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REGULAR PAPER

Fungal colonization of the invasive vine *Vincetoxicum rossicum* and native plants

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Background and aims – *Vincetoxicum rossicum* (Kleopow) Barbar., an aggressive European plant invader found in Eastern North America, has properties that enable it to establish large monocultures over time, displacing native species and their associated fauna. It has been proposed that *V. rossicum* also establishes associations with a subgroup of arbuscular mycorrhizal fungi (AMF) that are generalists described as having high infectivity and are particularly efficient with regard to phosphorus uptake. Such associations may also enhance seedling recruitment in mechanically disrupted areas due to the ability of these AMF to colonize roots directly via hyphal fragments as well as via direct spore germination. As well, invasive plants are also suggested to be fungal generalists, providing advantages when establishing in novel environments where fungi may be different from those in their native range.
Materials and methods – In a laboratory study we used molecular techniques to investigate the fungal associations of *V. rossicum* and local native plants goldenrod (*Solidago* spp.), wild ginger (*Asarum canadense*), and Canada anemone (*Anemone canadensis*) growing in dense patches of *V. rossicum*.
Key results – The findings indicate that *V. rossicum* forms associations with a broad array of fungal partners relative to proximal native plants, suggesting the likelihood of it being a fungal generalist. As well, *V. rossicum* was found to associate with a subgroup of described opportunistic AMF such as *Glomus intraradices*, *G. caledonium*, *G. fasciculatum* and *Glomus mosseae*, while natives growing within *V. rossicum* patches were not.
Conclusion – Our results suggest that *V. rossicum* is a fungal generalist, a trait that is beneficial in establishment and persistence. It is useful to understand the fungal association dynamics of *V. rossicum* when attempting to restore invaded sites, as inoculation with fungal species that are particularly beneficial to the establishment of *V. rossicum* may be avoided.

Key words – *Vincetoxicum rossicum* (Kleopow) Barbar., plant-fungal associations, invasions, molecular identification.

INTRODUCTION

Vincetoxicum rossicum (Kleopow) Barbar. (also *Cynanchum rossicum*) or Dog-strangling vine (DSV) is an invasive vine in the Asclepiadaceae (milkweed) family. An aggressive competitor, it has the ability to establish dense monocultures in invaded sites due to advantages such as polyembryonic propagules, anti-feedant compounds in the leaf and stem tissues, self-compatible gametes, and the ability to regenerate from even small desiccated fragments of the root crown (Cappuccino 2004, DiTommaso et al. 2005, Miller 2008, Mogg et al. 2008). It establishes dense, light-limiting nets of vines in invaded areas that render light, nutrient, and moisture conditions unsuitable for many understory and emergent plants (Lawlor & Raynal 2002, Cappuccino 2004).

Attempts to successfully control DSV in North America are currently being investigated and include mechanical, chemical, and biological applications (Averill et al. 2008, Milbrath 2010), but none have proven to be economically successful on a large scale basis. Further characterization of the fungal partners of DSV may be useful in understanding the invasion strategies employed by this species (Greipsson & DiTommaso 2006, Averill et al. 2008, Mogg et al. 2008, Smith et al. 2008). Endophytic arbuscular mycorrhizal fungi (AMF) form highly specialized 'nutrient-exchange' structures at the plant-fungus interface and are recognized as being a particularly beneficial group of root endophytes (Bon-

fante 2003, Brundrett 2004). As most crop plants are hosts to AMF, this association is potentially an important resource for agriculture (Sieverding 1991). Fungal partners may also mediate plant-to-plant transfer of carbohydrates and other nutrients via common mycorrhizal networks (Selosse et al. 2006, Egerton-Warburton et al. 2007).

It has been proposed that some AM fungi may be harnessed by aggressive invasive plant species in a way that can alter existing native soil fungal communities, thus disrupting the fungal associations normally established in native plant communities (Greipsson & DiTommaso 2006, Hawkes et al. 2006). Given that AMF plant-fungal associations have demonstrated host specificity (Bever 2002, Appoloni et al. 2008), disruptions to fungal communities by invaders could subsequently contribute to altering the existing plant population structure (Vandenkoornhuyse 2002). Greipsson & DiTommaso (2006) predicted that invasive species such as DSV will tend to associate with fast-growing fungal species, enabling rapid establishment of DSV relative to native plants. *Glomus* has been shown to comprise a subset of such AMF because they readily form anastomoses (direct connections between hyphae) with other conspecifics, and are also capable of colonizing via fragments of mycelium, while other AM fungi may be limited to infection via intact mycelia or spore germination (Biermann & Linderman 1983, Giovanetti et al. 1999, Helgason et al. 2002).

Our study employed restriction fragment analysis and sequencing methods to characterize the fungal communities colonizing the roots of DSV and neighbouring native plants. We hypothesized at the outset that the successful establishment and spread of DSV may be due in part to its ability to form associations with numerous ubiquitous AM fungi, and unlike a number of native plant species, DSV forms associations with a subgroup of highly beneficial AMF species.

MATERIALS AND METHODS

Study sites and sample collection

We collected root samples of DSV as well as co-occurring native plant species (natives-in-dsv) in mid July 2009 from woodland and field plots in two main sites that are in permanent neighbouring watersheds in Southern Ontario: East Highland Creek at the University of Toronto at Scarborough, and the Rouge River at the Toronto Zoo (table 1). Native plants included goldenrod *Solidago* spp. (UTSC and Zoo field), Canada anemone *Anemone canadensis* (UTSC woodland), and wild ginger *Asarum canadense* (Zoo woodland). Soil samples from all plots per site were sent to the University of Guelph Laboratory Services Division (Guelph, Ontario) for analysis, including both field and woodland samples.

Fungal community comparisons

We dug up the plants carefully to protect root hairs and to ensure that the entire root ball was extracted from the ground. Samples were transported to the lab within a few hours of collection, where they were stored at 4°C until processing the following day. We washed the roots extensively in running water, and then removed root sections for subsequent DNA extractions. Detailed descriptions of the molecular methods used for characterizing the fungal community can be found in table 2.

Table 1 – Plant collections from 2 sites: UTSC and ZOO. Native growing in DSV patches denoted by '-dsv' post script; samples are collected from 4 UTSC sites and 4 Zoo sites, totaling 24.

Site	Environment	Native plant species	Samples
UTSC 43.7803° N 79.1886° W	Field	*Solidago* spp.	3 goldenrod-dsv 3 DSV
	Woodland	*Anemone canadensis*	3 anemone-dsv 3 DSV
ZOO 43.8208° N 79.1852° W	Field	*Solidago* spp.	3 goldenrod-dsv 3 DSV
	Woodland	*Asarum canadense*	3 wild ginger-dsv 3 DSV

Data analyses

We normalized peak height data for each individual terminal restriction fragment (TRF) to a percentage of total peak height per sample. The normalized terminal restriction fragment electropherogram data were then organized using a macro created by Christopher Walsh (Rees et al. 2004) in Excel 2007 (Microsoft Corporation), and then subsequently used in downstream analyses. We exported the normalized dataset to R open source statistical analysis software, v 2.12.0 (R Development Core Team 2010) and used the Adonis function in the vegan package (Oksanen et al. 2012) and NMDS in the MASS package (Venables & Ripley 2002) for multivariate analyses.

We used NMDS (based on Bray-Curtis distances) and Adonis analyses as a means of data reduction and to provide further investigation of the underlying structure of the fungal phylotypes in each sample. NMDS is a common ordination method for T-RFLP community data that preserves the rank ordering of original distances among observations, and uses these ranks to map the objects in two-dimensional ordination space (Ramette 2007). Stress values of 0.20 or less indicate a biologically relevant plot (Rees et al. 2004). Adonis tests the null hypothesis that similarity between objects within a group is the same as the similarity within the groups (akin to ANOVA), and produces an R^2 test statistic between -1 and 1; significance is assessed using a permutation test. NMDS and Adonis are free from the assumption of normality (Rees et al. 2004). The comparison of fungal diversity between DSV and natives-in-DSV (using bp pair length variation of individual TRFs) was done using Student's t-test.

Nomenclature

Goldenrod, *Solidago* spp.; wild ginger, *Asarum canadense* L.; Canada anemone, *Anenome canadensis* L.; dog-strangling vine, *Vincetoxicum rossicum* (Kleopow) Barbar.

RESULTS

The soil samples are sandy loams, and had an average pH of 7.6 +/- 0.37, organic carbon of 2.8 +/- 1.43%, and phosphorus levels of 7.7 +/- 5.06 mg/g. The average P levels in both

Table 2 – Summary of molecular methods used for fungal community fingerprint analysis.

Procedure	Procedure	Materials	Additional steps
DNA extraction	Genomic DNA extracted from 200 mg root tissue, concentration est. with NanoDrop™ ND-1000 V3.7.0 (Thermo Fisher Scientific Inc., Wilmington, USA); visualized by gel electrophoresis	FastDNA® SPIN Kit (Q-Biogene, Carlsbad California); gel electrophoresis run on a 0.8% (wt/vol) agarose gel containing ethidium bromide in 0.5× Tris/Borate/EDTA buffer and quantified using a DNA ladder (GeneRuler™ 1kb DNA Ladder Plus, Fermentas, Burlington, Ontario)	Gels run at 100 V for 0.5 hour intervals until distinct bands were resolved. The average yield of DNA was 6.5mg of DNA per root sample
Amplification and digestion of ITS fragments	Reactions incubated in a PTC-100™ thermal cycler (MJ Research Inc., Waltham, Massachusetts): DNA polymerase initialization at 95ºC for 5 minutes, followed by 34 cycles at 94ºC for 50 seconds, 51ºC for 1 minute, 72ºC for 1 minute, final extension step at 72ºC for 10 minutes.	Primers ITS1F/ITS4 (ITS1F 5′-CTT GGT CAT TTA GAG GAA GTA A-3′ forward and ITS4 5′-TCC TCC GCT TAT TGA TAT GC-3′ reverse; Sigma Gensosys, Oakville, Canada) (Manter & Vivanco2007) 20 µL amplification reactions consisted of 10µl of HotStar Taq Plus Master mix, 2x (Qiagen, Canada), 0.4µl of each primer at 50µM, 7.8µl of RNase-free water, 0.4 µl bovine serum albumin (albumin solution from bovine serum, 20 mg/mL in H_2O, Sigma-Aldrich Canada, Oakville, Ontario) and 1µl of DNA template. Enzymes: *Eco*RII and *Fsp*BI (Fermentas Canada Inc., Burlington, Ontario) (Alvarado & Manjón 2009)	Amplicons were digested following the manufacturer's instructions using restriction enzymes *Eco*RII and *Fsp*BI for 2 hours at 37ºC. Digests contained 15 µl of PCR product, 2U each *Eco*RII and *Fsp*BI, 2 µl Tango™ 1X buffer, and 2.6 µl sterile water. All PCR products were visualized on a 2.5% (wt/vol) agarose gel stained with ethidium bromide.
Amplification and digestion of SSU fragments	Reactions incubated in a PTC-100™ thermal cycler (MJ Research Inc., Waltham, Massachusetts): Denaturation was at 95ºC for 5 minutes, followed by 34 cycles at 94ºC for 50 sec., 55.5 ºC for 50 sec., 72ºC for 60 sec., followed by a final extension step at 72ºC for 10 minutes.	Primers AML1/AML2 (AML1 5′-ATC AAC TTT CGA TGG TAG GAT AGA-3′ forward and AML2 5′-GAA CCC AAA CAC TTT GGT TTC C-3′ reverse; Sigma Gensosys, Oakville, Canada) (Lee et al. 2008) 20 µL amplification reactions as above. Enzymes: *Alu*I and *Hin*fI (Fermentas Canada Inc., Burlington, Ontario) (Querejeta et al. 2009).	Amplicons were digested following the manufacturer's instructions using restriction enzymes *Alu*I and *Hin*fI for 2 hours at 37ºC. Digests contained 15 µl of PCR product, 2U each *Alu*I and *Hin*fI, 2 µl Tango™ 1X buffer, and 2.6 µl sterile water. All PCR products were visualized on a 2.5% (wt/vol) agarose gel stained with ethidium bromide.
Molecular cloning of ITS and SSU fragments	Sequenced 28 woodland clones and 28 field clones in total from both study sites (4 DSV, and 4 natives): PCR products were cloned into vectors; plasmids extracted from transformants with QIAprep spin columns	pCR®4-TOPO® vectors with TOPO TA Cloning® Kit with (Invitrogen, Carlsbad, CA, USA) Use of ITS1F-ITS4 and AML1-AML2 primer pairs used, as above. Digestion with *Eco*RI was carried out to verify the presence of inserted amplicons. We used a total of 4 ITS clones and 3 SSU clones from each of 4 different DSV, 1 wild ginger, 2 goldenrod, and 1 anemone for sequencing = 28 woodland and 28 field clones (4 DSV, and 4 natives).	Plasmids containing inserts of interest were sequenced using M13 (-27) reverse primer by The Center for Applied Genomics (SickKids Hospital, Canada). Clone sequences analyzed using BLAST program from NCBI (http://blast.ncbi.nlm.nih.gov/).

Table 2 (continued) – Summary of molecular methods used for fungal community fingerprint analysis.

Procedure	Procedure	Materials	Additional steps
T-RFLP	Primers labeled with fluorescent dye. 10 µl of each restricted sample was analyzed on a 3730 DNA sequencer (Applied Biosystems Inc., Fredmont, California) for sizes and intensities (peak height) of the 5'-terminal fragment at the Laboratory Services Division at the University of Guelph (Guelph, Ontario).	Primers were labeled with phosphoramidite 6-FAM (forward label, labeled at the 5' end, Invitrogen Canada), and PCR products were visualized using a 1.0% (wt/vol) agarose gel, as above.	T-RFLP data were displayed graphically as individual fluorescence peaks that represent individual operational taxonomic units (OTUs), a proxy for species variants, or phylotypes. Fragment sizes ranged from 50-900 base pairs (bp) and included a range of fluorescence intensities.

Table 3 – Results of BLAST search on NCBI.
Accession numbers based on best BLAST matches. Dominant sequences from root fungi that were amplified by internal transcribed spacer (ITS) primer pairs and small-subunit (SSU) primer pairs. Putative ectomycorrhizal fungi (Rinaldi et al. 2008) denoted as 'ecto'; others are endophytes. † can have plant pathogens in group.

		Results of BLAST search on NCBI		
Plant, primers, sample code	Fungal Phylum	Accession number	Fungal Genus/class	Sequence similarity (%)
DSV-ITS	Basidiomycota			
C3		FJ554417	*Agaricomycetes* (class)	97
CPH9		DQ102399	*Ceratobasidium*	99
CPH3		JF519835	*Rhizoctonia* †	97
C2		AF516524	*Polyporus* † (ecto)	91
CPC11		FJ554155	uncultured	98
	Ascomycota			
CPF5		GQ219835	*Cadophora*	100
CPF6		GQ280341	*Fusarium* †	100
CPC8		FJ378720	*Leptodontidium* † (ecto)	97
CPF3		AF455451	*Nectria* †	96
DSV33		JN123359	*Phialocephala* (ecto)	94
CB16		DQ493934	*Plectosphaerella*	92
C4		AF384679	*Rhynchosporium* †	98
CPE17		GU32747	*Tetracladium*	97
	Glomeromycota		*Glomus*:	
CPC1		JF439109	*G. intraradices*	100
CPH1		JF820562	*G. irregulare*	97
CPE18		AJ972462	*G. diaphanum*	100
DSV-SSU	Glomeromycota		*Glomus*:	
A322		Y17635	*G. caledonium*	98
A3221		NG_017178	*G. mosseae*	97
A23		HM153423	*G. iranicum*	98
A22		HM153420	*G. iranicum*	98
A2		Y17640	*G. fasciculatum*	99
CPA6		FJ009599	*G. intraradices*	99
CPA7		FJ009602	*G. intraradices*	99
A1		EU232660	*G. intraradices*	99
CPA4		FJ009617	*G. irregulare*	99
CPA5		FJ009618	*G. irregulare*	99

Table 3 (continued) – Results of BLAST search on NCBI.

		Results of BLAST search on NCBI		
Plant, primers, sample code	Fungal Phylum	Accession number	Fungal Genus/class	Sequence similarity (%)
DSV-SSU	Glomeromycota		*Glomus*	
A3		AY903711	uncultured	98
CPA8		AY903740	uncultured	98
Goldenrod-ITS	Ascomycota			
I642, I321		EU883430	*Tetracladium*	97
I321, I643		EU883429	*Tetracladium*	99
I322, I643, I320		FJ803966	*Tetracladium*	99
I644		GU327473	*Tetracladium*	97
Goldenrod-SSU	Glomeromycota		*Glomus*	
32A4, 32A5		HM153423	*G. iranicum*	98
32A2, 32A6		EF393590	uncultured	99
32A3		AJ276082	*Paraglomus occultum*	95
32A1		AJ301862	*P. brasilianum*	95
Anemone-ITS	Ascomycota			
I575, I572, I571		EU883430	*Tetracladium*	97
I573	Glomeromycota	FJ769323	uncultured *Glomus*	99
Anemone-SSU	Glomeromycota		*Glomus*	
I574		HM153423	*G. iranicum*	98
I577, I578		EF393590	uncultured	99
Wild ginger-ITS	Ascomycota			
I393, I392		GU055705	*Tetracladium*	99
I391, I3918		EU883432	*Tetracladium*	99
Wild ginger-SSU	Glomeromycota			
39A2		HM153424	*Glomus iranicum*	98
39A4		HM153423	*G. iranicum*	98
39A1		FJ831601	uncultured	97

woodland sites were higher than the field sites (Zoo woods = 10.8 mg/g, UTSC woods = 9.8 mg/g, Zoo field = 4 mg/g, and UTSC field = 3 mg/g).

ITS amplicons of approximately 600-1000 bp and AML amplicons of 500-800 bp in length were successfully obtained from root tip DNA. PCR amplifications using the AML1-AML2 primer pair were successful on all plant samples. All ITS sequences were fungal, and all SSU sequences were Glomeromycota.

We obtained sequences for total fungal (ITS) and AMF (SSU) amplicons from DSV, goldenrod, wild ginger, and Canada anemone. The best sequence matches obtained from BLAST analysis indicated that DSV roots harboured species from three different fungal phyla (Ascomycota, Basidiomycota, and Glomeromycota), while natives-in-dsv had representation from only Ascomycota and Glomeromycota (table 3). All fungal sequences from high BLAST matches (> 90% query coverage) found in one or more natives-in-DSV samples were also represented in DSV except for *Paraglomus* spp., though the converse was not true. In DSV, 13 different genera of fungi were observed using both primer sets, while in natives-in-dsv, only 3 different genera were observed. While some *Glomus* sequences in table 3 are listed to the species level where strong BLAST matches supported this information, species level matches could include variability.

The AML1-AML2 primer pair showed high AMF specificity, amplifying only species belonging to the *Glomus* and *Paraglomus* genera (AMF) from all plant samples.

There was no representation of the described generalist *Glomus* spp. (Biermann & Linderman 1983, Giovannetti et al. 1999, Helgason et al. 2002) in the natives-in-DSV, but these species (i.e. *Glomus intraradices*, *G. caledonium*, *G. fasciculatum* and *G. mosseae*) were found to be associating with DSV in both the UTSC and Zoo sites.

TRF data from the DSV and natives-in-dsv samples showed significant differences between the number of different TRFs (determined by bp length) in DSV and natives-in-dsv ($p < 0.025$) using Student's t-test, with DSV yielding higher average numbers of individual TRFs.

NMDS and Adonis analysis results for both ITS and SSU normalized TRF data for the 24 samples (12 field and 12 woodland: 12 DSV, 6 goldenrod, 3 wild ginger, 3 ane-

Table 4 – **Summary of statistical analyses**.
NMDS and ADONIS analysis results for normalized TRF peak data for the groups 'plant', and 'type', where 'plant' includes four groups: DSV, wild ginger-in-DSV, Canada aneone-in-DSV, and goldenrod-in-DSV, and 'type' includes DSV and natives-in-DSV (all natives-in-DSV are pooled). TRFs from both primers are included (ITS and SSU).

	NMDS stress	ADONIS	
		p-value	R^2
ITS plant	0.20	0.01	0.24
ITS type	0.20	0.01	0.11
SSU plant	0.13	0.01	0.29
SSU type	0.13	0.03	0.11

mone) are shown in table 4. A comparison of 'plants' (DSV, goldenrod-in-dsv, Canada-anemone-in-dsv, and wild ginger-in-dsv) resulted in higher R^2 values relative to 'types' (DSV and natives-in-dsv), though comparison of the two 'types' also produced significant differences in the fungal community fingerprints. Figures 1 and 2 show NMDS plots for ITS and SSU data respectively. The broad fungal representation in the different plants (ITS TRFs or phylotypes) shows a tendency to sort by plant, though no tight clusters are apparent for any given plant, suggesting a range of phylotypes associating with the different plant species. The AM fungal representation (SSU) in the different plants shows DSV forming a relatively tight cluster with most of the samples, suggesting that this plant is likely forming associations consistently with specific AM fungi. Goldenrod shows similarities to the DSV for half of the samples, and is quite divergent for the other half. Field and forest plants have not separated according to environment (DSV-woodland, wild ginger, and anemone versus DSV-field and goldenrod), nor have the samples separated by site (DSV-utsc, goldenrod-utsc, and anemone versus DSV-zoo, goldenrod-zoo, and wild ginger). The goldenrod cluster distant from the DSV includes two Zoo samples and one UTSC sample (upon detailed examination of the raw data). There is an observable tendency to separate by plant species, but only the DSV is forming a tight cluster, suggesting some consistency in the AM fungal associations.

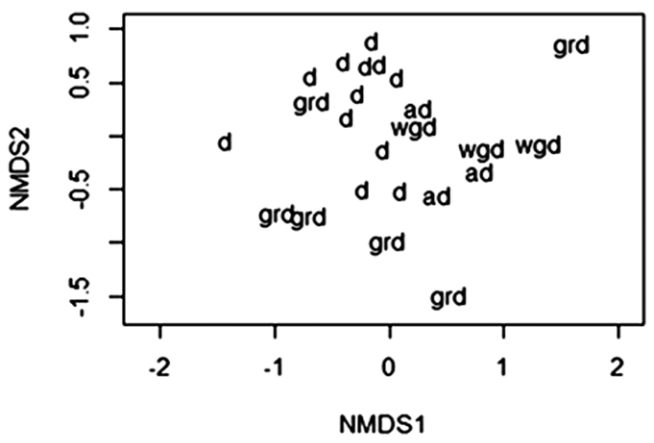

Figure 1 – NMDS plot derived from normalized ITS TRF data organized by plant group: d = DSV, ad = Canada anemone-in-dsv, grd = goldenrod-in-dsv, and wgd = wild ginger-in-dsv. The DSV tends to form a loose cluster while the natives-in-dsv collectively are more scattered.

DISCUSSION

DSV was found to associate with a broader range of fungal phylotypes relative to the natives-in-dsv considered in this study. However, findings reported for fungal colonizers associating with DSV and proximal native plants up until now have been based on fungal soil spore analysis or microscopy of arbuscles and vesicles found in DSV root samples. Our findings therefore represent preliminary data for characterizing fungal associations of DSV using molecular analyses.

We found that DSV formed associations with numerous AMF species based on the sequencing analysis, some of which are known to confer advantages such as increased phosphorus uptake, possibly leading to enhanced seedling recruitment. *Glomus intraradices* and *G. caledonium* were found to form associations with the DSV in both the UTSC and Zoo sites, but not with the native plants-in-dsv. We hypothesize that advantages could be conferred with regard to phospohorus uptake in DSV, as both species of *Glomus* have demonstrated efficient nutrient acquisition and reallocation of phosphorus (Graham & Eissenstat 1994, Giovannetti et al. 1999, Appoloni et al. 2008, Van Aarle et al. 2009). *Glomus fasciculatum* and *G. mosseae* were also found to be associating only with DSV in this study. While these species have not demonstrated high efficiencies with regard to phosphorus uptake, like *G. intraradices* and *G. caledonium*, they have been observed to form mycorrhizal associations directly from hyphal fragments, unlike other Glomeromycota (Biermann & Linderman 1983) that are limited to establishing associations from spore germination in the rhizosphere. This

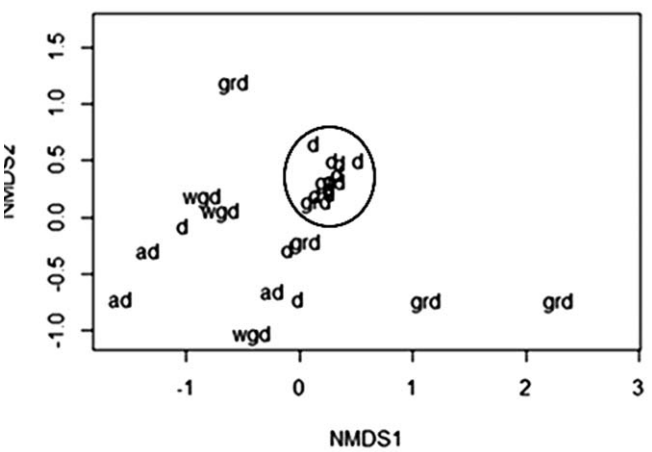

Figure 2 – NMDS plot derived from normalized SSU data organized by plant group: d = DSV, ad = Canada anemone-in-dsv, grd = goldenrod-in-dsv, and wgd = wild ginger-in-dsv. The DSV tends to form a cluster while the natives-in-DSV collectively are more scattered. The DSV tends to cluster (circled) more tightly for this data than the ITS data (figure 2), suggesting that AMF colonizing DSV are very consistent relative to those colonizing natives-in-dsv.

trait provides advantages to host plants attempting to establish in mechanically disturbed sites.

Given that the structure and dynamics of plant communities may be heavily dependent on mycorrhizal fungi (van der Heijden et al. 1998, Helgason et al. 2002, Klironomos 2003, Brundrett 2004), it is worth emphasizing that several highly advantageous AMF species (*Glomus intraradices*, *G. caledonium*, *G. fasciculatum* and *G. mosseae*) were absent in the native plants growing in DSV, but present in DSV.

It is apparent from the preliminary but novel findings of this study that DSV is most likely a fungal generalist, a characteristic that may provide advantages for establishment and spread in a novel environment. DSV is host to a greater variety of fungal colonizers relative to proximal native plants, and it forms associations with generalist *Glomus* species. Future research should include molecular techniques to compare natives growing in DSV with natives growing separately from DSV, and data sets should be expanded to produce robust density and diversity analyses. Investigation of the diversity and density of fungal colonizers during different stages of *Vincetoxicum rossicum* succession could also be investigated to see which species are supporting the establishment as well as the progression of this invasive species. As well, interaction between/among invading plant species, and the native community response to aggressive invaders needs to be elucidated, particularly with regard to native community structural shifts and associated fungal influences.

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