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Endogenous fungi isolated from three locoweed species from rangeland in western China

Hao Lu[1], Haiyun Quan[1], Qiwu Zhou[2], Zhenhui Ren[1], Ruixu Xue[1], Baoyu Zhao[1] and Rebecca Creamer[3]*

[1]College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, 712100, China.
[2]Department of Agriculture, Dianxi Science and Technology Normal University, Lincang, Yunnan, 67700, China.
[3]Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, Las Cruces, NM 88003, USA.

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Leguminous locoweeds cause toxicosis to grazing animals in western China and western USA. Swainsonine, a toxic alkaloid, is produced by the endophytic fungus *Alternaria* section *Undifilum* sp. living within the locoweed plants. Nothing is known of the other endogenous fungi associated with locoweed and it is unknown if the presence of *Alternaria* sect. *Undifilum* sp., a potential mutualist, in a locoweed influences the fungal microbiome associated with the plant. To help address these questions, endogenous fungi associated with three locoweed species (*Oxytropis glabra, Sphaerophysa salsula,* and *Astragalus variabilis*) collected from grasslands from western China were evaluated. Fungi were isolated from the tissues and identified by morphological features and sequencing of the internal transcribed spacer (ITS) regions. A total of 1209 fungal isolates were obtained from 1819 tissues for an isolation rate of 66.5%. *Alternaria* sect. *Undifilum oxytropis*, *Alternaria* spp. and *Fusarium* spp. were most commonly isolated. Plant host species, plant part, and environment influenced the endogenous fungal communities isolated from the locoweed plants. There were significant differences in the diversity of fungal species isolated from *O. glabra* from two sites, and no differences between the diversity of fungi isolated from *A. variabilis* from two sites. *Alternaria* sect. *Undifilum* was found most frequently associated with toxic locoweeds. Plants or plant parts that did not yield this endophyte had more plant pathogenic fungi associated with them. This is the first report of the diversity of fungi associated with these locoweeds and the first to suggest a beneficial role for *Undifilum*.

Key words: Fungal endophytes, *Undifilum* sp., locoweeds.

INTRODUCTION

Locoweeds are perennial, herbaceous, poisonous legume plants containing the fungal-produced toxic alkaloid, swainsonine, which causes the neurological disorder locoism in grazing animals (Dorling et al., 1980;

*Corresponding author. Email: creamer@nmsu.edu Tel: 15756463068.

James et al.,1981; Allred, 1991; Cook et al., 2009b). Locoweeds are primarily species of *Astragalus* and *Oxytropis* genera and have been reported from the Americas (western USA and South America) and Asia (Allred, 1991; Robles et al., 2000; Yu et al., 2010). Swainsonine is an indolizidine alkaloid that was first identified from *Swainsona*, a toxic legume that is found in Australia (Colegate et al., 1979). Swainsonine inhibits alpha-mannosidase and mannosidase II causing a lysosomal storage disease and neurologic impairment (Dorling et al., 1980). Locoism symptoms include a lack of muscular coordination and inability to eat or drink (James et al., 1970; Stegelmeier et al., 1999). Losses due to locoism depend on the severity of poisoning, but have been estimated at $300 million annually in the western United States due to poisoned cattle, sheep, and horses (Torell et al., 2000; Turner et al., 2012).

A large diversity of locoweeds is found in China. There are 46 species of locoweeds reported in China, including 23 species of *Oxytropis*, and 23 species of *Astragalus* (Lu et al., 2012b). Thirteen of the 46 species have been reported to cause severe damage to animal husbandry. Toxic locoweeds are primarily distributed over an area of 11 million ha in arid and semi-arid grasslands of Tibet, Inner Mongolia, Qinghai, Gansu, Xinjiang, Ningxia, Shanxi, and Sichuan provinces (Zhao et al., 2003; Lu et al., 2012b).

Toxic locoweeds from North America and China, and *Swainsona* from Australia, contain an endophyte, *Alternaria* section *Undifilum* spp. that produces the swainsonine (Braun et al., 2003; Wang et al., 2006; Pryor et al., 2009; Yu et al., 2010; Lu et al., 2012a; Creamer and Baucom, 2013; Grum et al., 2013; Woudenberg et al., 2013). Although not every plant in a population is toxic or contains the endophyte, all toxic plants that contain swainsonine have been shown to contain a swainsonine-producing fungal endophyte (Cook et al., 2011, 2012; Achata et al., 2012).

A fungal endophyte is defined as a fungus that lives within a plant. Fungal endophytes include saprophytic fungi, pathogenic fungi, and mutualistic fungi (Petrini, 1991). Fungal endophytes are widely found in roots, stems, and leaves of higher and lower plants. The diversity of fungal endophytes includes species diversity in colonization of host plants and diversity in the distribution of fungal endophytes within different tissues of host plants. This diversity could be due to differences in the host plants and natural factors, such as geographical location and climate, in addition to presence of other organisms (Tiina et al., 2013).

Different locoweed species have been associated with different *Alternaria* sect. *Undifilum* sp. *Oxytropis lambertii* and *O. sericea* harbor *A. U. oxytropis*, *Astragalus mollissimus* contains *A. U. cinerea*, and *Astragalus lentiginosus* contains *A. U. fulva* (Pryor et al., 2009; Baucom et al., 2012). *Alternaria* sect. *Undifilum* spp. can readily be cultured from dried plant tissue and most produce conidia in culture. The cultures are very slow growing, expanding less than an average of 0.2 mm/day (Pryor et al., 2009). They can be readily isolated from leaves, petioles, stems, flowers and seed (Braun et al., 2003). The fungi are vertically transmitted through seed, but found only in the seed coat and underlying layer (Oldrup et al., 2010).

Alternaria sect. *Undifilum* spp. can usually be cultured from locoweed samples with swainsonine concentrations greater than 0.01% swainsonine, and not from plants with lower swainsonine levels (Ralphs et al., 2008). While the endophyte has been found in all plant parts, it is highest in the crown, and has been difficult to isolate from roots (Cook et al., 2009a; Cook et al., 2011). Endophyte amounts change seasonally in above ground parts of plants until the plants reach maturity (Achata et al., 2012; Cook et al., 2012).

Gao et al. (2012) cultured *Alternaria* sect. *Undifilum oxytropis* from *Astragalus variabilis* and *Oxytropis glabra* plants collected from Inner Mongolia that contained swainsonine concentrations greater than 0.01%. Swainsonine was detected in only 2 of 50 samples of *Sphaerophysa salsula*, and at concentrations less than 0.01% and *A. U. oxytropis* was not cultured from any of the samples.

Only fungi have been demonstrated to produce swainsonine. In addition to *Undifilum* sp., fungi such as *Slafractonia* (*Rhizoctonia*) *leguminicola*, *Metarhizium anisopliae*, and an undescribed species of Chaetothyriales, have also been reported to produce swainsonine (Sim and Perry, 1997; Cook et al., 2014; Alhawatema et al., 2015).

While the role of *Undifilum* sp. in swainsonine production has been clearly established, the ecological role of *A.U. oxytropis* is not quite so clear. The fungus does not cause any obvious disease symptoms on the plant hosts and the plant host does not show a resistance response toward the fungus even at the cellular level (Reyna et al., 2012). The fungus does not deter invertebrate feeding, or aid in plant growth under heat stress or nitrogen deficiency (Thompson et al., 1995; Oldrup et al., 2010). Does *Alternaria* sect. *Undifilum* provide benefit to the locoweed plant, or does it function primarily as a commensal (Creamer and Baucom, 2013)? Perhaps it could help deter disease from pathogenic fungi. An ecological role for *Alternaria* sect. *Undifilum* could be suggested if it influenced the fungi associated with its host plant.

Highly stringent isolation procedures developed to culture the slow-growing *Alternaria* sect. *Undifilum* preclude the growth of other fungi (Braun et al., 2003). As a result, there is little known about the fungi, other than *Alternaria* sect. *Undifilum* sp., that is associated with locoweeds. Therefore, using lower stringency culture techniques, fungi were isolated from two toxic locoweeds,

O. glabra, *A. variabilis*, and a rarely-toxic locoweed, *S. salsula,* that were collected from semi-arid meadows in Ningxia Hui Autonomous Region and Inner Mongolia Autonomous Region in north central China. Fungi were identified based on morphological characteristics and phylogenetic analyses of rDNA ITS sequences and the diversity of the fungi were compared between plant species, locations, and plant parts. This study is the first to identify the diverse fungi associated with locoweeds and the first to suggest a beneficial role for *Alternaria* sect. *Undifilum*.

MATERIALS AND METHODS

Sample collection

Samples were collected from four areas, Yinchuan city in Ningxia Hui Autonomous Region and BayanHot, AoLun Prague, and Jilantai towns, in the Inner Mongolia Autonomous Region, which were selected to be representative of different environments (Table 1). *O. glabra* was collected from Yichuan city and Bayan Hot, *A. variabilis* was collected from AoLun Prague and Jilantai, and *S. salsula* was collected from BayanHot. One whole healthy-looking locoweed plant was collected from each of three sites within each of the four areas. At sampling, the type of vegetation, landscape, habitat, altitude, latitude, and climate were recorded and the location determined by GPS (Global Position System) (Table 1). The samples were dried with allochroic silica gel and maintained at-20°C.

Isolation of fungal endophytes

The dried plants were washed thoroughly with running tap water, then twice more with double distilled water to remove debris. The plants were cut into separate parts, i.e. leaves and petioles, roots, and seed pods were removed. Stems, leaves, and seeds of *O. glabra*, *A. variabilis* and *S. salsula* were assessed. Roots from *A. variabilis*, and petioles from *S. salsula* were also tested. Similar tissues from plants of the same species were combined, that is, *O. glabra* leaves harvested from the three plants collected from BayanHot were pooled for testing to maximize diversity from each plant species and location. The tissues were treated with a minimal cursory treatment of 30 s in 75% ethanol, followed by 1-3 min in 2%NaClO, and then 3-5 times in sterile water. Tissues were dried on sterile paper towels, and stems, petioles, and roots were cut into 5 mm sections, leaves were cut into 5 mm x 5 mm sections, and seeds were quartered. The tissues (6 to 7 sections) were placed in the same potato dextrose agar (PDA, Qingdao High-tech Industrial Park Haibo Biotechnology Co., Ltd, Qingdao, Shandong, China) plate at 28°C for 7 to 30 days. To determine if loosely associated microbes had been removed from the plant tissue surface, tissue prints were done with sterile water on control media. No bacterial contamination was observed, suggesting that cursory surface sterilization was effective. When hyphae grew from the cut tissues, the hyphae were removed to new media, and transferred 2 to 3 times. The hyphae were harvested and stored at 4°C.

Morphological identification of fungal endophytes

Hyphal tips of all fungi were plated onto PDA, and cultured in an inverted position at 28□C. Colonies were observed daily and size, color, texture, growth rate, edge, and shape were recorded. Subsequently, a third of a coverslip was inserted into the edge of the colonies in the medium at a 45° angle, and fungal mycelium, conidiophore structure, spore morphology, color, and ontology, were observed using light microscopy. Primary identification was carried out based on the taxonomic guides of Barnett and Hunter (1977), Shao et al. (1996), and Wei (1979). Ecological roles for fungi were determined using the USDA Fungal Databases from the Systematic Mycology and Microbiology Laboratory (http://nt.ars-grin.gov/fungaldatabases). Fungi were classified as pathogens, mutualists, or saprophytes based on the majority of reports for the fungus. Fungi not identified to species were not classified.

Identification of rDNA ITS sequences

Hyphae grown on PDA were collected and weighed. Genomic DNA was extracted from the fungi, with cetyltrimethylammonium bromide (CTAB) lysis buffer (Sun et al. 2006). Universal primers that amplify fungal rDNA, ITS1 (5'-TCCGTAGGTGAACCTGCGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), were used as upstream and downstream primers (White et al., 1990; Deng et al., 2006) to amplified the conserved ribosomal rDNA-ITS sequences using the genomic DNA as template. PCR reactions were carried out in a volume of 25 µL, containing 3 µL of DNA, 10 $\mu mol \cdot L^{-1}$ of ITS1 and ITS4 (1 µL each), 12 µL of 2X EasyTaq PCR SuperMix (TransBionovo, Beijing) and 8 µL of ddH_2O. Amplification was carried out in a Bio-RAD Gene Cycler™ PCR with an initial denaturation at 95°C for 30 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension of 72°C for 10 min. Amplification products (5 µL) were separated by electrophoresis on a 1.2% low-melting agarose gel, and the bands were visualized using a gel imaging system. Selected DNA fragments were purified from the agarose gel using a DNA extraction kit and sequenced (Sangon Biotech Co., Ltd., Shanghai). Species identification was based on sequence analysis of approximately 500 bp from the nuclear ribosomal internal transcribed spacer region (rDNA-ITS), (Arnold and Lutzoni 2007).

Phylogenetic relationship between different fungi

After sequencing, homology comparison was done using Blast and 5.8S rDNA-ITS sequence in Gen Bank, then the selected high similarity sequences were used to produce a phylogenetic tree by applying ClustalX 1.83 version and Neighbor-Joining method (MEGA5.0) with bootstrap support based on 1,000 replicates, and evolutionary distances computed using the Maximum Composite Likelihood method.

Data analysis

The isolated fungal communities were characterized for diversity by plant and by site. The α-diversity of the fungal community associated with locoweeds was analyzed using Shannon-Wiener diversity index ($H' = -\sum_{i=1}^{k} P_i \times \ln P_i$) and Simpson index ($D = -\sum (P_i)^2$) (*k*= the total species of fungi in certain plants, *Pi*= the proportion of a specific fungus out of all isolated fungi) (Gazis and Chaverri, 2010; Kharwar et al., 2011). The Evenness index (J=H/lnS) was used to estimate the evenness of species distribution

Table 1. Location and environment of locoweed collection sites.

Species/Locations	GPS (No.)	Altitude/m	Phenological condition	Land Forms and Soil
Oxytropis glabra (BayanHot, Inner Mongolia)	(1) 38°49' 805", 105°42' 021"	1593	Fruiting	*Ixeris denticulate, Herba taraxaci,* L. *Plantago depressa* Willd*., Phleum pratense, Agropyron cristatum* (Linn.) Gaertn, brown calcic soil
	(2) 38°49' 810", 105°41' 988"	1574	Flowering and early fruiting	*Medicago falcate* Linn, *Oxytropis aciphylla,* pine needle grass, brown calcic soil
	(3) 38°49' 803", 105°42' 015"	1593	Fruiting	*Ixeris denticulate, Agropyron cristatum* (Linn.) Gaertn. *Phleum pratense, L. Plantago depressa* Willd., brown calcic soil
Oxytropis glabra (Yinchuan, Ningxia province)	(1) 38°29' 870", 106°08' 288"	1108	Flowering and early fruiting	Lawn*, Agropyron cristatum* (Linn.) Gaertn*, Chenopodium album* Linn*, Ixeris denticulate, Thermopsis lanceolata* R. Br, gray soil
	(2) 38°29' 867", 106°08' 289"	1101	Flowering and early fruiting	Lawn, *Agropyron cristatum* (Linn.) Gaertn. *Ixeris denticulate. Thermopsis lanceolata* R. Br, *Medicago sativa* Linn*,* gray soil
	(3) 38°29' 979", 106°08' 268"	1116	Flowering and early fruiting	Lawn*, Agropyron cristatum* (Linn.) Gaertn, *Salix babylonica, Chenopodium album* Linn*,* Black spongy soils.
Astragalus variabilis	(1) 38°49' 812", 105°41' 988"	1584	Full bloom	*Ixeris denticulate, Agropyron cristatum* (Linn.) Gaertn*.,* pine needle grass, brown calcic soil
(Jilantai, Inner Mongolia)	(2) 38°49' 808", 105°42' 003"	1593	Full bloom	*Ixeris denticulate, Agropyron cristatum* (Linn.) Gaertn*.,* pine needle grass, brown calcic soil
	(3) 38°49' 813", 105°41' 983"	1599	Flowering and early fruiting	Desert*, Agropyron cristatum* (Linn.) Gaertn*., Phleum pratense*, grit, brown calcic soil
Astragalus variabilis	(1) 40°26' 874", 106°12' 878"	1036	Fruiting	Desert*, Agropyron cristatum* (Linn.) Gaertn*., Phleum pratense,* grit, brown calcic soil
(Aolun Prague, Inner Mongolia)	(2) 40°26' 930", 106°12' 763"	1038	Fruiting	Desert*, Agropyron cristatum* (Linn.) Gaertn*., Phleum pratense,* grit, brown calcic soil
	(3) 40°31' 593", 106°27' 083"	1066	Full bloom	Desert*, Agropyron cristatum* (Linn.) Gaertn. *,Peganum nigellastrum* Bunge*,* pebble beach, brown calcic soil
Sphaerophysa salsula (BayanHot, Inner Mongolia)	(1) 38°49' 803", 105°42' 015"	1593	Flowering and early fruiting	Lawn*, Agropyron cristatum* (Linn.) Gaertn.. *Ixeris denticulate. Thermopsis lanceolata* R. Br, *Medicago sativa* Linn*, Chenopodium album* Linn*.*
	(2) 38°49' 813", 105°42' 011"	1596	Flowering and early fruiting	*Agropyron cristatum* (Linn.) Gaertn., *Oxytropis glabra DC, Oxytropis coerulea,* pine needle grass*,* basic brown calcic soil
	(3) 38°49' 813", 105°41' 983"	1599	Flowering and early fruiting	*Ixeris denticulate, Herba taraxaci,* L. *Plantago depressa* Willd*., Phleum pratense, Agropyron cristatum* (Linn.) Gaertn., brown calcic soil

of fungi in the biotic community (*H*=the Shannon-Wiener diversity index, *S*=the total species) (Kharwar et al., 2011). The β-diversity of the fungi associated with locoweed species and sites was determined using two similarity indices, Sorenson Index (C_S = 2/(a+b)) and Jaccard Index (C_J =j/(a+b-j)), which were used to compare the similarity of fungal species composition between the two sites (*j*= the common species of fungi between the two sites, *a* and *b*= the species of fungi in the two sites, respectively) (Gazis

Table 2. Isolation of fungi from locoweeds through surface sterilization.

Locoweeds	Location	Parts	Number of tissues	Number of isolates	Isolation rate (%)
Sphaerophysa salsula	BayanHot, Inner Mongolia	Leaf	49	26	53.1
		Petiole	38	16	42.1
		Stem	71	23	32.4
		Seed	57	29	50.9
Oxytropis glabra	BayanHot, Inner Mongolia	Leaf	36	25	69.4
		Stem	52	35	67.3
		Seed	123	108	87.8
	Yinchuan, Ningxia	Leaf	126	85	67.5
		Stem	174	96	55.2
		Seed	342	236	69.0
Astragalus variabilis	Jilantai, Inner Mongolia	Leaf	46	26	56.5
		Stem	38	30	79.0
		Seed	60	46	76.7
		Root	253	183	72.3
	AoLun Prague, Inner Mongolia	Leaf	95	75	79.0
		Stem	85	75	88.2
		Seed	48	35	72.9
		Root	126	60	47.6

and Chaverri, 2010).

RESULTS

Isolation and identification of fungi associated with locoweeds

Fungi were isolated from all tissues using all combinations of bleach and ethanol treatments tested. Generally, fewer fungi were isolated from toxic locoweeds with increasing times of bleach and ethanol. Although only tested for *A. variabilis*, roots yielded the highest fungal isolation rates, 87%. Isolation rates from the nontoxic *S. salsula* were generally lower and more variable, ranging from a high of 41.6% from leaves to a low of 3.9% from seed.

One hundred sixty-eight fungal isolates were obtained from 211 tissues (leaves, stems, seeds) from *O. glabra* from BayanHot with an isolation rate of 79.6%, and 417 fungal isolates were obtained from 642 tissues from *O. glabra* from Yinchuan with an isolation rate of 65.0%. Fungal isolation rates from BayanHot and Yinchuan were 87.8 and 69.0% from seeds, 69.4 and 67.5% from leaves, and 67.3 and 55.2% from stems, respectively (Table 2). The fungi isolated from *O. glabra* differed between the two areas. Only five fungal species were isolated from plants from BayanHot, while 21 species were isolated from plants from Yinchuan.

Two hundred eighty five fungal isolates were obtained from 397 tissues (leaves, stems, seeds, roots) from the *A. variabilis* from Jilantai. The isolation rate was 71.8%. Two hundred forty five fungal isolates were obtained from 354 tissues of *A. variabilis* from AoLun Prague. The isolation rate was 69.2%, with higher isolation rates from stems and seeds than from leaves or roots from both locations (Table 2). The number of fungi isolated from *A. variabilis* was somewhat similar between

the two areas. Fifteen fungi were isolated from plants from Jilantai and 19 fungi were isolated from plants from AoLun Prague.

Ninety-four fungal isolates were obtained from 215 tissues of *S. salsula* (leaves, petioles, stems, and seeds) from BayanHot. The isolation rate was 43.7%, from leaves, 53.1% from seeds and 50.9% from stems (Table 2). The 94 cultures of fungi that were isolated included 19 species, distributed in 4 classes, 5 orders, 8 families, (2 families undetermined) and 10 genera on the basis of morphological characteristics and rDNA-ITS sequence analyses (Figure 1).

Overall there were 10 orders of fungi isolated, with the largest number of fungi within the Pleosporales and Hypocreales (Table 3). There were 9 species of *Alternaria*, 7 species of *Fusarium*, and 4 species of *Aspergillus* isolated. *Alternaria porri* was the only fungus isolated from all locations, while *Alternaria* sect. *U. oxytropis*, *Alternaria alternata*, and *Fusarium tricinctum* were each isolated from 4 locations.

Community composition and diversity of isolated fungi

The distribution of fungi isolated from *O. glabra* differed between BayanHot town and Yinchuan city. Only 5 fungal species were isolated from *O. glabra* from BayanHot, while 21 were isolated from the plants collected from Yinchuan. The endophyte, *A. U. oxytropis,* was distributed in all tissues of *O. glabra* from both locations except for the leaves in Yinchuan city. Relative isolation rates of *A. U. oxytropis* were all higher than 65.0% in *O. glabra* from BayanHot, with a relative isolation rates in seeds, leaves, and stems of 80.6, 80.0 and 68.6%, respectively. In Yinchuan, however, *A. U. oxytropis* was isolated only from the seeds and stems of *O. glabra*, and the relative isolation rates were 58.9 and 44.8%, respectively. Additionally, *Alternaria* spp., *Aspergillus* spp., and *Fusarium* spp. were isolated from most tissues of *O. glabra*, from both locations, and relative isolation rates were also higher (Table 4). The α-diversity (number of unrelated species of fungi isolated) for *O. glabra* (Figure 3) and *A. variabilis* (Figure 2) was similar and lower than for *S. salsula* (Figure 1).

Alternaria sect. *Undifilum* spp. were distributed in all the tissues of *A. variabilis* collected from both locations and was the dominant species in most tissues. The total relative isolation rates of *Alternaria* sect. *Undifilum* sp. from *A. variabilis* in Jilantai were 46.2, 60.0, 58.7 and 33.9% from leaves, stems, seeds, and roots, respectively, and in AoLun Prague was 62.7, 38.7, 68.6 and 20.0%, from leaves, stems, seeds, and roots, respectively. Hence, *A. U. oxytropis* was the dominant fungal species isolated from *A. variabilis* and *O. glabra. Alternaria* sect. *Undifilum gansuense*, instead of *A. U. oxytropis*, was isolated from roots of *A. variabilis* collected from AoLun Prague, and the dominant species was *Alternaria alternata*, which was similar to that isolated from leaves of *O. glabra* in Yinchuan (Table 4, Figure 2).

For the four tissues of *S. salsula*, *Alternaria* spp. were dominant in the stems, leaves, and seeds, while *Fusarium chlamydosporum* was dominant in petioles. The relative isolation rates of *Alternaria* spp. from stems, leaves, and seeds were 65.2, 46.2, and 44.8%, respectively, while the rate for *F. chlamydosporum* from petioles was 25.0% (Table 4).

Fungal diversity indices

According to the Shannon-Wiener index for the fungi isolated from the 3 locoweeds in the 4 ecological communities, the diversity of fungi varied among plants and locations (Table 5). The diversity of fungi from *O. glabra* in BayanHot was quite low (1.0) compared to that of *O. glabra* in Yinchuan (2.2). Enriched diversity of fungi from *A variabilis* was the same (2.0-2.1) in both sampling sites. The Shannon-Wiener index of fungi from the three tissues sampled from all plants (leaves, stems, seeds) for *S. salsula* was the highest at 2.5, followed by *A. variabilis* at 2.0-2.1, and *O. glabra* at 1.0-2.2.

The Simpson index (range 0 to 1) reflects the dominance in a community, so increases as diversity decreases. The Simpson index values for fungi isolated from locoweed tissues gave similar results as those calculated for the Shannon-Wiener index. The Simpson index of *S. salsula* was the lowest, and that for *O. glabra* was the highest, reflecting the highest and lowest diversity, respectively.

Evenness reflects the uniformity of distribution of different species in the community. The evenness of distribution of fungi was not homogenous in the sampling sites. The evenness index of fungi was highest for the community isolated from *A. variabilis* and lowest for the community from *S. salsula*. However, the evenness of distribution of fungal endophytes in *O. glabra* differed between the two sampling sites (Table 5).

The Jaccard and Sorenson indices provide an estimate of similarity or shared species between samples. The Jaccard indices were 0.4 or less comparing fungi isolated from *S. salsula* to those from *O. glabra* or *A. variabilis*, suggesting low similarity. However, the Sorenson indices were 0.6 for fungi from stems and leaves of *O. glabra* from Bayan Hot and leaves, stems, and leaves of *A. variabilis* from Jilantai compared to the fungi from *S. salsula* leaves, suggesting more similarity among those populations.

For fungi isolated from *O. glabra* collected from BayanHot, the Sorenson and Jaccard indices among stems and leaves, seeds and leaves, and seeds and

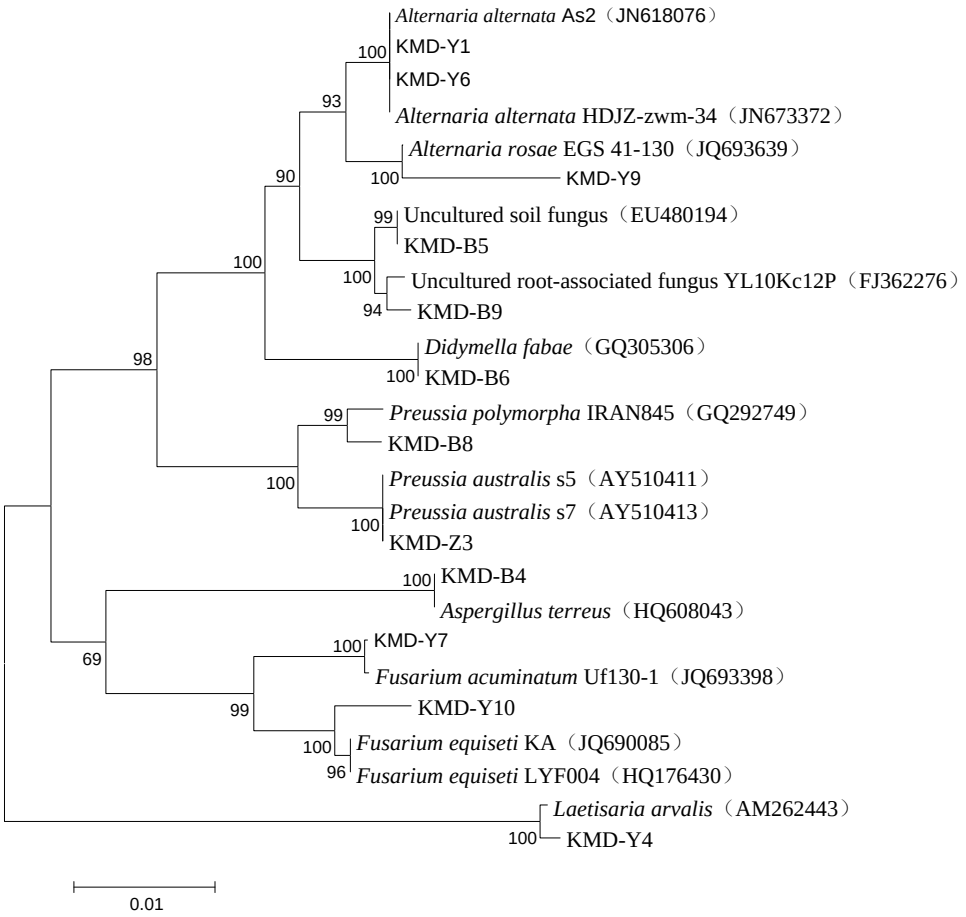

Figure 1. Phylogenetic tree constructed using Neighbor-joining (NJ) based on 5.8S rDNA-ITS sequences of fungi isolated from *Sphaerophysa salsula.*

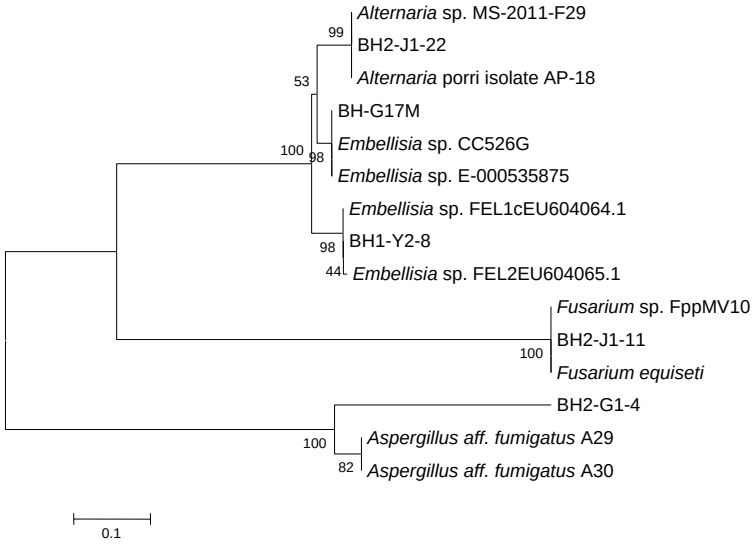

Figure 2. Phylogenetic tree constructed using Neighbor-joining (NJ) based on 5.8S rDNA-ITS sequences of fungi isolated from *Astragalus variabilis.*

Table 3. Species of fungi isolated from Locoweeds.

Species	Order	Number of isolations from aerial plant parts/roots					Ecological Role - Host
		O. glabra		*A. variabilis*		*S. salsula*	
		BayanHot	Yinchuan	Jilantai	AoLun Prague	BayanHot	
Acremonium sp.	Hypocreales		4				S
Alternaria alternata	Pleosporales		58	14	21/14	22	P- bean/many hosts[1]
A. brassicae	Pleosporales		1				P- many hosts
A. infectoria	Pleosporales			2			P/S – wheat
A. porri	Pleosporales	17	61	6/25	4	4	P-onion
A. rosae	Pleosporales			6		3	P - rose
A. solani	Pleosporales			2		3	P- tomato
A. tenuissima	Pleosporales		13		7	10	P/S-pigeonpea[2]
A. sect. *Undifilum gansuense*	Pleosporales				0/12		P–*Astragalus adsurgens*[3]
A. sect. *Undifilum oxytropis*	Pleosporales	131	182	57/62	100		M- locoweeds
Aspergillus candidus	Eurotiales		3	2			S
A. fumigatiaffinis	Eurotiales	6	3		4/6		M
A. lentulus	Eurotiales		3				S
A. terreus	Eurotiales			0/17	2/3	2	S
Bipolaris neergaardii	Pleosporales			4			S
Chaetomium globosum	Sordariales		5				S
Chloridium sp.	Sordariales					3	S
Cladosporium sp.	Capnodiales					5	P/S - mixed
Cochliobolus lunatus	Pleosporales				5		P - sugarcane
Colletotrichum cereale	Glomerellales			0/10			P-wheat
C. gloeosporiodes	Glomerellales		5				P - citrus
Coniolariella hispanica	Xylariales		6				S
Curvularia coicicola	Pleosporales	I		0/13			S
Didymella fabae	Pleosporales					2	P-beans[4]
Diplodia sp.	Botryosphaeriales					1	P-mixed
Fusarium acuminatum	Hypocreales					4	P-pine
F. brachygibbosum	Hypocreales				7		P-mixed
F. chlamydosporum	Hypocreales			0/23			P-lentil/many hosts[5]
F. equiseti	Hypocreales	11		0/10	5/8	19	P- many hosts[5]
F. oxysporum	Hypocreales			0/23			P-alfalfa/many hosts[5]
F. proliferatum	Hypocreales		15				P/S-locoweed[6]
F. tricinctum	Hypocreales	3	30	12	9/10		P-many hosts[5]
Gibberella moniliformis	Hypocreales				1		P-cereals
Humicola fusocoatra					1		S

Table 3. Cont'd.

Laetisaria arvalis	B - Corticiales				3	M
Microascus sp.	Microascales	3				S
Myrothecium verucaria	Hypocreales				2	P-mixed
Paecilomyces lilacinus	Eurotiales		2	3		S
Phaeosphaeria nodorum	Pleosporales	2				P-wheat
Phoma glomerata	Pleosporales	5				S
Preussia australis	Pleosporales				3	S
P. polymorpha	Plesoporales			3	1	S
Xylaria sp.	Xylariales	6				S

P=pathogen, S=saprophyte, M=mutualist; [1] Tu (1985), [2]Sharma et al. (2012), [3]Liu et al. (2016), [4]Hernandez-Bello et al. (2006), [5]Asan (2011) and [6]Zhou et al. (2012).

Table 4. Quantitative distribution of fungi isolated from locoweeds (ME=mutualist endophyte, P=pathogen, S=saprophyte).

Locoweed	Location/# species	Tissue type	Fungi isolated（number）	Ecological Roles/ # species	Dominant species
Oxytropis glabra	BayanHot (5 species)	Leaf	*Alternaria sect. Undifilum oxytropis* (20) M, *Alternaria porri* (3) P, *Aspergillus fumigatiaffinis* (2) M	88% ME 12% P 3 species	*A. U. oxytropis* (80.0%)
		Stem	*Alternaria sect. Undifilum oxytropis* (24), *Alternaria porri* (7) P, *Fusarium equiseti* (4) P	68% ME 31% P 3 species	*A. U. oxytropis* (68.6%)
		Seed	*Alternaria sect. Undifilum oxytropis* (87), *Alternaria porri* (7) P, *Fusarium equiseti* (7) P, *Aspergillus fumigatiaffinis* (4) M, *Fusarium tricinctum* (3) P	84% ME 15% P 5 species	*A. U. oxytropis* (80.6%)
	Yinchuan (19 species)	Leaf	*Alternaria porri* (54) P, *Alternaria alternata* (17) P, *Xylaria* sp. (6) S, *Alternaria tenuissima* (4) P, *Aspergillus candidus* (3) S, *Alternaria brassicae* (1) P	89% P 11% S 6 species	*Alternaria porri* (63.5%)
		Stem	*Alternaria sect. Undifilum oxytropis* (43), *Alternaria alternata* (15) P, *Fusarium tricinctum* (11) P, *Alternaria porri* (7) P, *Dothideomycetes* sp. (7), *Chaetomium globosporum* (5) S, *Aspergillus lentulus* 7(3) S, *Phoma glomerata* (5) S,	34% P 19% S 8 species	*A. U. oxytropis* (44.8%)
		Seed	*Alternaria sect. Undifilum oxytropis* (139), *Alternaria alternata* (26) P, *Fusarium tricinctum* (19) P, *Fusarium proliferatum* (15) P, *Alternaria tenuissima* (9) P, *Coniolariella hispanica* (6) S, *Colletotrichum gloeosporioides* (5), P, *Dothideomycetes* sp. (5), *Acremonium* sp (4) S, *Microascus* sp. (3) S, *Aspergillus fumigatiaffinis* (3) M, *Phaeosphaeria nodorum* (2) P	60% ME 32% P 6% S 12 species	*A. U. oxytropis* (58.9%)

Table 4. Cont'd.

Astragalus variabilis	Jilantai (16 species)	Leaf	*Alternaria sect. Undifilum oxytropis* (12), *Alternaria porri* (6) P, *Fusarium tricinctum*(5) P, *Alternaria solani* (2) P, *Paecilomyces lilacinus* (1) S	50% P 46% ME 4% S 5 species	*A. U. oxytropis* (46.2%)
		Stem	*Alternaria sect. Undifilum oxytropis* (18), *Alternaria rosae* (6) P, *Alternaria alternata* (3) P, *Alternaria infectoria* (2) PS, *Fusarium tricinctum* (1) P	60% ME 33% P 7% S 5 species	*A. U. oxytropis* (60.0%)
		Seed	*Alternaria sect. Undifilum oxytropis* (27), *Alternaria alternata* (11) P, *Fusarium tricinctum* (6) P, *Ascomycota* sp. (2)	59% ME 37% P 4 species	*A. U. oxytropis* (58.7%)
		Root	*Alternaria sect. Undifilum oxytropis* (62), *Alternaria porri* (25) P, *Fusarium oxysporum* (23) P, *Fusarium chlamydosporum* (23) P, *Aspergillus terreus* (17) S, *Curvularia coicicola* (13) S, *Fusarium equiseti* (10) P, *Colletotrichum cereale* (10) P	50% P 34% ME 16% S 8 species	*A. U. oxytropis* (33.9%)
	AoLun Prague (20 species)	Leaf	*Alternaria* sect. *Undifilum oxytropis* (47), *Fusarium tricinctum* (9) P, *Alternaria alternata*(7) P, *Paecilomyces lilacinus* (3) S, *Preussia polymorpha* (3) S, *Dothideomycetes* sp. (2), *Aspergillus candidus* (2), *Gibberella moniliformis* (1) P, *Humicola fuscoatra* (1 S)	63% ME 23% P 12% S 9 species	*A. U. oxytropis* (62.7%)
		Stem	*Alternaria* sect. *Undifilum oxytropis* (29), *Fusarium brachygibbosum* (7) P, *Alternaria tenuissima* (7) P, *Alternaria alternata* (6) P, *Cochliobolus lunatus* (5) P, *Fusarium equiseti* (5) P, *Bipolaris neergaardii* (4) S, *Alternaria porri* (4) P, *Dothideomycetes* sp. (3), *Ascomycota* sp. (2), *Aspergillus terreus* (2) S, *Aspergillus fumigatiaffinis* (1) M	45% P 40% ME 8% S 12 species	*A. U. oxytropis* (38.7%)
		Seed	*Alternaria* sect. *Undifilum oxytropis* (24), *Alternaria alternata* (8) P, *Aspergillus fumigatiaffinis* (3) M	77% ME 23% P 3 species	*A. U. oxytropis* (68.6%)
		Root	*Alternaria alternata* (14) P, *Alternaria* sect. *Undifilum gansuense* (12) P, *Fusarium tricinctum* (10) P, *Fusarium equiseti* (8) P, *Phoma chystallifera* (7) S, *Aspergillus fumigatiaffinis* (6) M, *Aspergillus terreus* (3) S	73% P 17% S 10% ME 7 species	*Alternaria alternata* (23.3%)
Sphaerophysa salsula	BayanHot (17 species)	Leaf	*Alternaria tenuissima* (10) P, *Fusarium equiseti* (7) P, *Fusarium acuminatum* (4) P, *Laetisaria arvalis* (3) M, *Alternaria rosae* (2) P	88% P 11% ME 5 species	*Alternaria tenuissima* (38.5%)
		Petiole	*Fusarium chlamydosporum* (4) P, *Aspergillus terreus* (2) S, *Didymella fabae* (2) P, Uncultured soil fungus (1), *Preussia polymorpha* (1) S, *Myrothecium verrucaria* (2) P, Uncultured root-associated fungus (2), *Alternaria solani* (1) P, *Alternaria rosae* (1) P	63% P 25% S 9 species	*Fusarium chlamydosporum* (25.0%)

Table 4. Cont. Cont.

	Stem	*Alternaria alternata* (9) P, *Alternaria porri* (4) P, *Alternaria solani* (2) P, *Cladosporium* sp. (2) PS, *Fusarium equiseti* (5) P, *Chloridium* Lk. (1) S	91% P 9 % S 6 species	*Alternaria* (39.1%)	*alternata*
	Seed	*Alternaria alternata* (13) P, *Fusarium equiseti* (7) P, *Preussia australis* (3) S, *Cladosporium* sp. (3) PS, *Chloridium* Lk. (2) S, *Diplodia* sp.(1) P	78% P 22% S 6 species	*Alternaria* (44.8%)	*alternata*

stems were not less than 0.6, suggesting that the similarity of fungi among leaves, stems, and seeds at this location was very high. For fungi isolated from *O. glabra* collected from Yinchuan, the Sorenson indices for stems and leaves and seed and leaves was less than 0.5, suggesting that there was lower similarity in fungal species.

Comparing the fungi isolated from tissues of *O. glabra* at two sampling sites and *A. variabilis* at two sampling sites gave generally low similarity, with a few exceptions. The Sorenson index was 0.6from stems of *A. variabilis* collected from AoLun Prague compared with *O. glabra*, and 0.6 for stems of *O. glabra* collected from Yinchuan compared with *A. variabilis* (Table 4).

Among the fungi isolated from leaves, stems, and seeds of *A. variabilis* collected in Jilantai, both Sorenson and Jaccard indices were higher than 0.6. In contrast, the Sorenson and Jaccard indices were lower for AoLun Prague. The Jaccard index was less than 0.5 among seeds, leaves and stems of *A. variabilis* collected in AoLun Prague. The Sorenson index was more than 0.5, indicating that similarity of fungi was higher in *A. variabilis* collected from AoLun Prague (Table 6).

Ecological roles of isolated fungi

The relative rate of isolation of mutualist endophytes significantly impacted recovery of pathogenic fungi (Table 4). There was an inverse relationship between isolation of *A. U. oxytropis* and plant pathogenic fungi. *O. glabra* from BayanHot yielded very high levels of *A. U. oxytropis* (68.6- 80.6% of total fungal isolations) and very low levels of plant pathogens (12-15% of total fungal isolations). In the absence of *A. U. oxytropis*, the pathogen levels were very high; *O. glabra* leaves from Yinchuan yielded 89% pathogens. Overall the relative pathogen and saprophyte levels from *O. glabra* from Yinchuan were higher and *A*. *U. oxytropis* levels lower than from *O. glabra* from BayanHot. The leaves from Yinchuan had 89% pathogens and 11% saprophytes, the stems 34% pathogens and 19% saprophytes, and the seeds 32% pathogens and 6% saprophytes. *A. variabilis* yielded moderate pathogen levels at both locations. Pathogen levels from leaves, stems, seeds, and roots from Jilantai were 50, 33, 37 and 50%, respectively, and at AoLun Prague were 23, 45, 23 and 73%, respectively. Saprophyte levels were higher in roots than other tissues from both locations, 16% from Jilantai and 17% from AoLun Prague. *S. salsula* yielded high levels of pathogens from all tissues, 88, 63, 87 and 72% from leaves, petioles, stems, and seeds, respectively. Higher levels of saprophytes (25%) were only isolated from petioles.

DISCUSSION

Plant host species, plant part, and environment influenced the endogenous fungal communities isolated from the locoweed plants. Diverse fungi were found associated with the locoweed plants. While one fungal species (*Alternaria porri*) was found associated with all three plants tested, generally different species of plants were colonized by different fungi. There were a few dominant species found, particularly *Alternaria* sect. *U. oxytropis* from *O. glabra* and *A. variabilis.* There were also abundant rare fungal species found in small numbers from a single plant species, for example 12 for *O. glabra* from Yinchuan, 8 from *S. salsula* from BayanHot and 7 from *A. variabilis* from AoLun Prague. Since there are very few reports of fungi associated with locoweeds, for many of the fungi isolated, this constitutes the first report of most of the association of those fungal species with these plant species. Only *A. U. oxytropis*, *A. U. gansuense*, and *Fusarium proliferatum* have been previously reported to be associated with locoweeds in China (Gao et al., 2012; Zhou et al., 2012; Liu et al., 2016). Other than these three fungal species, only 8 species found in this study have even been reported to be a pathogen of any legume (Tu, 1985; Hernandez-Bello et al., 2006; Asan, 2011; Sharma et al., 2012).

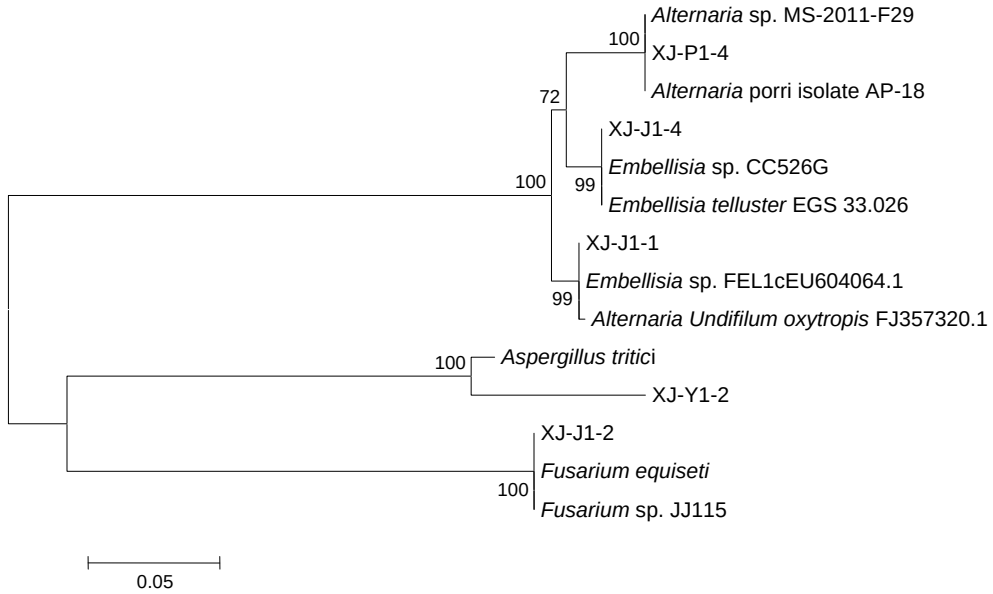

Figure 3. Phylogenetic tree constructed using Neighbor-joining (NJ) based on 5.8S rDNA-ITS sequences of fungi isolated from *Oxytropis glabra*

Table 5. Diversity index of fungi from locoweeds and sampling sites.

Locoweed	Location	Diversity index		Evenness
		Shannon-Wiener (H')	Simpson (D)	
Sphaerophysa salsula	BayanHot	2.5	0.1	0.5
Oxytropis glabra	BayanHot	1.0	0.5	0.6
	Yinchuan	2.2	0.2	0.7
Astragalus variabilis	Jilantai	2.0	0.2	0.8
	AoLun Prague	2.1	0.2	0.8

Table 6. Similarity index of fungi isolated from different locoweed tissues and species.

Locoweed tissues		*Sphaerophysa salsula*			*Oxytropis glabra*			*Astragalus variabilis*		
		Leaf	Stem	Seed	Leaf	Stem	Seed	Leaf	Stem	Seed
S. salsula	Leaf		0.4	0.3	0.2 0.2*	0.4 0.2*	0.4 0.2*	0.3 0.2*	0.3 0.2*	0.3 0.2*
	Stem	0.6		0.8	0.3 0.2*	0.3 0.3*	0.3 0.2*	0.3 0.2*	0.3 0.2*	0.3 0.2*
	Seed	0.4	0.8		0.3 0.1*	0.3 0.4*	0.3 0.3*	0.3 0.3*	0.3 0.3*	0.3 0.1*
O. glabra	Leaf	0.3 0.5*	0.5 0.3*	0.4 0.1*		0.6 0.2*	0.6 0.2*	0.2 0.2*	0.2 0.2*	0.2 0.5*
	Stem	0.6 0.4*	0.5 0.5*	0.4 0.4*	0.8 0.3*		0.6 0.5*	0.3 0.5*	0.3 0.5*	0.3 0.3*
	Seed	0.6 0.3*	0.5 0.3*	0.4 0.4*	0.8 0.3*	0.8 0.6*		0.3 0.3*	0.3 0.3*	0.4 0.3*

Table 6. Contd.

A. variabilis	Leaf	0.6 0.3*	0.5 0.3*	0.4 0.4*	0.3 0.3*	0.5 0.6*	0.4 0.4*		0.6 0.5*	0.6 0.3*
	Stem	0.6 0.3*	0.5 0.3*	0.4 0.4*	0.3 0.3*	0.5 0.6*	0.4 0.4*	0.8 0.6*		0.6 0.3*
	Seed	0.6 0.3*	0.5 0.3*	0.4 0.3*	0.3 0.5*	0.5 0.5*	0.6 0.5*	0.8 0.5*	0.8 0.5*	

"*" means the second sampling site (Yinchuan or AoLun Prague); the number represents Jaccard index (above diagonal) and Sorenson index (below diagonal).

Interestingly, most of the pathogens found have not been reported to infect legumes, including *A. porri*, a pathogen of onion, which was found associated with all three plant species. Most of the saprophytic fungi isolated are considered ubiquitous, found in soil or on decaying plant material of many different plant types and environments (Petrini, 1991). Our experimental objective was to screen for culturable strains of fungi associated with locoweeds. It is likely that other nonculturable fungi are associated with the locoweeds, but not identified in this study.

Environment had an influence on the endogenous fungal communities, particularly for the two toxic locoweeds, for which location and environment appeared to strongly influence the fungal community. The isolation rates and species of fungal endophytes differed within *O. glabra* collected from different locations, in which, five species of fungi were isolated from *O. glabra* in BayanHot town, while 21 species were isolated from Yinchuan city and 12 of those 21 species were unique to that site. BayanHot is in Inner Mongolia and Yinchuan is in Ningxia province, thus, providing different locations and environmental factors. The environmental factors that differed between the two sites include surrounding flora, habitat, soil type, rainfall, temperature, and altitude. Because of the limited number of sampling sites, the quantitative relationship between a particular environmental factors and fungal endophyte species could not be definitively determined. In contrast, the numbers of fungi isolated from *A. variabilis* from Jilantai (14, with 4 unique species) were similar to that isolated from AoLun Prague (17, with 7 unique species). Both collection sites for *A. variabilis* are located in Inner Mongolia. The distribution of fungi within a plant species can be influenced by location, season, age, climate, and geographical conditions. Arnold and Lutzoni (2007) analyzed the distribution of communities of fungal endophytes in forests between the Canadian arctic to the central part of Panama, and found that the colonization rate of fungal endophytes is significantly influenced by latitude. However, within a narrow scope of latitude, fewer fungal endophyte species were found in arid areas than in semi-deciduous forests (Hoffman and Arnold, 2008). Siles and Margesin (2016) found that the relative size fungal communities associated with alpine forests increased with altitude and the composition changed as well. The authors noted that changes in fungal richness and diversity and community structure were primarily influenced by pH and carbon/nitrogen in the soil, which was the result of environmental factors at the sites tested.

For α-diversity index, Shannon-Wiener and Simpson indices were used in this study. Two factors influence the Shannon-Wiener index: diversity (variety and amount) and uniformity of fungal localization among species. In this study, the Shannon-Wiener index of *S. salsula* collected from BayanHot of the Inner Mongolia Autonomous Region was the highest, while that of *O. glabra* was the lowest. These results could be because the presence of the fungal endophyte, *Alternaria* sect. *U. oxytropis* has an impact on other fungi infecting the same plant tissues. The Simpson index is also an indicator of diversity, with a larger value indicating greater diversity, but is a dominance index giving more weight to common species, such that a few rare species do not affect the diversity value. The Simpson diversity index of fungal endophytes in *O. glabra* in the two locations was significantly different, but that of *A. variabilis* was not significantly different between locations. In contrast, the Shannon-Wiener index of fungal endophytes isolated from *A. variabilis* collected in AoLun Prague was higher than in Jilantai. Compared to forage plants in similar ecological environments in other countries, the diversity index we found was slightly low (Porras-Alfaro et al., 2008; Khidir et al., 2009). This may be due to the small number of samples.

β-diversity, as measured through the Jaccard and Sorenson indices, is the difference in the composition of species between different communities in different habitats, or the replacement rate of species along environmental gradients, which is also called between-habitat diversity. The main ecological factors controlling β-diversity are thought to be soil, topography, and interference of external factors. Fewer common species are found in different communities or different sites in the same environmental gradient and hence, β-diversity is

larger. The β-diversity index can indicate the degree to which species are isolated by habitat, which can be used to compare habitat diversity in different areas, and constitutes the overall diversity or the biological heterogeneity in certain locations together with the α-diversity index. For β-diversity index, Jaccard and Sorenson diversity indices were used to compare the degree of similarity of composition of species of fungal endophytes between two locations. In this study, the species and quantity of distribution of fungal endophytes differed between hosts and tissues. Compared to the results calculated with Jaccard and Sorenson indices, there were uniform patterns of distribution of fungal endophytes in different tissues from the same plant, and the same plant at different sample sites. Stems and seeds showed the highest index (0.8) in similarity of flora in *S. salsula* collected in BayanHot, followed by petioles and seeds, indicating a similarity in flora of fungal endophytes among stems, petioles, and seeds in *S. salsula*. The Sorenson and Jaccard indices between the tissues colonized by fungi, in *O. glabra* and *S. salsula* and between all the tissues colonized by fungi in *A. variabilis* and *S. salsula*, were low, suggesting that there are no similarities in the community of fungi between *O. glabra* or *A. variabilis* and *S. salsula*.

The fungal communities differed somewhat by plant part. Although roots were only sampled from *A. variabilis*, they yielded higher proportions of saprophytes than the aerial portions of the plants. Similar results were demonstrated for roots and aerial parts of wheat (Comby et al., 2016). David et al. (2016) demonstrated that the influence of location and host species on fungal endophyte community composition also depended on plant part, in that environment and host species were very important in endophyte community composition from leaves of beach grasses, but only the sand dune environment was important for determining the endophyte community from roots.

Alternaria sect. *U. oxytropis* was consistently isolated from the seeds of the toxic locoweeds and less consistently with the leaves and stems. This colonization is consistent with transmission of *A.U. oxytropis*, which is maternally transmitted through seed (Oldrup et al. 2010). Braun et al. (2003) found that slow growing *Alternaria* sect. *Undifilum* spp. were isolated from *A. mollissimus*, *O. lambertii*, and *Oxytropis sericea* locoweed populations collected from New Mexico. They found that the infection rates of fungal endophytes in stems, leaves, flowers, and seeds were 97.2, 72.9, 100 and 93.1%, respectively. Fast-growing fungi were also isolated from roots when sterilization was minimal, but they were not characterized. Cook et al. (2009a) quantified fungal endophytes in 10 plants each of *O. sericea*, *A. mollissimus*, and *A. lentiginosus*, using quantitative PCR, and showed that the amount of *Alternaria* sect. *Undifilum* sp. differed significantly among the plant species and between individual plants within a species.

The isolation rate of *Alternaria* sect. *U. oxytropis* from plant sections coincided with fewer plant pathogens isolated from the same sections. Fewer plant pathogens and a higher isolation rate of *A. U. oxytropis* were obtained from *O. glabra* from BayanHot than from Yinchuan. Comparing plant species collected from BayanHot, the non-toxic *S. salsula*, which did not yield *A. U. oxytropis*, had a much higher relative proportion of pathogens, than did *O. glabra*. The diversity of the fungi was also much lower from *O. glabra* than *S. salsula* from BayanHot. Together this suggests that *A. U. oxytropis* may influence the decrease in pathogen isolations. This could be due to competition for space or nutrients or it might be related to its swainsonine toxin production. Since swainsonine data was not obtained from these plants, this can't be verified. Infection with fungal endophytes has been shown to change microbial species composition and reduce pathogens (Clay, 1990; Arnold et al., 2003; Pan and May, 2009; Busby et al., 2016). Infection with *E. coenophiala*, a shoot-specific fungal endophyte of tall fescue influenced soil fungal communities, decreasing the relative abundance of Ascomycota and increasing the abundance of Glomeromycota (Rojas et al., 2016). Fungal endophyte infection in mature leaves of the tropical tree *Theobroma cacao* prevented infection by *Phytophthora* sp. pathogens (Arnold et al., 2003). The nature of the beneficial interaction needs to be assessed with other locoweeds and species of *Alternaria* sect. *Undifilum* to determine if this is a universal characteristic or specific only to this situation.

Conclusion

The diversity of fungi associated with locoweeds, the sampling sites, plant part and isolation rates were considered in this study. Location/environment, plant species, and presence/absence of the fungal endophyte *Alternaria* sect *Undifilum* all had strong impacts on the diversity of fungi associated with the locoweed plants. This initial catalog of fungi associated with locoweeds lays the foundation for future research on the microbial community associated with these toxic plants. The endogenous fungal community associated with locoweeds in other locations such as the western USA and associated with other plant species containing different *Alternaria* sect. *Undifilum* spp. should be examined to determine if the community profile is ubiquitous. This is the first report of a beneficial role for this fungal endophyte. The beneficial role for *Alternaria* sect. *Undifilum* sp. found here has implications for management strategies, since this endophyte has been found associated with a variety of plants in the western USA and Australia, as well as China. Current efforts to

manage the toxicosis associated with grazing plants infected with the fungus have ranged from using herbicide to kill the plant, to avoiding grazing on infected (toxic) plants, and using fungicide to cure locoweeds of the toxic endophyte. If the beneficial role for the endophyte is linked to its production of swainsonine, then efforts to develop endophyte-free plants should be pursued. If the beneficial role for the endophyte is not tied to swainsonine production, then development of a fungal mutant that did not produce swainsonine might possibly help its plant host survive pathogen infection.

Conflict of Interests

The authors have not declared any conflict of interests.

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