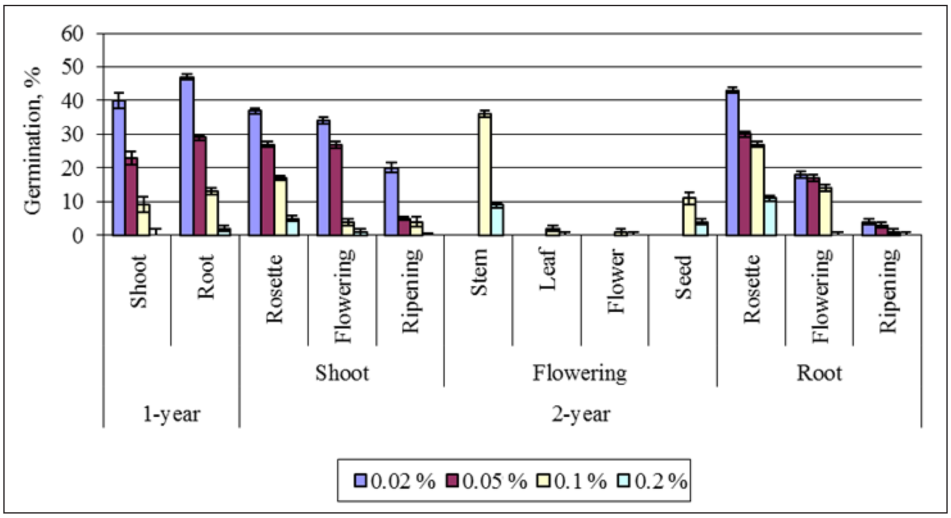

Fig. 1. Effects of *H. sosnovskyi* extracts on rapeseed germination (mean ± SE)

germination was observed in 0.2% extract and ranged between 0% (in both shoot and root extracts at flowering-ripening stages) and 9% (in shoot extract at rosette stage) or 10% (in root extract at rosette stage). The lowest germination inhibition was observed at the lowest concentration (0.02%) extract. Germination ranged between 19–30% in shoot and 9–33% in root extracts at ripening-rosette growth stages.

Considering the acceptor species, rapeseed had lower germination than ryegrass (Figs. 1 and 2). Consequently, rapeseed exhibited higher sensitivity to *H. sosnovskyi* aqueous extract in comparison with ryegrass. The germination results were better for rapeseed than for ryegrass possibly due to different seed structure and their biological peculiarities.

Though a strong inhibition was observed across the extracts produced at flowering stage, various parts of *H. sosnovskyi* were peculiar with rather different inhibitory effects (Fig. 2). Rapeseed germination was 0% in 0.2% extract of leaf, flower and seed, whereas, it was 36% in 0.1% extract of stem. Complete inhibition of ryegrass germination was observed only in 0.2% extracts of leaf and seed. The response of ryegrass germination to 0.1% extracts of 2-year *H. sosnovskyi* was significantly weaker than 0.2% extracts and ranged between 5% (in stem extract) and 11% (in flower and seed extracts).

Furthermore, these germination rates varied with extract concentration and their phenols concentration. Our results corresponded with those of

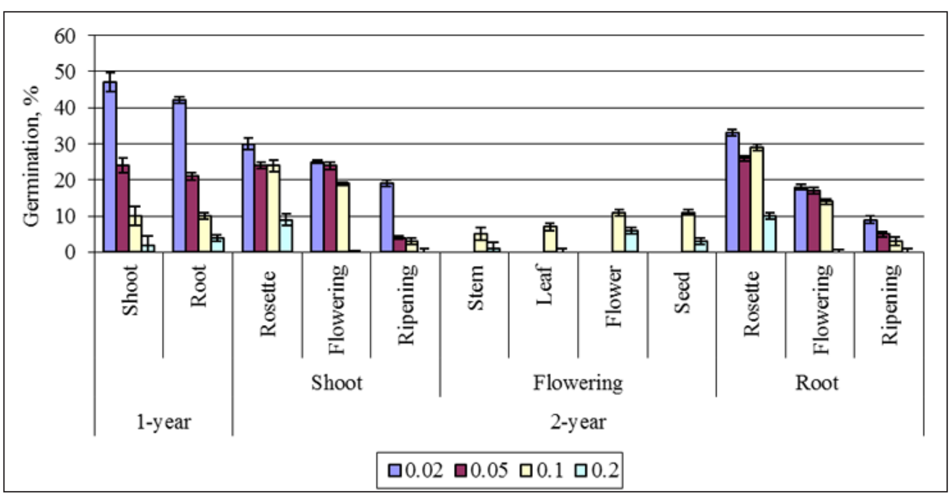

Fig. 2. Effects of *H. sosnovskyi* extracts on perennial ryegrass germination (mean ± SE)

Tesio et al. (2011) who reported that Jerusalem artichoke phenols reduced the lettuce germination. The shoot extracts of *H. sosnovskyi* were stronger inhibitory to germination than root extracts due to their higher content of TPC (Fig. 4). Indeed, the different germination reaction may be also related to acceptor-seed size and seed structure and differences in seed coat permeability (Moise et al., 2005). More specifically, seed germination and seedling emergence are results of a sequence of biological events initiated by water permeability followed by enzymatic metabolism of storage nutrients. All these events are influenced by the environment and the seed quality (Farahani et al., 2011). The rapidly germinating rapeseed needs a short period for G_{50}, whereas ryegrass needs longer time for G_{50}. The seed coat (*testa*) structure scarification responds to germination. The thick, lignified seed coats of ryegrass were impermeable to extracts, thereby reduced the phenols availability to embryo. Hence, ryegrass seeds were more resistant to germination than rapeseed. Thus, in the tested extracts seed germination was less in ryegrass than in rapeseed.

Phenolics content in *H. sosnovskyi*

The results of this study showed that all parts of *H. sosnovskyi* produced phenols (Fig. 3). The phenolic compounds inhibited the seed germination and their concentrations varied in plant materials due to genotype, harvest time, growing environment, etc. (Inderjit et al., 2009). The results of this study indicated that the phytotoxic impact of *H. sosnovskyi* aqueous extracts on the seeds germination of acceptor plant depended on plant age, plant parts, growth stage and extract concentration.

The variability in phytotoxicity of root and shoot extracts could be due to variations in their phenol contents (Fig. 4). As shoot is more stressed due to aerial conditions than underground parts, shoot extracts are rich in phenols (20.94–5.27 mg ml^{-1}) than root extracts (5.92–0.22 mg ml^{-1}). Due to higher TPC values in shoot extracts, these were more harmful than root extracts. It could be explained that plant aboveground parts are more accessible for empathic stress of ambient factors which induce production of bioactive compounds including phenolics (Nielsen et al., 2005).

These variations in content of phenolic compounds agree with Liu et al. (2010) results that metabolic balance correlates between the biosynthesis and further catabolism of plants and external environmental condition. Moreover, stronger phytotoxicity of plant shoot might negatively affect the neighbouring species and thus facilitate the establishment of newcomer *H. sosnovskyi* in a new habitat (Pyšek, Hulme, 2011). Consequently, this alien species may influence the populations of specific native species through allelochemicals. Our experimental design cannot determine if successful invasion of *H. sosnovskyi* is a direct result of solely allelopathic effects on native flora or it should be also attributed to the escape from parasites and pathogens present in their native ranges, etc. (Mitchell,

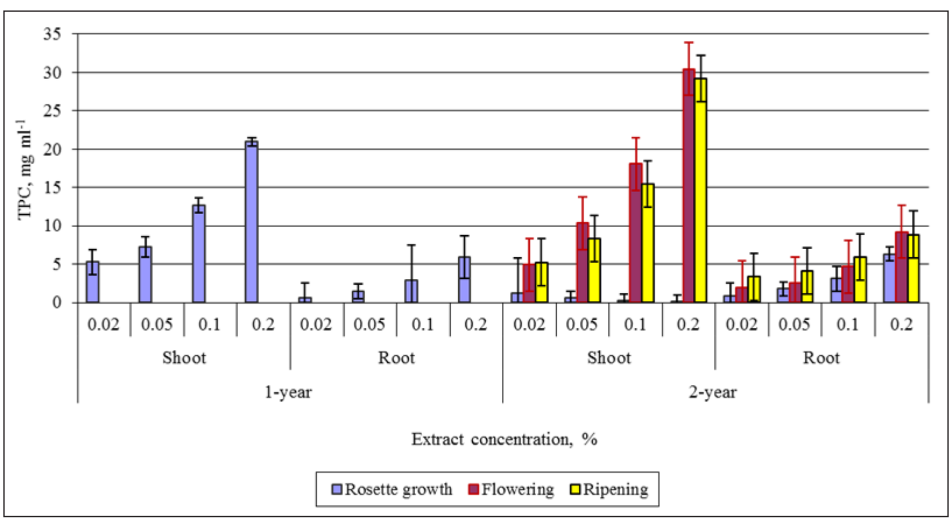

Fig. 3. Total phenolics accumulation (TPC) comparison in 1- and 2-year *H. sosnovskyi* vegetative parts at rosette stage (mean ± SE)

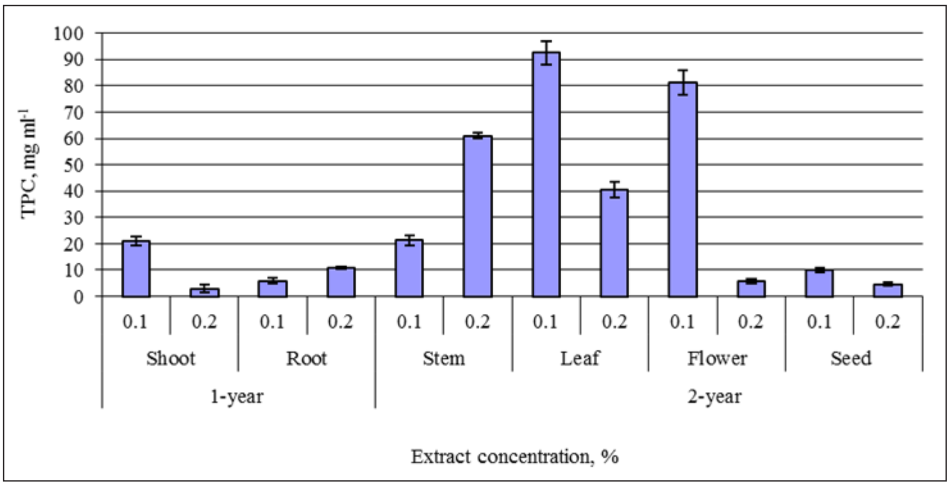

Fig. 4. Comparison of total phenols accumulation in various parts of different maturity *H. sosnovskyi* (mean ± SE)

Power, 2003). The observed significant allelopathic effects of invasive *H. sosnovskyi* have undoubtedly facilitated the invasion into the new habitats by stimulating the species competitive abilities to spread.

As confirmed by chemical analysis, the inhibition level of various concentrations and plant parts of 2-year *H. sosnovskyi* depended on variations in the TPC content during plant growth (Fig. 5). The 2-year *H. sosnovskyi* was exposed to low temperatures during winter, hence, accumulated higher TPC than did 1-year *H. sosnovskyi*. As Meng et al., 2009 and Padda, Picha, 2008 argue, the low-temperature stress increases the build-up of phenolics in plants. At chilling temperatures, the permeability of cell membranes and the activity of membrane bound enzymes are changed, thus leading to accumulation of toxic intermediates in cells. This creates physiological stress in plant cells and considerably increases the level of phenylalanine ammonia lyase (PAL) enzyme (involved in phenolic biosynthesis catalysing the reductive deamination of phenylalanine to form cinnamic acid). Subsequently more phenolic compounds are accumulated.

As phenolic compounds are involved in many plant interactions with their biotic and abiotic environment, these substances can accumulate in different plant parts, tissues and cells during ontogenesis and under the influence of various environmental stimuli. Moreover, the various phenolics are localised in cell walls, vacuoles and cell nuclei (Hulme, 2007). Certain variations found in phenolic concentration in 2-year old *H. sosnovskyi* plants affirmed the influence of growth stage, plant part and extract concentration on the production and release of these metabolites. The highest amounts of TPC (30.42 and 29.19 mg ml^{-1}) were accumulated in 0.2% shoot extracts produced at flowering and ripening stages of 2-year *H. sosnovskyi*. The lowest TPC (0.17–1.20 mg ml^{-1}) were obtained in 0.02–0.2% extracts from the ripening stage.

Our findings suggest that TPC was lower in all root extracts than respective values in shoot extracts of 2-year old *H. sosnovskyi* plants during the vegetative growth. Consequently, lower TPC in root might result in less inhibitory effects on neighbour plants than shoot. In roots, the highest TPC content was at flowering (9.22 mg ml^{-1}) and ripening (8.86 mg ml^{-1}) stages during the most intensive conversion of synthesized materials.

As it has been noticed for other species (Baležentienė, Sampietro, 2009; Inderjit et al., 2009; Meng et al., 2009), the highest content of phytotoxins in stem, leaf, blossom and seed of 2-years old *H. sosnovskyi* caused maximum inhibition in germination. The phenolic compounds are produced naturally by most plant species and usually synthesized in leaves (Grodzinsky, 1990). These statements correspond with our findings of TPC variation across different plant parts at the flowering stage (Fig. 4). Thus we found out that the lowest TPC content (4.68 mg ml^{-1}) was observed in 0.1% root extract. While the highest TPC content (92.61 mg ml^{-1}) was found in extracts of 0.2% leaves and blossoms (81.03 mg ml^{-1}).

In general, different TPC of *H. sosnovskyi* showed that these allelochemicals contents vary both

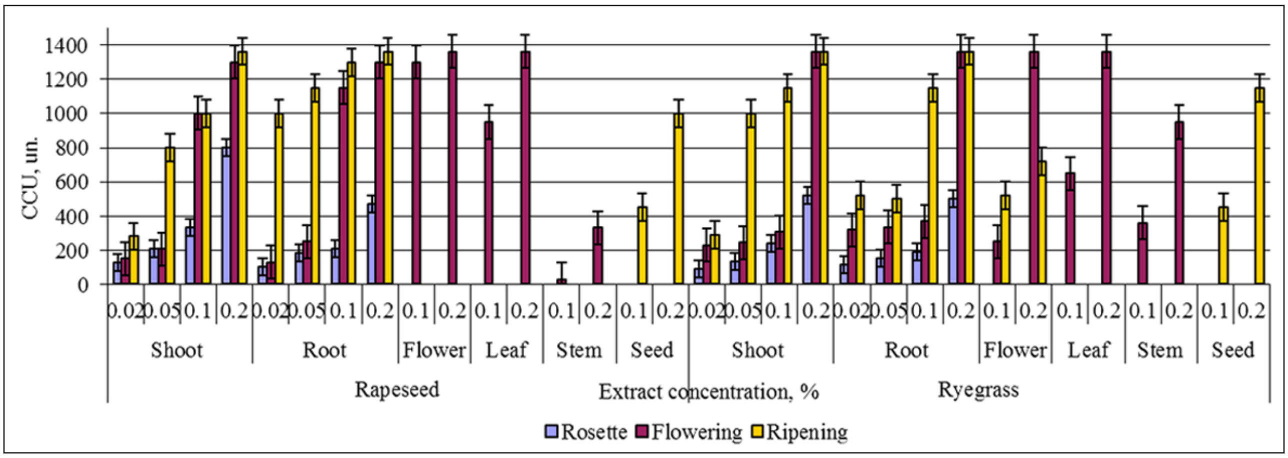

Fig. 5. CCU content in 2-year *H. sosnovskyi* according to acceptor germination (mean ± SE) Shoot and root extracts produced at rosette stage; flower, leaf and stem extracts produced at flowering stage

with growth stage and plant parts. These variations of TPC in *H. sosnovskyi* throughout the vegetation period and across different plant parts influenced the acceptors plants germination. Our research suggested that the allelochemical suppression maintained by *H. sosnovskyi* against the neighbouring plants eventually occur in the invaded communities. Thus prospective research for use of *H. sosnovskyi* allelopathic effects in environment may have important implications on the plant communities susceptible to invasion as it was noticed for other invaders (Hierro, Callaway, 2003). Moreover, secondary metabolites (high content of phenolics, etc.) deter the herbivores thus providing protection to invader species (i. e. *H. sosnovskyi*) with successful spread in new territories (Ridenour, Callaway, 2011). Consequently, suitable manipulation of allelopathy for improvement of environmental protection through eco-friendly control of synthesis of novel agrochemicals based on natural products has gained prominent attention of scientist engaged in allelopathy research.

CCU content in *H. sosnovskyi* extracts

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. We assessed that the content of CCU varied in accordance with TPC. CCU content of 1-year *H. sosnovskyi* decreased from maximum 1 364 in 0.2% shoot extract (acceptor-rapeseed) down to 105 in 0.02% root extract (acceptor-ryegrass) (Table).

CCU content of 2-year old *H. sosnovskyi* plants ranged between 100 in 0.02% root extracts at rosette stage (for acceptor-rapeseed) and 1 364 in 0.2% shoot, root, flower and leaf extracts at flowering or ripening stages (for both acceptor-species) (Fig. 5).

Among the reasons of species invasiveness success in new environment may be its chemical interaction with the recipient community due to the absence of tolerance of resident flora to new chemicals produced by invader, e. g. *H. sosnovskyi*. The hypothesis that allelopathy is expected to be an important mechanism in the plant invasion may encourage development of general research models of invasive

Table. **CCU content in 1-year *H. sosnovskyi* at rosette stage according to acceptor germination**

Extract concentration, %	Rapeseed		Ryegrass	
	Shoot	Root	Shoot	Root
0.02	115	84	84	105
0.05	251	191	241	280
0.1	520	400	500	500
0.2	1364	1150	1150	1000

susceptibility in ecosystems. Invasive species *H. sosnovskyi* exhibited high biochemical activity due to accumulation of phenolics. The species phytotoxicity (0% germination of acceptor seed) was strongest in shoots of 2-year plant at flowering stage due to the highest phenolics content (30.42 mg ml^{-1}). Therefore, it is not only *H. sosnovskyi* giant size, vigorous uptake of nutrients, and reproduction of high seed yield but also biochemical activity that substantially contributes to the success of this invasive plant. The results suggested that invasive plant species may acquire spreading advantage in new land by using 'novel weapons' to inhibit germination as it was observed for other species (Ridenour, Callaway, 2011). Nonetheless, species evidence for allelopathic effects should not be restricted to analysis of plant extracts in lab, but also should be based on research in natural environment.

CONCLUSIONS

Among reasons of species invasiveness success in new environment may be considered its chemical interaction with the recipient community determined by intolerance of resident flora to new chemicals produced by invader, in this particular case by *H. sosnovskyi*. Invasive species *H. sosnovskyi* exhibited high biochemical activity due to accumulation of phenolics. Total phenols content of *H. sosnovskyi* demonstrated that these allelochemicals are both growth stage – and plant parts–variant. Aboveground plant parts usually are stressed to a higher degree by more labile aerial conditions than underground parts, therefore higher phenols content was obtained in shoot extracts (20.94–5.27 mg ml^{-1}) than that in root extracts (5.92–0.22 mg ml^{-1}) of 1-year *H. sosnovskyi*. Due to higher TPC values in shoot extracts, these were more harmful than root ones. TPC was lower in all root extracts if compared to respective values in shoot extracts for 2-year *H. sosnovskyi* during vegetation. In roots the highest TPC content accumulated at flowering (9.22 mg ml^{-1}) and ripening (8.86 mg ml^{-1}) stages during the most intensive conversion of synthesized materials. The lowest total phenols content (4.68 mg ml^{-1}) was observed in 0.1% root extract and the highest was determined in 0.2% extracts of leaves (92.61 mg ml^{-1}) and blossoms (81.03 mg ml^{-1}). The species phytotoxicity (0% germination of acceptor seed) was observed the strongest in shoots of 2-year plant at flowering stage due to highest phenolics content (30.42 mg ml^{-1}). Therefore, it is not only *H. sosnovskyi* giant size, vigor uptake of nutrition material, and reproduction of high seed yield but also biochemical activity that makes a substantial contribution to the plant invasiveness.

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INVAZINIO *HERACLEUM SOSNOVSKYI* MANDEN ANTRINIŲ METABOLITŲ KAUPIMASIS IR FITOTOKSIŠKUMAS

S a n t r a u k a

Didėjantis žmonijos aktyvumas lemia ryškų svetimžemės monokarpinės žolinės rūšies *Heracleum sosnovskyi* migracijos didėjimą ir plitimą naujose augavietėse slopinant vietines rūšis milžinišku dydžiu, intensyviu dauginimusi išauginant gausų sėklų derlių bei biocheminiu aktyvumu.

Buvo tiriamas įvairių augimo tarpsnių (rozetės, žydėjimo ir brandos) augalo ir jo dalių 0,02, 0,05, 0,1 ir 0,2 % ekstraktų biocheminis aktyvumas spektroskopiškai įvertinant bendrąjį fenolių kiekį, taip pat buvo tiriamas šių antrinių metabolitų fitotoksiškumas skirtingų biologinių savybių akceptorių (žieminio rapso ir daugiametės svidrės) daigumui.

Preliminarus biotyrimas *ex situ* rodo skirtingą 1 ir 2 metų augalo ekstraktų fitotoksiškumą dėl įvairaus susikaupusių fenolių kiekio. Ekstraktai stabdė sėklų dygimą ir tai priklausė nuo jų koncentracijos. Fenolių kiekis skyrėsi priklausomai nuo augalo amžiaus (0,22–81,03 mg ml^{-1}), augimo tarpsnių (0,17–81,03 mg ml^{-1}) ir augalo dalių (2,97–92,61 mg ml^{-1}), stabdydamas augalų daigumą. Rezultatai rodo, kad invazinis *Heracleum sosnovskyi* yra alelopatiškai aktyvus, todėl galėtų plisti naujose teritorijose dėl alelochemikalų, tačiau alelopatiniam efektui patvirtinti reikėtų atlikti tyrimus *in situ*.

Raktažodžiai: alelopatija, fitotoksiškumas, aplinka, daigumas, *H. sosnovskyi* Manden, fenoliai