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SHORT COMMUNICATIONS

Abundance and Diversity of Arbuscular Mycorrhizal Fungi in Invasive *Solidago canadensis* and Indigenous *S. virgaurea*

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Mycorrhiza formation may be one of the critical processes contributing to the expansion of plant species ranges, including those of invasive plants. Available data on this process in invasive plants are contradictory, showing that its activity is often reduced (Stinson et al., 2006; Endresz et al., 2013; Betekhtina and Veselkin, 2015), but successful invaders may be highly mycorrhizal (Kovács and Bagi, 2001; Kovács and Szigetvári, 2002; Fumanal et al., 2006; Veselkin and Prokina, 2015). Invasive plants may have an effect on local communities of arbuscular mycorrhizal fungi (AMF), reducing their diversity and abundance (Stinson et al., 2006; Bunn et al., 2013) or specifically modifying their composition (Zhang et al., 2010). In general, significant variation is observed both in the prevalence of arbuscular mycorrhizas (AMs) in invasive plants and in the strategy of their interaction with AMF, which may be determined geographically (Callaway et al., 2008).

The purpose of this study was to analyze specific features in the development of mycorrhiza and AMF communities associated with two goldenrod species, indigenous *Solidago virgaurea* L. and invasive *S. canadensis* L., growing in neighboring habitats of Belarusian Polesye. Our initial hypothesis was that *S. canadensis* (alien to the study region) is less adapted to the interaction with local AMF and that their diversity and AM abundance are lower than in indigenous *S. virgaurea*. The species of the genus *Solidago* were used as model plants taking into account the importance of analyzing the ecological and biological properties of *S. canadensis* as an invasive species of North American origin that has spread in Europe, Asia, Australia, and New Zealand (Weber, 2001; Lu et al., 2007). It is noteworthy that this species in its secondary range has shown activity toward native AMF (Zhang et al., 2007; Yang et al., 2014).

Solidago virgaurea and *S. canadensis* are herbaceous perennial rhizomatous plants of the family Asteraceae, both of them form AMs (Wang and Qiu, 2006). With respect to cenotic distribution, *S. virgaurea* is a forest–meadow plant, while *S. canadensis* in Belarus actively expands over roadsides, field margins, abandoned surface mines, and other disturbed habitats (Gusev, 2015).

The material was collected near the city of Gomel, Belarus, in habitats similar in edaphotope, microclimate, and the composition and structure of plant communities. *Solidago virgaurea* was collected in a disturbed meadow at 5 m from the edge of pine forest (52°23′24″ N, 30°54′45″ E), at a site with mechanically disturbed soddy podzolic yellowish sandy soil and groundwater table depth of 2–3 m. The plant community had 90% coverage and was dominated by *Achillea millefolium*, *Calamagrostis epigeios*, *Elytrigia repens*, *Poa pratensis*, and *Trifolium pratense*. *Solidago canadensis* was collected from a plant group at an early succession stage growing in the middle part of sand pit slope, on sandy soil, at 500 m from the habitat of *S. virgaurea*. The community had 70% coverage and was dominated by *S. canadensis,* proportions of other species were insignificant. They included *Achillea millefolium*, *Artemisia campestris*, *A. vulgaris*, *Calamagrostis epigeios*, *Conyza canadensis*, *Corynephorus canescens*, *Helichrysum arenarium*, *Jasione montana*, *Koeleria glauca*, *Oenothera biennis*, *Poa pratensis*, *Saponaria officinalis*, *Trifolium arvense*, *T. pratense*.

Parameters of the development of arbuscular mycorrhiza and the diversity of mycorrhizal fungi associated with *Solidago virgaurea* and *Solidago canadensis*

Parameter	Species	
	Solidago virgaurea	*Solidago canadensis*
Colonization ($m \pm SE$), %:		
total	99.3 ± 0.9	93.4 ± 2.4
by arbuscules	96.0 ± 1.7	45.8 ± 9.1
by vesicles	65.3 ± 11.2	48.8 ± 11.2
Occurrence of root hairs ($m \pm SE$), %	3.4 ± 1.6	44.0 ± 4.9
Number of cloned sequences	19	20
Richness, number of virtual taxa	4	8
Diversity, Shannon's index	0.90	1.96
Dominance, Berger–Parker index	0.68	0.25

Analysis was performed with five plants of each species, with undamaged root systems, collected as herbarium specimens in early August 2014. Each plant was evaluated for mycorrhization by examining 15 randomly selected 1-cm fragments of fine roots of the last two orders. The fragments were macerated in KOH solution for 1 h and stained with aniline blue. Squashed preparations (Selivanov, 1981) were examined under a Leica DM 5000 microscope at 100× magnification to record AMF structures in 375 microscopic fields per species and the proportion of microscopic fields where root hairs were observed. The degree of AM development was estimated from the proportions (%) of microscopic fields with any fungal structures (*F*, total colonization) and with vesicles (*V*, vesicular colonization) or arbuscules (*A*, arbuscular colonization).

An informative method for evaluating the species diversity of AMF in plant roots and soil is sequencing of marker genes or their fragments (18S rRNA and 25S rRNA genes, transcribed intergenic spaces, or their combination) amplified from the corresponding material (Gorzelak et al., 2012). Analysis of the sequences of 18S rRNA gene fragment allows identification of virtual taxa (VT) of AMF, which comprise phylogenetically related groups of sequences sharing more than 97% homology that correspond to the species level (Opik et al., 2010).

Fragments of the last-order fine roots (100–200 mg) from each individual plant were ground in liquid nitrogen and homogenized in lysis buffer (0.1 M Tris-HCl, pH 8.0, with 1.4 M NaCl, 2% CTAB, and 20 mM EDTA). DNA was isolated by precipitation with a twofold volume of 95% ethanol in the presence of NaAc and EDTA followed by washing with 70% ethanol. PCR amplification of a 18S rRNA gene fragment (about 530 bp) was performed with primers NS31 (TTGGAGGGCAAGTCTGGTGCC) and AML2 (GAACCCAAACACTTTGGTTTC) (Simon et al., 1992; Lee et al., 2008) in a 25-μL reaction mixture using a Tertsik thermal cycler (DNK Tekhnologiya, Russia). PCR conditions were as follows: initial denaturation at 95°C for 3 min; 42 cycles of denaturation at 95°C for 10 s, annealing at 64°C for 10 s, and extension at 72°C for 15 s; and final extension at the same temperature for 5 min. Amplification products were separated by agarose gel electrophoresis and stained with ethidium bromide. They were then purified using a DNK-sorb AM kit (InterLabService, Russian), cloned in pAL-TA vector (Evrogen, Russia), and transformed in competent *Escherichia coli* Top10, with subsequent white-blue selection for transformants. Ten clones were selected for each sample, with some of them containing nontarget sequences. The target fragments were sequenced in two directions with the ABI 310 Prism genetic analyzer (Applied Biosystems, United States) using a BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's protocol.

A phylogenetic tree was constructed and genetic distances were calculated with the MEGA 6 program (Tamura et al., 2013). Virtual taxa numbers were assigned in correspondence with type sequences deposited in Maarj*AM* database (http://maarjam.botany.ut.ee) (Opik et al., 2010). Differences in parameters of mycorrhiza and root hair development were evaluated by *t*-test after arcsine transformation of fractional values. The diversity of AMF VT was estimated for the plant species as a whole. Shannon's indices were compared as described (Magurran, 1992).

The results showed that the degree of AM development in the roots of *S. canadensis* was lower than in *S. virgaurea* (table). This is evident from comparison between the values of F ($t = 2.34$, $P = 0.047$; for *t*-test, $dF = 8$ in all cases) and A ($t = 5.99$, $P < 0.001$). Differences in A values are almost twofold, while those in V lack statistical significance ($t = 1.09$; $P = 0.308$). Arbuscules are the main structures of this mycorrhiza type, and the conclusion about reduced activity of AM formation in the invasive species, compared to the

indigenous species, can therefore be considered reliable. The frequency of root hairs was significantly higher in *S. canadensis* than in *S. virgaurea* ($t = 7.89$, $P < 0.001$).

We obtained 39 cloned DNA sequences of AMF 18S rRNA gene fragment (523–529 bp) isolated from 10 samples of *S. virgaurea* and *S. canadensis* roots and, for each plant, 1–5 transformants containing target inserts of 18S rRNA gene fragment from fungi of the division Glomeromycota. These sequences belonged to 11 VTs of the genera *Acaulospora* (VT28), *Claroideoglomus* (VT276), and *Glomus* (VT113, VT115, VT130, VT137, VT156, VT166, VT172, VT214, VT423). Because of certain disagreement between AMF classifications by morphological and molecular genetic characters, species identification of VTs was successful in only three cases: *Glomus fasciculatum* (VT113), *G. vesiculiferum* (VT115), and *Acaulospora longula* (VT28). The dendrogram plotted on the basis of these sequences is shown in the figure.

The diversity of fungal VTs associated with *S. virgaurea* was lower than in the case of *S. canadensis*. Only one taxon (VT115) was common to both species not dominant in any of them. Along with low diversity, VTs in *S. virgaurea* were characterized by a high dominance level: the proportion of the most abundant taxon (VT113) reached 68%, compared to 25% (VT156) in *S. canadensis*. Sequences obtained from two *S. canadensis* plants (three from one and five from another) were identical and belonged to taxa VT423 and VT156, which were not found elsewhere. Other plants were colonized by more than one AMF taxa. Shannon's diversity index was also significantly lower in *S. virgaurea* than in *S. canadensis* ($t = 4.29$, $dF = 34$; $P < 0.001$).

An assessment of genetic distances (D) within and between the groups of 18S rRNA gene sequences revealed significant differences in AMF diversity between the two *Solidago* species. The average distance (±SE) within *S. virgaurea* was $D = 0.024 \pm 0.003$, compared to $D = 0.049 \pm 0.005$ in *S. canadensis;* i.e., the diversity of AMF community in the invasive species proved to be two times higher. The average genetic distance between AMF sequences from *S. virgaurea* and *S. canadensis* reached $D = 0.069 \pm 0.008$, indicating differentiation between these AMF communities.

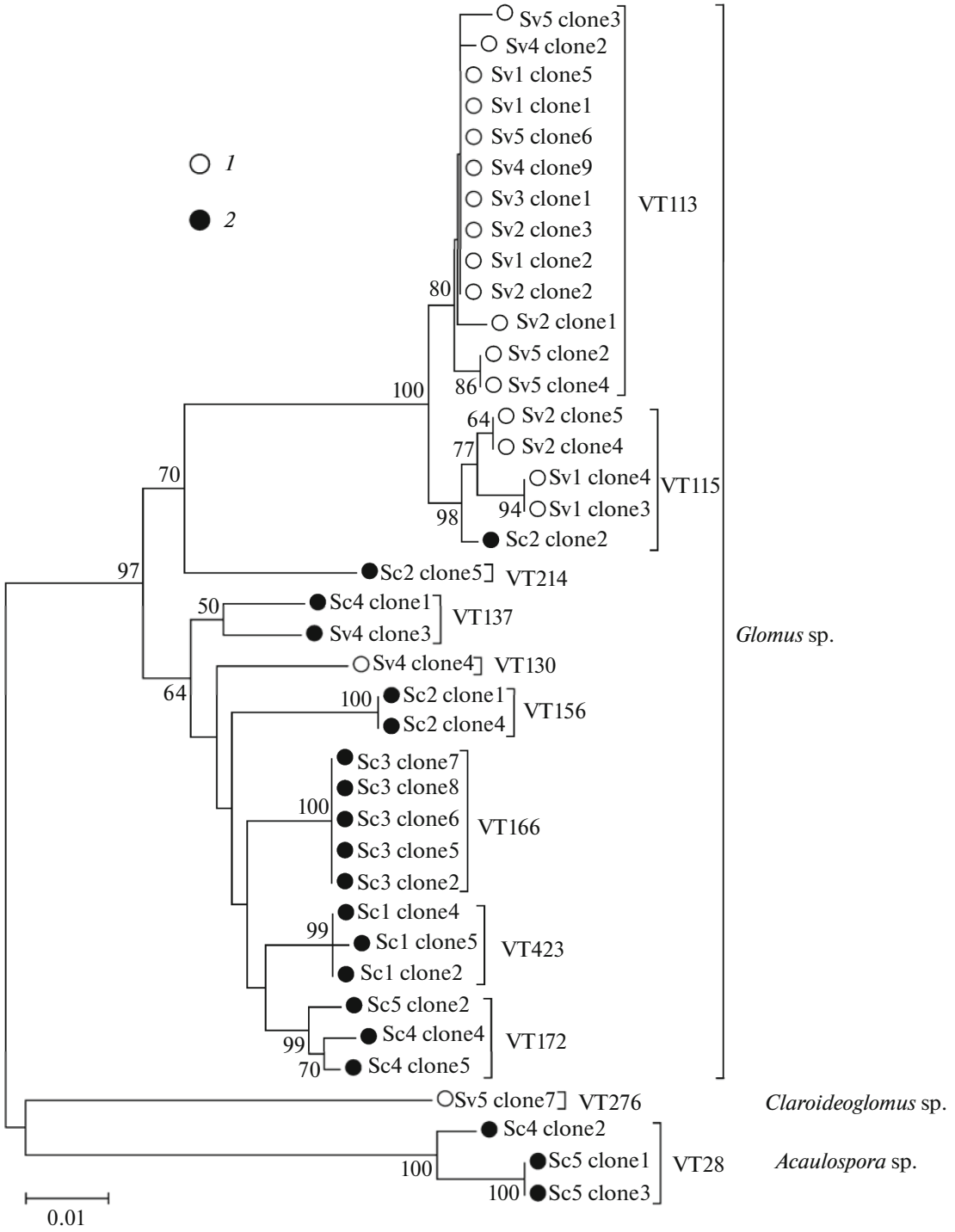
Differences between *S. virgaurea* and *S. canadensis* plants in the degree of mycorrhization and the composition and diversity of AMF associated with them are not accidental. The observed reactions are consistent with each other and agree with certain published data.

The reduced rate of AM formation in *S. canadensis,* compared to *S. virgaurea*, is concordant with low AMF abundance in the roots of invasive plants, a large proportion of nonmycorrhizal species among them, and their low responsiveness to the interaction with AMF (Fitter, 2005; Pringle et al., 2009; Shah et al., 2009; Endresz et al., 2013; Betekhtina and Veselkin, 2015). Thus, our results confirm the initial hypothesis as regards reduced mycorrhization success in invasive plants. This is consistent with higher frequency of root hairs in invasive *S. canadensis,* since AM development may be negatively correlated with their development (Muthukumar et al., 1999).

The diversity of AMF in *S. canadensis* is higher than in indigenous *S. virgaurea*. However, the AMF community in *S. virgaurea* consists mainly of *Glomus fasciculatum* and *G. vesiculiferum,* phylogenetically close and widespread species (Moora et al., 2011), while there are no distinct dominants in the diverse AMF community of *S. canadensis*. The wide spectrum of AMF species involved in the interaction with the invasive species contradicts the initial hypothesis as it concerns reduced diversity of mycorrhizal fungi in such plants. However, this result agrees with published data: for example, Bongard et al. (2013) revealed such differences between invasive *Vincetoxicum rossicum* and local native plants.

Differences in the species composition of AMF associated with the indigenous and invasive species can be explained differently. First, it is conceivable that certain fungal species selectively colonize *S. canadensis* due to the absence of stable connections between this plant and local dominant fungal species. Second, the invasive species may alter the composition of AMF in the soil by stimulating the development of certain species and inhibiting other species. The possibility of such an effect was demonstrated experimentally (Zhang et al., 2007): *S. canadensis* (an invasive species in China) was found to alter the composition of soil AMF community (probably by releasing some allelopathic substances) and to stimulate increase in the abundance of *Glomus geosporum* and *G. etunicatum* relative to that of *G. mosseae,* the main dominant in the AMF community of indigenous *Kummerowia striata* (Zhang et al., 2010; Yang et al., 2014). The transformed AMF community provided for more active absorption of water and salts from the soil, thereby giving *S. canadensis* competitive advantage over the indigenous plant. Our results are in complete agreement with the data on the specificity of AMF composition in the invasive plant obtained by Chinese scientists (Zhang et al., 2007, 2010; Yang et al., 2014).

Thus, the results of this study provide evidence for ecological individuality of the two *Solidago* species, indigenous *S. virgaurea* and invasive *S. canadensis,* in the belowground sphere. These species significantly differ in all characters included in analysis: qualitative parameters of AM development, the frequency of root hairs, and the species richness and composition of associated AMF communities. These facts confirm the significance of mycorrhizal interactions for the course and success of plant invasions.



Phylogenetic tree constructed by the neighbor-joining method based on partial AMF 18S rRNA gene sequence (529 bp): (*1*) clones isolated from *Solidago virgaurea*, (*2*) clones isolated from *S. canadensis*.

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