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Cite this article: Radušienė J, Marksa M, Karpavičienė B (2018) Assessment of *Solidago* × *niederederi* Origin Based on the Accumulation of Phenolic Compounds in Plant Raw Materials. Weed Sci 66:324–330. doi:10.1017/wsc.2018.8

Received: 30 August 2017
Accepted: 1 February 2018

Associate Editor:
Muthukumar V. Bagavathiannan, Texas A&M University

Key words:
Chemotaxonomy; invasion of *Solidago* spp.; natural hybrid; principal component analysis

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Assessment of *Solidago* × *niederederi* Origin Based on the Accumulation of Phenolic Compounds in Plant Raw Materials

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Abstract

This study provides the first phytochemical characterization of the morphologically identified natural hybrid *Solidago* × *niederederi* Khek compared with the native *Solidago virgaurea* and two invasive species, Canada goldenrod (*Solidago canadensis* L.) and giant goldenrod (*Solidago gigantea* Aiton). The phenolic compounds, namely, chlorogenic acid, rutin, isoquercitrin, hyperoside, and quercitrin, were detected in leaves and inflorescences by the high-performance liquid chromatography–photodiode array detector/ultraviolet (PAD/UV) method. All analyzed *Solidago* species contained all of the phenolic compounds investigated. The quantitative phytochemical differentiation among *Solidago* taxa was shown by principal component analysis. The results indicated that *S. gigantea* plants were characterized by significantly different quantities of phenolic compounds compared with three other *Solidago* taxa, which formed a separate cluster in the space of the principal component model, indicating the high similarity of their profiles. An additional multivariate analysis of the three species studied revealed a chemical gradient from *S. canadensis* to *S. virgaurea* with a slightly overlapping zone on the score plots presented by *S.* × *niederederi* and *S. virgaurea* accessions. The results showed that *S.* × *niederederi* was closely related to *S. virgaurea*. This result is suggestive of a hybrid origin with significant contributions from the native species. However, *S.* × *niederederi* was significantly different from its parental species with respect to chlorogenic acid and quercitrin in leaves and rutin with isoquercitrin in inflorescences. Conversely, samples indicating intermediate chemical composition between native *S. virgaurea* and invasive *S. gigantea* were not distinguished. The comparison of phenolic compound accumulation in *Solidago* plants supported the additional identification of the origin of *S.* × *niederederi*.

Introduction

Invasive species are viewed as a significant component of global environmental changes that threaten biological diversity and functioning of ecosystems. The Delivering Alien Invasive Species Inventories for Europe (DAISIE) database contains 2,843 records of plant species alien to Europe, most of which are naturalized in new habitats (Hejda et al. 2009; Pyšek et al. 2012). Canada goldenrod (*Solidago canadensis* L.) and giant goldenrod (*Solidago gigantea* Aiton) are exceptionally successful worldwide invaders from North America and have a great potential to invade new habitats in the future (Weber 2001). Invasive *Solidago* spp. are well adapted to a relatively wide range of habitats with variable soil conditions that are more conducive to their growth and lead to the development of monodominant stands and unification of the landscape over large areas (Fenesi et al. 2015; Karpavičienė et al. 2015; Scharfy et al. 2009). In this way, the spread of alien *Solidago* spp. disturbs spontaneous successions and reduces plant diversity. In addition, alien species invade habitats of native congeners, which affects the origin of new taxa. The hybridization of invasive species with native plants is common and leads to the transfer of adaptations among invasive species. This process increases their invasive potential and can cause a loss of genetic diversity, fitness, threat of extinction of native species, and even evolutionary changes in spontaneous flora (Ellstrand and Schierenbeck 2006; Hovick and Whitney 2014). The spontaneous hybrids becoming stabilized are considered as new alien species (Pyšek et al. 2004).

The natural hybrid *S.* × *niederederi* Khek between the native to Europe *S. virgaurea* and alien *S. canadensis* was first recorded more than a century ago in Austria (Khek 1905). Currently, a comparatively rapid spread of *S.* × *niederederi* in European countries is observed (Jaźwa et al. 2018). The hybrid origin of *S.* × *niederederi* has been estimated using plant morphological characters, achene development, and pollen viability characters (Karpavičienė and Radušienė 2016; Migdałek et al. 2014). The complication of hybrid identification arises due to extremely diverse morphology of *Solidago* species, which causes taxonomic confusion within the genus (Semple et al. 2015). Consequently, alien *Solidago* plants with pubescent stems

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and leaves and inflorescences with ascending lower branches may be considered as one complex taxon *S. canadensis* s.l. (Weber 1997). *S. gigantea* appears to be one of the more clearly defined taxa; however, it also refers as a whole complex of varieties (Weber and Jakobs 2005). Furthermore, the European *S. virgaurea* is also highly polymorphic and divided into several closely related taxa (Kiełtyk and Mirek 2014). Taking into consideration the geographical differences in origin and high intraspecific morphological diversity within *Solidago* species, it can be assumed that the identification of hybrids and their parental species solely based on morphological traits can be misleading. Recently, the first genetic identity of hybrids has been confirmed using molecular methods (Pliszko and Zalewska-Gałosz 2016). According to Desjardins (2008), in addition to genetic, classical morphological and other nonmorphological methods, phytochemical evaluation can provide supplementary information on species identification.

The primary objectives of this research were to (1) assess the origin of morphologically identified *S.* × *niederederi* on the basis of phenolic compound accumulation and (2) to reveal chemotaxonomic value of some phenolics for identification of *Solidago* taxa. As far as we know, this study provides the first chemical characterization based on a comparison of the phenolic compound profiles of *S.* × *niederederi* and other *Solidago* species growing in close sympatry with the hybrid.

Materials and Methods

Sample Collection and Preparation

Nineteen accessions of *S. virgaurea*, 17 of *S. canadensis*, 11 of *S. gigantea*, and 24 of *S.* × *niederederi* were collected from 6 mixed wild populations in which all taxa were growing together. The sample of harvested plant material consisted of the three tops of two to three shoots of the same clonal plant in the flowering phase. The botanical identification of *Solidago* taxa was carried out by morphological diagnostic features defined in our previous work (Karpavičienė and Radušienė 2016). The herbarium vouchers of the accessions were deposited at the Herbarium of the Institute of Botany, Vilnius, Lithuania.

Collected plants were dissected into inflorescence and leaf parts and dried at 25 C. Air-dried material was mechanically ground to obtain a homogenous powder, and then samples of approximately 0.1 g were extracted in 10 ml of 70% methanol by ultrasonication at 25 C for 50 min. The solutions were filtered and stored at 4 C until analysis.

Chemicals

Solvents were of high-performance liquid chromatography (HPLC) grade and supplied by Roth GmbH (Karlsruhe, Germany). The reference substances, chlorogenic acid (≥ 95.33%), rutin trihydrate (97.11%), and isoquercitrin (≥ 94.16%), were purchased from HWI Analytik GmbH (Ruelzheim, Germany); quercitrin (≥ 98.0%) was obtained from Roth GmbH. Water was filtered through the Millipore (Billerica, MA, USA) HPLC-grade water-preparation cartridge.

HPLC-PDA Analysis

HPLC analysis was performed using a Waters Alliance 2695 (Waters, Milford, USA) separation module system equipped with a Waters 2487 UV/Vis and Waters 996 PDA photodiode-array detector. The separation of the compounds was carried out on YMC-Pack ODS-A column (3.0 µm, 150 mm by 4.6 mm i.d.) with a guard cartridge (3.0 µm, 10 mm by 4.0 mm). The analytical conditions were performed according to the method described in Radušienė et al. (2015).

Peaks of compounds were identified at a wavelength range of 210–550 nm by comparing their UV-Vis spectra and retention times to those of authentic reference standards. The samples were analyzed twice. The chromatograms of standards and flower extracts are shown in Figure 1. The quantification of detected compounds was carried out by the external standard method. Standard stock solutions for chlorogenic acid, rutin, hyperoside, isoquercitrin, and quercitrin were freshly prepared in 70% methanol and diluted to six different concentrations to establish calibration equations. Three injections per concentration were performed to determine linearity. A linear regression equation for all calibration curves was calculated by the least-squares method. The regression coefficients were $R^2 > 0.999$, confirming the linearity of the concentration ranges.

Data Analysis

Multivariate statistical approaches using the Statistica v. 10.0 (StatSoft) software package were performed. One-way ANOVA and a post hoc Sheffe's multiple-comparison test were used to identify and specify significant differences of phenolic compound quantities among the investigated taxa. The relationship between variables was analyzed using Spearman's rank correlation. Principal component analysis (PCA) was used to detect groupings, similarities, or differences among all analyzed accessions according to statistically independent variables, which represent the phenolic metabolite quantities in *Solidago* species. PCAs were based on eight standardized variables (the concentrations of chlorogenic acid, rutin, isoquercitrin, and quercitrin in leaves and inflorescences). The data sets of phenolic compounds in leaves and inflorescences were combined and used in PCA, yielding more compelling results than separate PCAs for leaves or inflorescences. Each sample had a score along PCA components, which showed its location in the space of the principal component model. The data for hyperoside concentration were eliminated from the PCA, as this variable had little explained variance and contributed to noise, reducing the quality of the model.

Results and Discussion

Interspecific Differences in Phenolics

Phenolic compounds, namely, chlorogenic acid, rutin, isoquercitrin, hyperoside, and quercitrin, were detected in leaves and inflorescences of *S. virgaurea*, *S. canadensis*, *S. gigantea*, and *S.* × *niederederi*. Leaves showed considerably higher mean values for chlorogenic acid compared with inflorescences, whereas inflorescences showed significant accumulation of isoquercitrin. All analyzed *Solidago* species shared the same common phenolic constituents.

ANOVA revealed high significant differences ($P \leq 0.001$) for the mean quantities of all phenolic compounds in leaves and inflorescences among the investigated species. Post hoc multiple comparisons of mean quantities specified that *S. gigantea* significantly differed for all compounds among all species. The highest values of phenolics were found in the leaves and inflorescences of *S. gigantea* compared with other species, except for rutin. Meanwhile, the rutin content in both the leaves and inflorescences of other species greatly exceeded that of *S. gigantea*, showing the highest accumulation in *S.* × *niederederi* leaves and in

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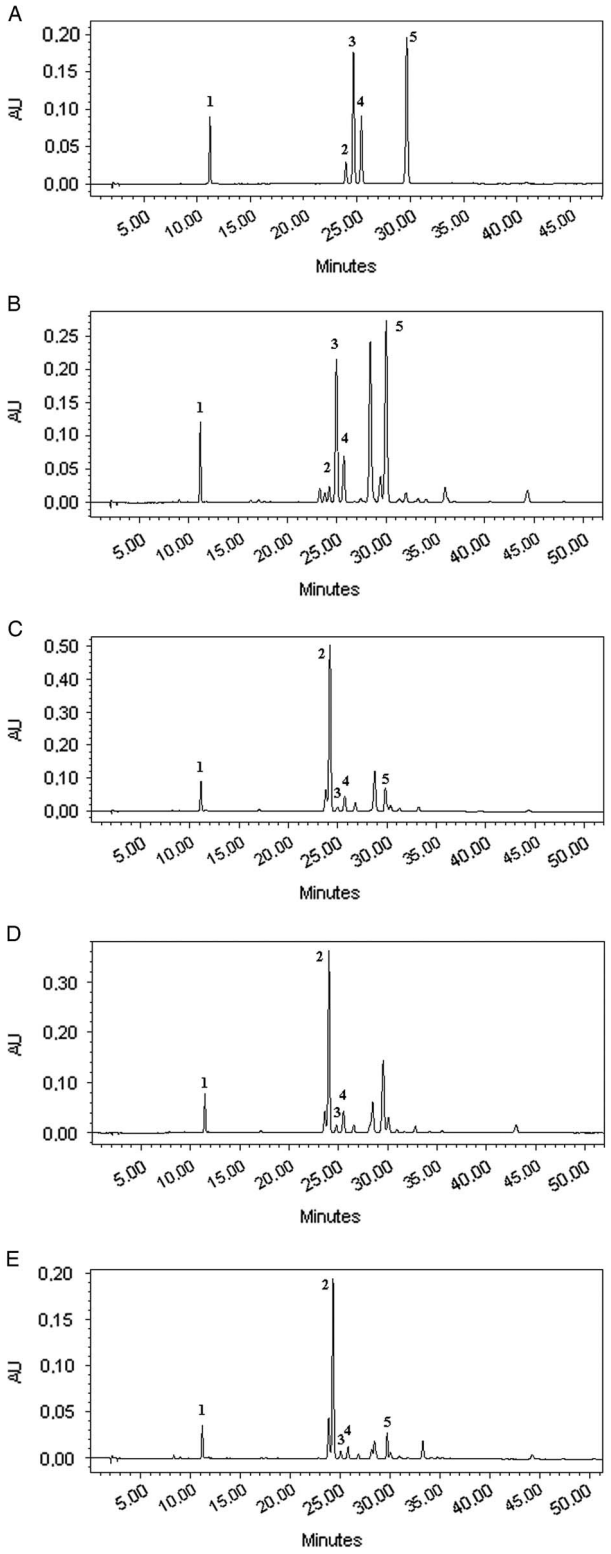

Figure 1. Chromatograms of reference standards (A) and inflorescence extracts of *Solidago* (B), *S. canadensis* (C), *S. virgaurea* (D), and *S.* × *niederederi* (E) detected by HPLC-PAD. Peak identified: 1, chlorogenic acid; 2, rutin; 3, hyperoside; 4, isoquercitrin; 5, quercitrin.

S. canadensis inflorescences. Furthermore, the differences for phenolics were analyzed between *S.* × *niederederi* and putative parental species *S. virgaurea* and *S. canadensis*. *Solidago gigantea* was excluded from the multiple-comparison test because the remaining taxa were not different for most phenolic compounds when *S. gigantea* was included. Significant differences among the three taxa were observed for chlorogenic acid and quercitrin in the leaves and for chlorogenic acid, rutin, and isoquercitrin in the inflorescences (Tables 1 and 2). The leaves of *S.* × *niederederi* significantly accumulated the highest ($P < 0.01$) level of chlorogenic acid, whereas the flowers of *S. canadensis* showed significant accumulation of this compound in comparison with the other two species. However, the amount of chlorogenic acid in the flowers did not differ significantly ($P > 0.05$) between *S.* × *niederederi* and *S. virgaurea*. The rutin content in the inflorescences of *S. canadensis* greatly exceeded that in the flowers of *S.* × *niederederi* and *S. virgaurea*. The highest amount of quercitrin was accumulated in the leaves and inflorescences of *S. canadensis*, and the mean content of this compound was found in leaves and inflorescences of *S.* × *niederederi*. Meanwhile, quercitrin was detected in minor quantities or not found at all in the flowers of *S. virgaurea*. Species significantly varied for content of isoquercitrin in the inflorescences, the highest levels of which were found in *S. virgaurea* followed by *S.* × *niederederi* and *S. canadensis*. However, the amount of hyperoside did not differ significantly among the species for both plant parts.

Table 1. Differences of phenolic compound accumulation in leaves among four *Solidago* species.[a]

Compounds	*S. virgaurea* ($n = 19$)	*S. canadensis* ($n = 17$)	*S.* × *niederederi* ($n = 24$)	*S. gigantea* ($n = 11$)
	Quantities, mg g^{-1} DM[b]			
Chlorogenic acid	17.65 ± 2.86b	18.78 ± 6.43b	22.42 ± 5.90a	37.38 ± 6.86
Rutin	13.69 ± 3.33a	14.00 ± 6.92a	17.35 ± 7.20a	0.49 ± 0.30
Isoquercitrin	0.08 ± 0.09a	0.11 ± 0.14a	0.13 ± 0.24a	2.50 ± 0.74
Hyperoside	0.28 ± 0.19a	0.41 ± 0.23a	0.52 ± 0.56a	0.93 ± 0.40
Quercitrin	0.07 ± 0.22c	1.08 ± 0.58a	0.33 ± 0.31b	39.48 ± 6.83

[a]Differences among four species for all compounds were significant at $P \leq 0.001$. n = the number of accessions analyzed. Values (mean ± SD) followed by different letters among first three species are significantly ($P \leq 0.05$) different according to Scheffe's test. *Solidago gigantea* was excluded from the multiple-comparison test because the remaining taxa were not different for most phenolic compounds when *S. gigantea* was included.
[b]Abbreviation: DM, dry mass.

Table 2. Differences of phenolic compound accumulation in inflorescences among four *Solidago* species.[a]

Compounds	*S. virgaurea* ($n = 19$)	*S. canadensis* ($n = 17$)	*S.* × *niederederi* ($n = 24$)	*S. gigantea* ($n = 11$)
	Quantities, mg g^{-1} DM[b]			
Chlorogenic acid	5.80 ± 1.69b	9.34 ± 2.48a	6.67 ± 1.74b	13.72 ± 3.52
Rutin	6.50 ± 1.50c	18.22 ± 6.96a	9.20 ± 3.74b	1.42 ± 0.34
Isoquercitrin	1.98 ± 0.65a	0.34 ± 0.28c	1.25 ± 0.39b	12.78 ± 3.71
Hyperoside	0.74 ± 0.24a	0.66 ± 0.43a	0.78 ± 0.50a	1.59 ± 0.49
Quercitrin	0.00b	0.23 ± 0.39a	0.02 ± 0.08b	18.10 ± 4.91

[a]Differences among four species for all compounds were significant at $P \leq 0.001$. n = the number of accessions analyzed. Values (mean ± SD) followed by different letters among three first species are significantly ($P \leq 0.05$) different according to Scheffe's test. Differences among four species for all compounds were significant at $P \leq 0.001$. *Solidago gigantea* was excluded from the multiple-comparison test because the remaining taxa were not different for most phenolic compounds when *S. gigantea* was included.
[b]Abbreviation: DM, dry mass.

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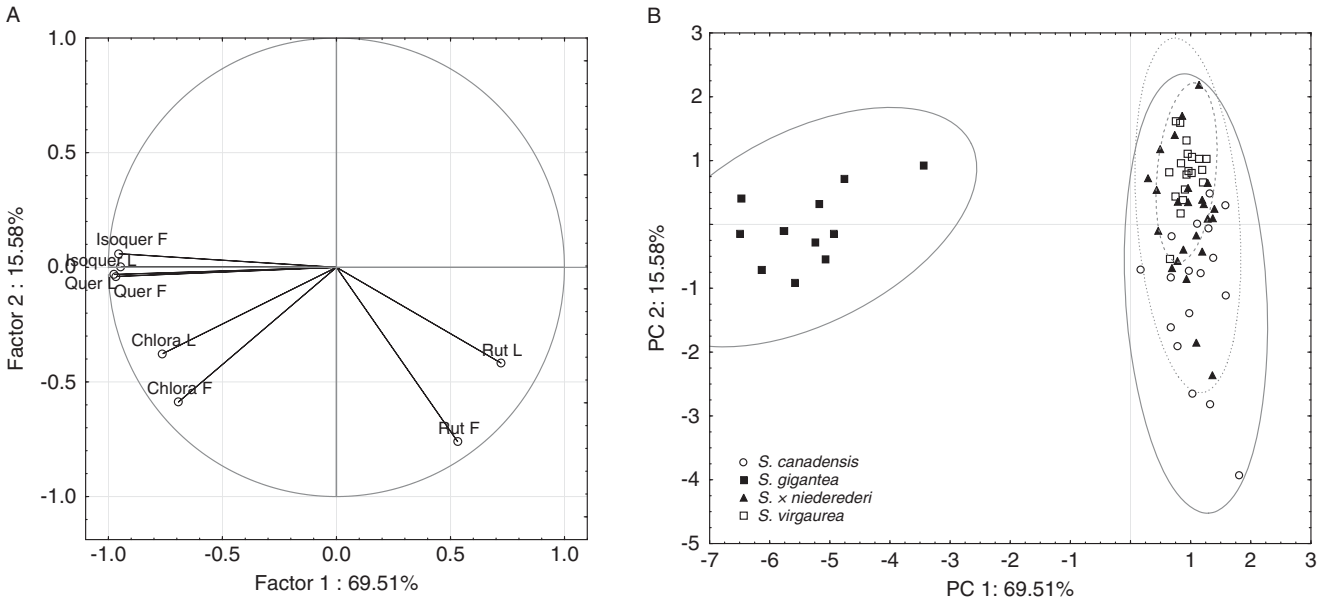

Figure 2. PCA1 representing four *Solidago* taxa for accumulation of phenolic compounds. (A) Loading plots for compound variables contributing to PC1 and PC2. (B) Score plots for the testing accessions with 95% confidence ellipses limit for each species.

Consequently, *S. virgaurea* accumulated the lowest quantities of phenolic compounds compared with *S. canadensis* and *S.* × *niederederi*, with the exception of isoquercitrin in leaves. In contrast, among the three *Solidago* species investigated, for the most part *S. canadensis* contained the highest amounts of the phenolic compounds. *Solidago* × *niederederi* showed average values of phenolics except for having the highest concentration of chlorogenic acid in leaves.

Principal Component Analysis

The first PCA1 model that represented the two-dimensional scatter plots showed differentiation between the sets of chemical data of the four *Solidago* species studied. The score plots for the first two PCs explained 85% of the total variance and represented enough visualization for any possible patterns of the data. PC1 accounted for 69.51% of the observed total variability and was strongly associated with negative loadings of quercitrin (Quer F, Quer L) and isoquercitrin (Isoquer F, Isoquer L) in flowers and leaves and chlorogenic acid (Chlora L) in leaves as well as the positive variable of rutin (Rut L) in leaves. The PC2 model explained 15.58% of the total variance and was characterized by negative loadings of rutin (Rut F) and chlorogenic acid (Chlora F) in flowers (Figure 2A). The PCA1 score plots with 95% confidence limit ellipses for the four species showed an arrangement of accessions into two distinct groups (Figure 2B). Within the right-hand plots, one group was formed by three overlapping ellipses along PC2 covering all accessions of *S. canadensis*, *S. virgaurea* and *S.* × *niederederi*. The location of accessions on the score plots can be explained by the position of variables on the loading plots. Rut F, Rut L, and Chlora F, with higher negative loading on PC2, were found to have high to moderate values in corresponding samples, while Quer F, Quer L, Isoquer F, and Isoquer L, with high negative loading on PC1, were accumulated in low quantities in all accessions of this group. The second group of accessions within the left-hand plots brought together all *S. gigantea* samples, which were closely clustered along negative PC1. In contrast to the first group within the right-hand plots, Quer L, Quer F, Isoquer L, and Isoquer F, scoring high in PC1, were detected as having the highest contents in those accessions; conversely, Rut L and Rut F, scoring low in PC1, were found in minor quantities. Chlora L and Chlora F, loading high to moderately in both PCs, were associated with higher levels of chlorogenic acid, especially in flowers. The results clearly suggested that accessions of *S. gigantea* were highly different with respect to phenolic content compared with the first group, which was formed by accessions of the other three *Solidago* species. The PCA1 model did not clearly separate morphologically different taxa as *S.* × *niederederi*, *S. virgaurea*, and *S. canadensis*. Consequently, the PCA2 model for the three species was used, in order to focus more specifically on differentiation among *S.* × *niederederi*, *S. virgaurea*, and *S. canadensis* accessions.

In the new PCA2, the first two PCs explained more than 56% of the total variance in the data set and presented much better separation between accessions of corresponding species. PC1 accounted for 37% of the observed variance and was best characterized by positive loadings of Chlora F, Rut F, and Quer L variables and negative Isoquer F. PC2 was highly associated with negative loadings of Chlora L and Rut L and moderate with positive Quer F (Figure 3A). Graphical representation of the score plots showed separation of all *S. canadensis* accessions in a separate cluster on the right-hand plot (Figure 3B). The positioning of *S. canadensis* samples coincided with higher loadings of Chlora F, Rut F, Quer L, and Quer F, indicating the greater influence of those variables on the colocated scores. The opposing location of Isoquer F to Chlora F, Rut F Quer L, and Quer F displayed negative correlations, which reflect opposite values of corresponding compounds in the accessions. One sample in the lower right-hand plot was separated from all of the others within the ellipses, suggesting that its composition differs significantly from the other accessions. Indeed, this sample contained the highest values of rutin and chlorogenic acid in both leaves and inflorescences. Meanwhile, the scores of *S.* × *niederederi* and *S. virgaurea* represented two overlapping ellipses. Additionally, *S.* × *niederederi* samples were much more scattered on PC1 versus PC2 along with Rut L and Chlora L loadings, displaying high

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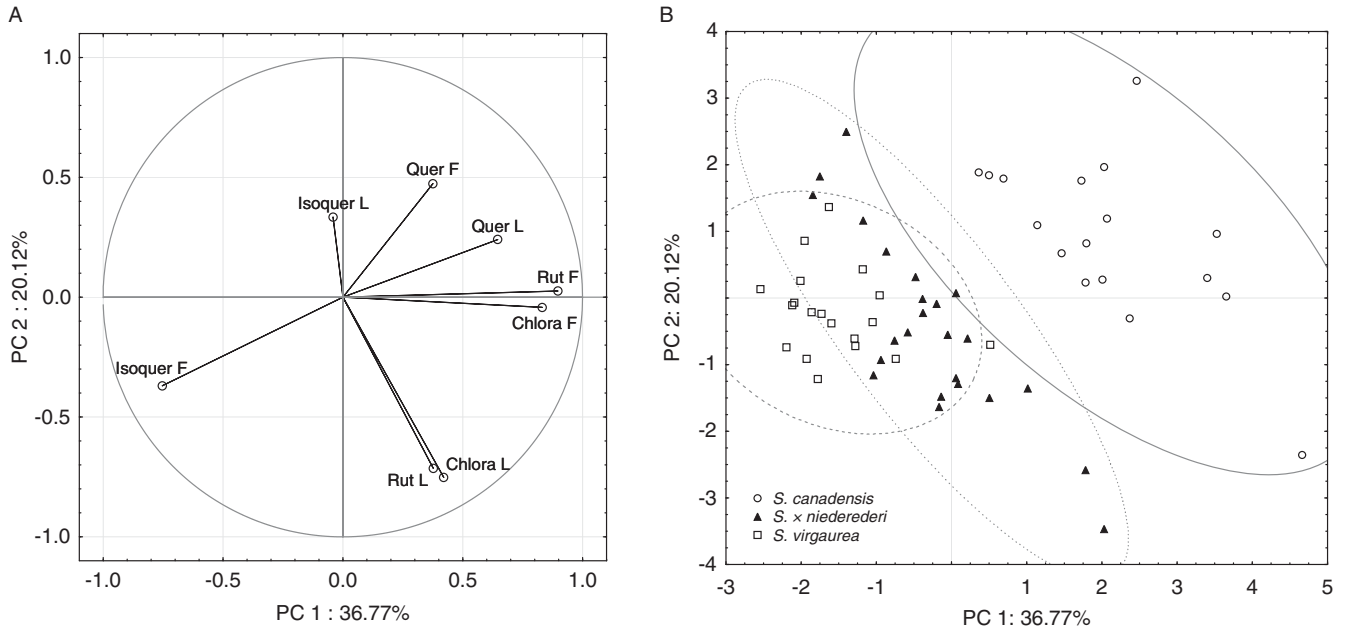

Figure 3. PCA2 representing three *Solidago* taxa for accumulation of phenolic compounds. (A) Loading plots for compound variables contributing to PC1 and PC2. (B) Score plots for the testing accessions with 95% confidence ellipses limit for each species.

variation in rutin and chlorogenic acid content in leaves. For these compounds, *S.* × *niederederi* was similar to *S. canadensis*. Some of *S.* × *niederederi* accessions exposed a tight position with *S. virgaurea*, indicating a great similarity in chemical content. Some hybrid samples demonstrated a remote position from all the others, scoring a high negative in PC2 and a low positive in PC1, which suggested higher amounts of chlorogenic acid and rutin in their leaves. The opposing location of some accessions on the upper left-hand score plot associated with low values of Chlora L and Rut L in those samples. Meanwhile, Quer F, scoring moderately in PC2, was found in mean quantities in previous samples. Isoquer F, scoring high in PC1 and moderately in PC2, had the greatest influence on colocated accessions and associated mean content of isoquercitrin in flowers of both species. Moreover, negative correlation of Isoquer F with Chlora F, Rut F, and Quer L suggested low values of these compounds in the corresponding samples. Consequently, the PCA2 model demonstrated that *S. canadensis* accessions formed a separate cluster, indicating a quantitative composition of phenolics highly different from that of from *S.* × *niederederi* and *S. virgaurea*. Conversely, *S.* × *niederederi* and *S. virgaurea* accessions did not reveal the separation of two well-defined groups, with high overlapping on the score plots.

In the current research, the main phenolic compounds in *Solidago* spp. plant materials, the quercetin-3-*O*-glycosides: quercitrin, isoquercitrin, rutin, and hyperoside, together with chlorogenic acid, were used to ascertain relationships and differences between *S.* × *niederederi* and other *Solidago* species growing in mixed populations. In general, the phenolic compounds are the most widely used of all secondary constituents in chemotaxonomic studies, mainly due to their ubiquitous occurrence in plants and their structural variability and chemical stability (Braunberger et al. 2015; Clark et al. 2014; Švehlíková et al. 2002). Furthermore, the phenolics listed above are considered principal bioactive compounds in *Solidago* spp. and are used for plant-derived medical preparations (Melzig 2004; Sabir et al. 2012). The present research on the comparison of four taxa demonstrated that all analyzed *Solidago* spp. exposed the same investigated phenolic constituents, which seems to be characteristic of the whole *Solidago* genus. On the other hand, the data obtained showed the significant quantitative differences in phenolics among corresponding taxa. The highest values of all studied phenolic compounds, with the only exception being rutin content in leaves and flowers, were found in *S. gigantea*. Further, chlorogenic acid was the only compound whose content in the leaves of *S.* × *niederederi* significantly exceeded that found in parental species, while the content of other compounds was intermediate or did not differ significantly. The accumulation of high amounts of phenolics may provide a competitive advantage via suppression of neighboring plants (Kim and Lee 2011). Moreover, phenolic compounds are known to be particularly important for plant interactions with abiotic and biotic environments and constitute one of the most common defense groups against herbivores (Mallikarjuna et al. 2004; Treutter 2006). Furthermore, chlorogenic acid is known to be associated with plant resistance to insects, fungi, bacteria, and viruses (Leiss et al. 2009; Niggeweg et al. 2004). It should be noted that chlorogenic acid and rutin in the leaves of *S.* × *niederederi* was highly variable. Moreover, chemical variability provides abundant material for environment-mediated selection and may play a role in superior resistance to herbivores and pathogens. Therefore, this variability may contribute to a higher hybrid fitness and persistence in a different environment (Oberprieler et al. 2011).

The chemistry of hybrids varied not only qualitatively, but also quantitatively (Orians 2000). Generally, hybrids express parental chemicals, but parental compounds are sometimes missing, or novel compounds are present. The survey by Cheng et al. (2011), based on 1,112 secondary metabolites of different hybrids, showed that the frequency of metabolite novelty accounted for 5.5% of all studied compounds. The probability of occurrence of novel compounds increases in polyploids, especially in F_2 and later-generation hybrids (Orians 2000). Accordingly, we have not detected any novel chemicals in *S.* × *niederederi*, which is diploid and apparently only a first-generation hybrid (Karpavičienė and Radušienė 2016).

PCA has been used here as an exploratory method to describe data sets without a priori knowledge of the data structure and to

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allow the visualization of the similarities and differences within analyzed data to indicate the compounds most responsible for separation of different plant groups. In this regard, the position of *S. gigantea* accessions on PC1 versus PC2 plots at a distance from others represented a greater difference in chemical composition as determined by the study of all phenolic compounds. Meanwhile, the other three taxa formed a separate cluster, indicating similarities in their quantitative accumulation of phenolics. It can be assumed that interspecific chemical variability among *Solidago* species should be genetically based. However, the mode of qualitative inheritance of most chemical compounds is Mendelian with dominance; if both or one of the parents produce a chemical, the hybrids almost always produce it as well (Orians 2000). It can be assumed that the genes responsible for the synthesis of phenolics are constitutively, but differently expressed in the analyzed species (Cheng et al. 2011). Although morphologically and phylogenetically *S. canadensis* is closer to *S. gigantea* than to *S. virgaurea* (Semple 2016), invasive *Solidago* spp. differ in their ploidy level. *S. gigantea* is tetraploid ($2n = 36$), whereas *S. canadensis* is diploid ($2n = 18$), as are *S. virgaurea* and *S.* × *niederederi* (Karpavičienė and Radušienė 2016). The ploidy level is known to have a significant effect on the intraspecific variation in concentrations of metabolites in *S. gigantea* (Hull-Sanders et al. 2009). In this context, tetraploid *S. gigantea* showed high mean phenolic content, while diploid *Solidago* spp. exhibited comparatively lower content for the compounds analyzed.

The additional PCA for the three species studied, with the exception of *S. gigantea*, demonstrated that the parental species *S. virgaurea* and *S. canadensis* were well distinguished from each other based on the analyzed phenolics. Although interspecific hybrids can often be clearly distinguished from parental species based on their biochemical phenotypes (Kirk et al. 2012), *S.* × *niederederi* accessions did not show itself to be a clearly defined group compared with the parental species, being in close position with *S. virgaurea*. They clustered less tightly and displayed greater variation in chemical composition, with some of the samples falling outside the range of the parental species. These results are similar to those reported previously by Kirk et al. (2005) for chemical composition of two *Senecio* species and their F_1 hybrids, which did not cluster intermediately to parents on the basis of PCA. According to Orians (2000), parental chemicals in hybrids are mostly either expressed at concentrations similar to one of the parents or at intermediate concentrations. However, *S.* × *niederederi* significantly exceeded the parental species in mean chlorogenic acid content in leaves, but the range of variation of this compound in the hybrid was similar to that of *S. canadensis*. The results agree with other studies showing that hybrid plants have much more genetic variation than the parental species (Ellstrand and Schierenbeck 2006; Zalapa et al. 2010), which leads to higher phenotypic diversity. Overall, in our research, *S.* × *niederederi* was characterized by the accumulation of phenolic compound content intermediate or similar to that of *S. virgaurea*, suggesting its hybrid origin.

The sparse chemical diversity of invasive species can be explained by the assumption that invasive species undergo one to several bottlenecks when they are introduced to new areas, which leads to genetic uniformity in plant populations (Müller-Schärer and Schaffner 2008). In addition, there exist some data about the lack of differentiation in DNA content observed in native *S. virgaurea* (Szymura et al. 2015) that may be associated with low chemical diversity. However, in contrast to low chemical diversity, high intraspecific morphological variations have been observed in the analyzed *Solidago* species (Karpavičienė and Radušienė 2016; Kiełtyk and Mirek 2014; Semple et al. 2015). Based on the hypothesis of Bossdorf et al. (2005), high plasticity of morphotype allows introduced species to naturalize across different environments, revealing the potential for their invasiveness. Therefore, the use of only morphological descriptions to examine taxa with large intraspecific variation may result in wrong conclusions and taxonomic confusion, as has been the case with *Solidago* taxa. Consequently, the phytochemical pattern in the present study complemented the evidence of hybrid *S.* × *niederederi* origin between native *S. virgaurea* and invasive *S. canadensis*. However, it can be assumed that *S.* × *niederederi* is continuously formed in mixed *Solidago* spp. populations, because the phytochemical intermediate of the hybrid is not fixed, suggesting that it is not a completely stabilized hybrid derivative leading to the creation of new genotypes and evolutionary novelty. *Solidago* × *niederederi* has occurred rather frequently in mixed *Solidago* spp. populations; therefore, it has the potential to spread and increase due to viable seed production (Migdałek et al. 2014) as well as through vegetative propagation by clonal growth (Pliszko and Kostrakiewicz-Gierałt 2017). However, accessions exhibiting an intermediate chemical composition between spontaneous *S. virgaurea* and invasive *S. gigantea* were not detected. This can be explained by the differing number of chromosomes of these species, which leads to more complicated hybridization. On the other hand, *S.* × *snarskisii* Gudž. & Žaln., the spontaneous putative hybrid between *S. virgaurea* and *S. gigantea*, has been recently described (Gudžinskas and Žalneravičius 2016). Furthermore, the appearance of a new hybrid indicated the growing impact of invasive *Solidago* spp. on local flora.

Although statistical analysis revealed the presence of phenolic compounds such as the chlorogenic acid and quercitrin in leaves and rutin with isoquercitrin in inflorescences of *S.* × *niederederi* at significantly different quantities from its parental species, high variability of these components and their overlapping ranges between species impact clear identification of hybrids in the wild. Nevertheless, the present study provided the first examination of expression of phenolic compounds in *S.* × *niederederi*. It is important to further assess its inheritance of phenolic compound expression in the second and later generations of the hybrids, which is very likely, because according to Pliszko and Kostrakiewicz-Gierałt (2017), hybrids are able to generate their own offspring by sexual reproduction. On the other hand, the implication of phenolic compounds does not preclude the importance of other secondary metabolites in species differentiation. Additional analysis of these species could show some differences for minor components, which could expose their importance in chemotaxonomy and the evolutionary dynamics of invasion. Overall, *Solidago* is a suitable model genus for the study of the plant invasion process, and the present study provides a basis for further research on the role of genetic diversity and expression of secondary metabolites during hybridization on invasive potential.

Acknowledgment. This research was funded by a grant from the Research Council of Lithuania (no. MIP-50/2013). No conflicts of interest have been declared.

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https://doi.org/10.1017/wsc.2018.8 Published online by Cambridge University Press

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https://doi.org/10.1017/wsc.2018.8 Published online by Cambridge University Press