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ALLELOPATHIC ACTIVITY OF INVASIVE SPECIES *SOLIDAGO CANADENSIS* L.

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Solidago canadensis L. (*Asteraceae*) spread throughout the world and also is enlisted in the National list of invasive species (2012). Here the allelopathic activity of the species was assessed in terms of further understanding of their distributions over the range of native spread. The invasion success of S. *Canadensis* was tried to base on the estimation of the total phenolics content (TPC) in the plant aqueous leachates at different growth stages. Allelopathic impacts (total phenolics content, total concentration and dynamic, conventional coumarine units, CCU) of *S. canadensis* were examined during 2012-2013 in Laboratory of raw materials, agro and zootechnics research, Aleksandras Stulginskis University. The plants were sampled in spring (May, rosette), summer (June, flowering) and autumn (September, seed maturity) for preparing the aqueous extracts. The biochemical (allelopathic) characteristics of *S. canadensis* aqueous extracts were examined at different plant growth stages. Principal (0–9) and secondary (0–9) growth stages as per universal BBCH scale description and coded using uniform two-digit code of phenologically similar growth stages of all mono- and dicotyledonous plant species.

The TPC ranged between 0.968 mg ml^{-1} to 23.591 mg ml^{-1} depending on the plant ontogenetic stage, plant part and extract concentration (r = -0.7). Due to accumulated allelochemicals, the invasive *S. canadensis*, might acquire distribution advantage in new territories outside the native habitat range, through the inhibitory effects on germination of native plant species.

Keywords: Solidago canadensis, allelopathy, phenolics.

INTRODUCTION

An invasive species' peculiarities to tolerate a wide range of environmental conditions and rapid spread by different means are often between the characteristics clarified how they can quickly colonize an existing communities and become pervasive outside of their native ranges. Although many ecologists have concentrated on the traits of the invader as determinants of invasion success, regional conditions, including climate and landscape spatial heterogeneity, play an important role in determining invasive species initial establishment and subsequent spread (With 2002; Clergeau, Quenot, 2007).

However, the next primary hypothesis for the astonishing success of many exotics as community invaders relative to their importance in their native communities is that they have escaped the natural enemies that control their population growth – the 'natural enemies hypothesis'. Moreover, many of the worldwide most common and ecologically devastating exotic invaders are not as successful at their native ranges. Nonetheless, currently someone considered that the biochemical compounds emergence by the invader that have harmful effects on the species of the recipient plant community, i.e., allelopathy. This non-resource mechanisms should be considered as potential mechanism for explaining the significant success of some invasive species.

Allelopathy is not 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.

Since Fraenkel (1959) (Iason et al., 2012) recognized that the secondary plant metabolites were not simply plant waste products but served to defend them against insects, herbivores, a number of ecological roles for these intriguing compounds have been established, viz. as defence means against a broad ranges of herbivores and pathogens, mediators of interactions with competitors and mutualists, and as a defence against abiotic stress. Plant' numerous secondary metabolites have the allelochemicals potential and supress both seed germination and seedling growth. They effect the neighbouring species of native flora and other organisms (Baležentienė, Renćo, 2014). However, the phenolic compounds are the most important and common plant allelochemicals (Iason et al., 2012). The phenolics participate in regulation of seed development, germination and plant resistance to various stresses. It is known that phenolics represent the main allelopathic compounds that inhibit different physiological processes of plant, and thereof result in changes of floristic composition within a plant community and dominance of one plant species over others (Djurdjevic et al., 2004). Nonetheless, their content and composition might be different depending on plant species, parts, tissues and cells during ontogenesis and under the influence of various environmental stimuli (Inderjit, Duke, 2003). 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 plant biochemical studies are needed to expand the allelopathy hypothesis and to evaluate its relative importance. *Solidago canadensis* L. or Canada goldenrod (*Compositae,* syn. *Asteraceae*) native to North America, is successful invader worldwide. Highly aggressive plant, which reduces the species diversity and out-competes all native plants. *S. canadensis* is perennial herb spreading by wind-dispersed seeds forming large colonies that reduces the abundance of native vegetation (Semple, Cook, 2006). The *Solidago* L. genus has ca. 100 herbaceous species in South America, Europe and Asia. All goldenrods are late bloomers, flowers in late summer into the fall and common along the edges of moist forests, roadsides and meadows. It was introduced into Europe in 17th century and now has become most aggressive invader (Weber, 1998) naturalised in Lithuania and has spread in slopes and along international highways.

Here, an alelochemical potential study for *Solidago canadensis*, an invasive North American forb in Lithuania, and assessment of general evidence for allelopathic activity of this invasive species is presented. The main object of this research was to measure the total phenolics content (TPC) in the plant aqueous leachates at different growth stages

MATERIALS AND METHOD

Allelopathic drivers and impacts (total phenolics content, total concentration and dynamic, influence on seed germination) of *S. canadensis* were examined during 2012–2013 at Laboratory of raw materials, agro and zoo research, Aleksandras Stulginskis University.

Lithuanian is situated in the southern (4 A hardiness zone) and central part (5 hardiness zone), specific with a temperate climate with mean precipitation of 627 mm, and mean summer (winter) temperature of 17 °C (-4 °C). Summer heats with temperatures above 30 ºC might occur. Winters can be very cold with temperatures ranging in between freezing and −25 ºC in January and February. Lithuanian climate suppose favourable conditions for invasive *S. canadensis* spread and incorporation in native habitats.

The plants were sampled in spring (May, rosette), summer (June, flowering) and autumn (September, seed maturity) for preparing the aqueous extracts. The biochemical (allelopathic) characteristics of *S. canadensis* aqueous extracts were examined at different plant growth stages: rosette (39 BBCH; end of May), flowering (65 BBCH; end of June) and milky stage (76 BBCH; end July). Principal (0-9) and secondary (0-9) growth stages as per universal BBCH 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. Plants leaves, stems, blossoms, seeds and roots were separated and chopped into 0.5 cm long pieces before extraction.

Total phenolics content (TPC) was determined by Singleton and Rossi’s method (1965) in leachate samples of 0.02, 0.05, 0.1 and 0.2 % (w/v) concentrations. In determining the TPC, standard curve with chlorogenic acid ($C_{16}H_{18}O_9$, C3878, Sigma, Aldrich, Germany) was introduced. One ml of 0.2–0.02 % leachate solution was mixed with 45 ml of distilled water. One ml of F-C reagent (Merck, Darmstadt, Germany) was added and mixed thoroughly.. The absorbance was measured at 760 nm. Samples were analysed in two replications. Identification and quantification of individual target polyphenolic compounds was performed by UV-Vis spectrophotometry (Bechman DU-40, Germany).

To evaluate the effects of selected chemicals as a standard equivalent on total phenolics in *S. canadensis*, the content was calculated on the basis of 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 leachates and each concentration TPC of *S. canadensis* was expressed on a fresh weight basis as milligram per gram chlorogenic acid equivalent.

The allelopathic potential of aqueous extracts expressed in conventional coumarine units (CCU). A universal index of allelochemicals activity of CCU was evaluated by a nomogram, which depends on the coumarine allelopathic activity.

Statistical analysis: The confidence intervals of the estimates were based on Student theoretical criterion. Standard deviation (SD) was calculated at $p<0.05$. Correlation coefficient between TPC and germination was calculated in order to evaluate their interaction. Significant differences among the means were determined using Tukey’s honest significant difference test. The results of allelopathic effects were statistically evaluated by using the statistical package STATISTICA of Stat Soft. The results regarding germination, phenols concentration and CCU are presented as mean ± SD of 4 independent analyses at the $p<0.05$ significance level.

RESULTS AND DISCUSSION

Correspondingly to former findings (Iason et al., 2012), phenolic compounds are the most important and common plant allelochemicals. Furthermore, they play a major role in ecosystems functionality and are involved in many plant-plant and their biotic and abiotic environment interactions (Gents et al., 2005). Additionally, the phenolic compounds are remarkable pattern of metabolic plasticity supporting plants adaptation to the changing biotic and abiotic environment (Boudet, 2007). Moreover, the content of secondary compounds in plant is subject to ontogenesis (genetically limited), plant part or season-contingent environment (Pawlaczyk et al., 2009). These findings correspond with the research data. Significant variations in phenolic compounds accumulation were observed in *S. canadensis* roots and shoots throughout the vegetation period (Figure 1).

In aqueous extracts of *S. canadensis*, TPC ranged between 0.968 mg ml^{-1} and 23.591 mg ml^{-1} and significantly corresponded to plant growth stage, plant part and extract concentration (r=-0.7). The highest TPC of 23.591 mg ml^{-1} was observed in leachates produced of plant leaves at flowering stage.

Comparing TPC in different plant parts, it was higher in roots (10.33 mg ml^{-1}) than in shoots (6.42 mg ml^{-1}). As Blumenthal (2005) revealed, the differences in TPC accumulation in roots and shoots depend on species genetically limited biological peculiarities. TPC content increase gradually from 9.08 mg ml^{-1} at rosette to 10.86 mg ml^{-1} at flowering and 11.06 mg ml^{-1} at seed maturity stage.

Meanwhile, the mean TPC rate of shoot extract was lower than the root extracts, showed a decrease from 7.56 mg ml^{-1} at rosette to 6.01 mg ml^{-1} at flowering and 5.68 mg ml^{-1} at maturity. Lower phenolic range in shoot might be explained as result of low-temperature stress, which is pointed out increasing the synthesis of phenolics in plants (Pawlaczyk et al., 2007). At winter 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 induces 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, high TPC was found in *S. canadensis* roots since in early growth stage (rosette).

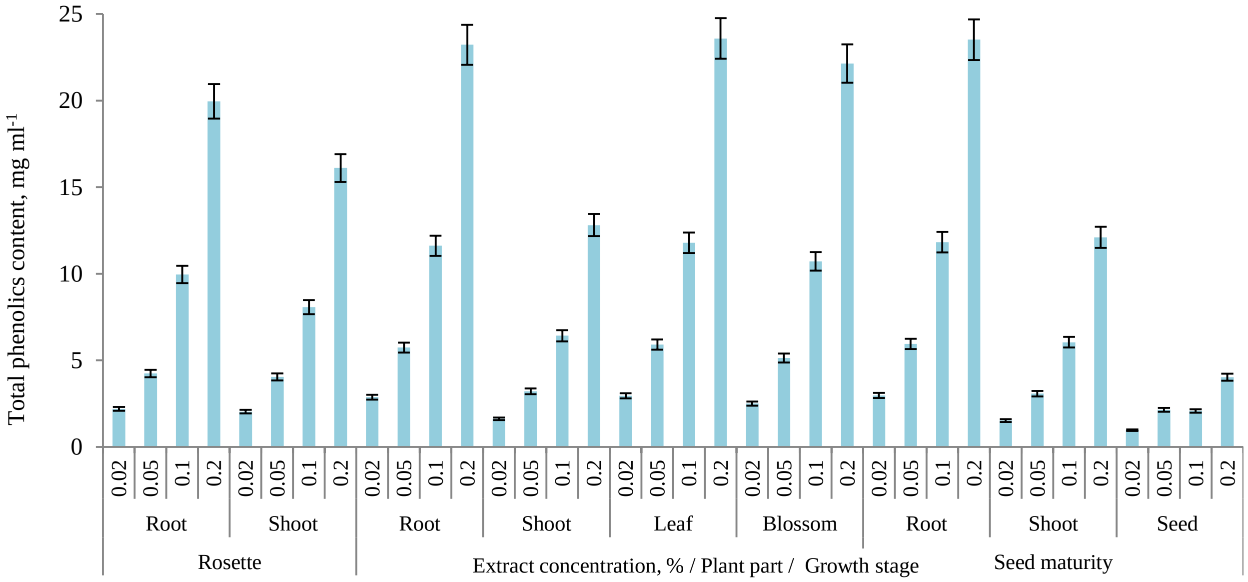

Figure 1. Content of total phenolic compounds in leachates of *Solidago canadens* (mean ±SE; p<0.05)

The phenolic compounds are relevant in plant' metabolic plasticity, and thus supporting their adaptation to changing biotic and abiotic environment conditions. Moreover, phenolics may improve species resistance to different stresses (Boudet, 2007). Additionally, phenolics remain important in terms of control of many eco-physiological processes including seed development and germination and thus regulating initial species recruitment or regeneration in plant communities (Mallik, 2003). The universal expression of TPC, namely conventional coumarine units (CCU), might be used to compare the biochemical activity of various plant parts or species influenced by different phenolic compounds. The CCU content is an universal index of extract bioactivity varied in accordance with TPC and germination rate in aqueous extracts of *S. canadensis*. It was assessed that content of CCU varied in accordance with TPC.

CCU content ranged between 16 and 1300 calculated for rapeseed and between 45 and 1300 for ryegrass (Figure 2). The highest CCU content was calculated for the shoot (1300 for rapeseed and 1130 for ryegrass at rosette; 1300 for rapeseed at seed maturity stage), leaf (1300 at flowering) and blossom leachates (1300 for rapeseed) of the highest concentration 0.2 % at different growth stages in which the TPC was the highest. This tendency revealed the highest phytotoxicity of these leachates. Moreover, the assessed significant (p<0.05) positive correlation approved a medium-strong correlation between extract CCU and TPC level (r=0.4 for rapeseed and r=0.6 for ryegrass). Nonetheless, the CCU was different for tested acceptors possibly to their differences of seed coats and also related to acceptor-seed size and seed structure (Hodgson, Mackey, 1986). The scarification of seed coat (*testa*) structure responds to permeability. The thick, lignified seed coats of ryegrass were impermeable to extracts, thereby reduces the water and TPC availability to embryo, thus resulted in lower CCU content. Hence, ryegrass seeds were most resistant to TPC than rapeseed. Thus, CCU content was less of ryegrass seed than that of rapeseed in tested extracts.

Besides acceptor-seed coat peculiarities and higher TPC accumulation in shoot than in root, the CCU content confirmed that *S. canandensis* contains phenolics that are phytotoxic for seeds germination of different systematical groups, e.g. Monocots (ryegrass) and Dicots (rapeseed).

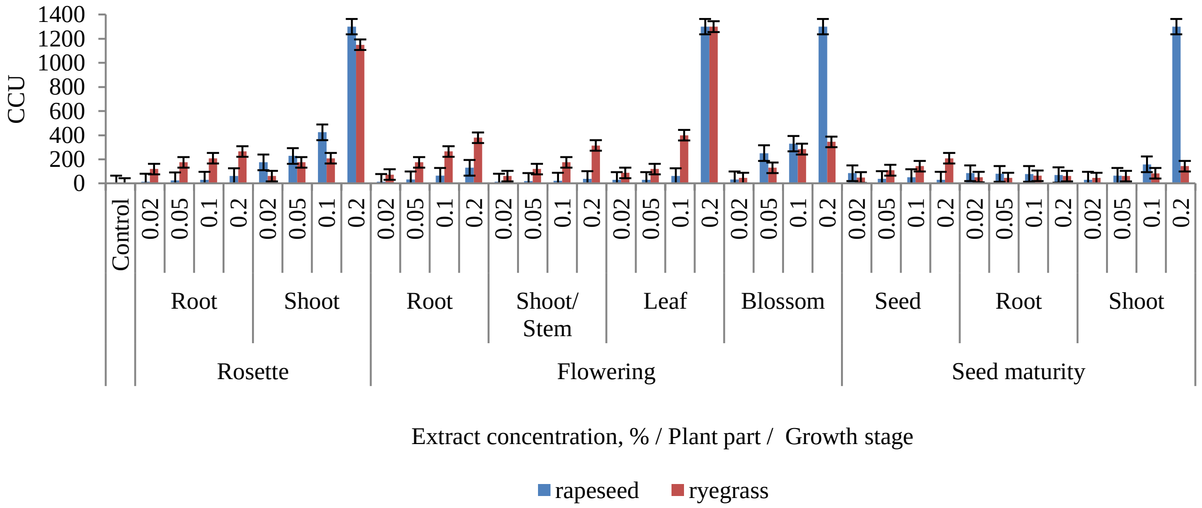

Figure 2. Content of universe conventional coumarine units (CCU) for different acceptors in leachates of *Solidago canadens* (mean ±SE; p<0.05)

In general, the different acceptor-species response to TPC possibly related with different anatomy and permeability of their seed coats, and thus determined different phenolics through inside seeds and calculated CCU content.

CONCLUSION

In general, the content of phenolic compounds was estimated different in aqueous extracts of *S. canadensis* due to dissimilar their accumulation in plant parts (root and shoot), growth stage and leachate concentration. As phenolics affect various physiological processes, and thus altering the physiological and growth patterns of plants, the revealed TPC exhibited different allelopathic potential of *S. canadensis* leachates. Thus, the TPC and CCU data suggest, that *S. canadensis* might provide the considerably allelopathic influence on the species diversity or local out-competion of the native plants in new habitats. The revealed allelochemical potential of *S. canadensis* should be considered as a partial explanation of high aggressiveness of species in invaded areas. Moreover, the hypothesis that allelopathy increases the invasive potential of *S. canadensis* may contribute a great deal to general models of invasive susceptibility in natural systems. Nonetheless, species evidence for allelopathic effects should not be restricted to TPC and CCU analysis of plant leachates in lab, but also grounded on future research in natural ecosystems.

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