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Swainsonine and Endophyte Relationships in *Astragalus mollissimus* and *Astragalus lentiginosus*

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ABSTRACT: Locoweeds are defined as *Astragalus* and *Oxytropis* species that induce locoism due to the toxic alkaloid swainsonine. Swainsonine was detected in all parts of *Astragalus lentiginosus* and *Astragalus mollissimus*, with greater concentrations found in the aboveground parts. *Undifilum oxytropis*, a fungal endophyte responsible for the synthesis of swainsonine, was detected in all plant parts of *A. lentiginosus* and *A. mollissimus*. The amount of endophyte within a plant part does not always correspond to the concentration of swainsonine in the same part. Plants of *A. mollissimus* and *A. lentiginosus* can be divided into two chemotypes: those that contain swainsonine (>0.1%; chemotype 1) and those that contain little or no detectable swainsonine (<0.01%; chemotype 2). Chemotype 1 plants in both species had quantitatively higher amounts of endophyte compared to chemotype 2 plants. Swainsonine and endophyte amounts were not uniformly distributed within stalks of the same plant. For that reason, repeated sampling of stalks from the same plant during one growing season may provide misleading results. Sequence variants of *U. oxytropis* exist within populations of *A. mollissimus*, *A. lentiginosus*, and *Oxytropis sericea* and do not correlate with chemotype. These findings suggest several possible reasons for differential concentrations of swainsonine that will be tested in future work.

KEYWORDS: Locoweed, swainsonine, endophyte, *Undifilum*, qPCR, ITS sequence variants

INTRODUCTION

Locoweed poisoning is a widespread poisonous plant problem in the western United States, occurring in most plant communities.[1] Locoweeds are defined as *Astragalus* and *Oxytropis* species that induce locoism due to the toxic alkaloid swainsonine.[2] Locoweed intoxication is not limited to North America; *Astragalus* and *Oxytropis* species that contain swainsonine have poisoned animals in Asia and South America.[3] Additionally, other plants have been documented to contain swainsonine, including *Swainsona canescens* in Australia [4] and some *Ipomoea*, *Sida*, and *Turbina* species in South America and Africa.[5−7]

The fungal endophyte *Undifilum oxytropis* found in locoweeds, previously described as *Embellisia oxytropis*, has been shown to be responsible for the synthesis of swainsonine.[8−10] *U. oxytropis* grows endophytically without causing any apparent symptoms to the plant; however, the exact nature of the plant−endophyte interaction has yet to be determined.[8] *U. oxytropis* has not been observed growing outside the plant or sporulating on plant material. The endophyte has been detected via microscopy, culturing, and polymerase chain reaction (PCR); of these, PCR is the most sensitive.[11−13]

Locoweeds vary greatly in their swainsonine concentrations. For example, populations of *Oxytropis sericea*, *Oxytropis lambertii*, and some *Astragalus* species may contain plants in which swainsonine may not be detected or have concentrations ranging from 0.001 to 0.38%.[11,14,15] Additionally, it has been shown that *O. sericea* plants with swainsonine concentrations of >0.01% had greater amounts of endophyte than *O. sericea* plants with swainsonine concentrations of <0.005%, suggesting that endophyte and swainsonine concentrations were related and thus possibly explaining the highly variable concentration of swainsonine in locoweeds.[15]

This research is part of a systematic study to determine if observations from *O. sericea*[15] can be extrapolated to other locoweed species represented by the *Astragalus* genus. *Astragalus mollissimus* and *Astragalus lentiginosus* were chosen because of their historical significance in poisoning episodes and because they have different growth habits when compared to *O. sericea*.[16] *O. sericea* has an acaulescent growth habit: all aboveground parts (scapes, leaves, flowers, and pods) arise from the crown. *A. lentiginosus* has a caulescent growth habit: leaflets, flowers, and pods arise from the multibranched stem. In contrast, *A. mollissimus* is subcaulescent to shortly caulescent.[16] We speculate that these differences in growth habit may influence the distribution of swainsonine and the endophyte.

To determine if observations from *O. sericea*[15] could be extrapolated to *Astragalus*, the objectives of this study were (1) to determine if *A. mollissimus* and *A. lentiginosus* plants in which swainsonine is not detected, or detectable at concentrations at or near the detection threshold (0.001%), have lower amounts of endophyte than plants with quantitatively higher amounts of swainsonine; (2) to determine the concentration of swainsonine and amount of endophyte in the different plant parts of *A. mollissimus* and *A. lentiginosus* at a single developmental stage from a single population; (3) to determine if swainsonine and endophyte

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amounts vary between stalks within the same plant of *A. mollissimus* and *A. lentiginosus*; and (4) to determine if the internal transcribed sequence (ITS) of the endophyte, *U. oxytropis*, is different between swainsonine-containing plants and those with little or no detectable swainsonine from *A. mollissimus*, *A. lentiginosus*, or *O. sericea*.

MATERIALS AND METHODS

Plant Materials. Fifty plants of *A. mollissimus* Torrey var. *mollissimus* (wooly loco) were collected in Union County, New Mexico (N 36° 32.161′, W 103° 31.838′). Thirty plants of *A. lentiginosus* Douglas ex Hooker var. *wahweapensis* (freckled milk-vetch, Wahweap milk-vetch) were collected in Garfield County (Henry Mountains), Utah (N 38° 05.199′, W 110° 58.729′). The plants collected were in full flower/early pod and divided into their corresponding parts representing the root (underground axis of the plant, tap root), crown (persistent base of a herbaceous perennial, nonphotosynthetic woody tissue extending from ground level to tap root), leaves (odd-pinnate with leaflets being opposite), stem (aboveground axis of plant bearing leaves and floral parts), flowers, and pods (green dehiscent fruits). These plants were used to investigate objectives 1, 2, and 4. In addition, whole plants of *A. lentiginosus* var. *wahweapensis* that had been previously collected from the same site as above were used to investigate objective 4. *O. sericea* Nuttall plants (n = 8) collected previously for use in ref 15 representing chemotype 1 and 2 plants were used to investigate objective 4.

Ten plants of *A. mollissimus* var. *earlei* were collected from Jeff Davis County, Texas (N 30° 34.635′, W 103° 43.357′). Ten plants of *A. lentiginosus* var. *wahweapensis* were collected, five from the site described above and five from another site in Wayne County (Henry Mountains), Utah (N 38° 11.573′, W 110° 44.915′). These plants were divided into five equal stalks; each stalk contained leaves, stems, and flowers/pods and was used to investigate objective 3. Five aliquots from a control sample, representing a ground composite of a single *A. lentiginosus* plant, were extracted to determine the range, mean, and relative standard deviation associated with the method of extraction and analysis for swainsonine and endophyte content as part of objective 3.

Immediately after collection all plant material was bagged and frozen on dry ice. Upon return to the laboratory the plants were freeze-dried and ground through a 2 mm screen in a Wiley mill. Swainsonine and DNA were extracted from this plant material for further analyses.

DNA Extraction. DNA was extracted from freeze-dried, ground plant material (~20 mg) using the DNEasy Plant Mini Kit (Qiagen Inc., Valencia, CA). Extractions were performed according to the manufacturer's instructions. DNA was quantified with the ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE).

PCR Primers. The PCR primers used have successfully detected the presence of the fungal endophyte *U. oxytropis* in *Oxytropis* and *Astragalus* species.[11−13,15] The primers used were ITS 5 (5′ GGA AGT AAA AGT CGT AAC AAG G 3′) and OR1a (5′ GTC AAA AGT TGA AAA TGT GGC TTG G 3′), which amplify the internal transcribed spacer (ITS) region. Primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA).

Quantitation of the Fungal Endophyte. The fungal endophyte *U. oxytropis* was quantitated via qPCR, and the identity of the endophyte was verified by a restriction enzyme diagnostic digest as described previously.[12,15]

Swainsonine Analysis. Swainsonine detection and concentration was measured using a modification of a previously published procedure[17] in the following manner. A measured quantity (50 mg) of dried plant material was placed in a 2 mL screw-cap microcentrifuge tube. Samples were finely ground using a Retsch MM301 mixer mill set at a frequency of 16.0 for 5 min. The ground plant material was then extracted in 1.5 mL of 2% acetic acid for 16 h with agitation. After extraction, the samples were centrifuged and 0.05 mL of extract was added to 0.95 mL of 20 mM ammonium acetate in a 1 mL autosampler vial. Samples were then analyzed by LC-MS as previously described.[14] The detection limit of swainsonine was 0.001% of dry weight.

DNA Sequencing. PCR products were prepared for sequencing with the QIAquick PCR purification kit (Qiagen Inc.) according to the manufacturer's instructions. The ITS region was sequenced using both the forward and reverse primers used for amplification. DNA sequencing was performed at the Genomics Core Facility, Center for Integrative Biology, Utah State University, Logan, UT.

Data Analysis. Swainsonine and endophyte concentrations were examined in plant parts using a general linear model of SAS (PROC GLM) (SAS Institute, Cary, NC). Mean comparisons among plant parts after a significant ($P < 0.05$) F test were done with preplanned comparisons using the PDIFF procedure in SAS. Initial data analysis revealed two potential chemotypes of *Astragalus*: one containing swainsonine concentrations well above the detection threshold for swainsonine (>0.1%; chemotype 1) and another with little or no swainsonine (<0.01%; chemotype 2); therefore, plants from these two chemotypes were compared for endophyte and swainsonine concentration using the GLM procedure with least-squares means for unbalanced sample sizes. Means were compared using the PDIFF procedure in SAS. If no treatment × part interaction was observed or the comparison between chemotypes could not be made, the means were compared using Duncan's multiple-range test. The variation in swainsonine concentration among stalks was determined using descriptive statistics, including the relative standard deviation (RSD, %), which is the absolute value of the coefficient of variation.

RESULTS AND DISCUSSION

Swainsonine in Chemotypes and Plant Parts. Plants of both species were separated into two groups for analysis based upon externally determined criteria from previous observations:[11,15] chemotype 1 plants containing swainsonine concentrations of >0.10% in leaves and chemotype 2 plants containing swainsonine concentrations of <0.01% in leaves. Swainsonine was detected at concentrations of >0.10% in the leaves of 47 of 50 *A. mollissimus* plants (94%) and in 29 of 30 *A. lentiginosus* plants (97%).

Twelve plants from each species representing chemotype 1 plants (>0.10% swainsonine in the leaves) were selected using a stratified selection based upon the concentration of swainsonine in leaves. These plants were analyzed for swainsonine content in each plant part. Mean swainsonine concentrations ranged from 0.03% in roots to 0.26% in flowers of *A. mollissimus* (Figure 1A). Mean swainsonine concentrations ranged from 0.04% in roots to 0.24% in leaves of *A. lentiginosus* (Figure 1B). Swainsonine concentrations were similar between the two species. Swainsonine was found in all plant parts, and its concentrations were approximately 10 times greater in aboveground tissues than in underground tissues ($P < 0.001$) (Figure 1); similar observations were also made in *O. sericea*.[15] Swainsonine concentrations differed in their accumulation in the aboveground parts of each species. For example, in *A. mollissimus* the floral parts (flowers and pods) had the highest concentrations of swainsonine, whereas in *A. lentiginosus* the leaves had the highest concentrations of swainsonine as was observed in *O. sericea*.[15,18] We have no explanation as to why swainsonine accrues differently in different plant parts of *A. mollissimus* when compared to *A. lentiginosus* and *O. sericea*; these plants were all collected at a similar developmental stage, and apparent differences in endophyte amounts between parts or growth habits of the plant do not readily explain these observations.

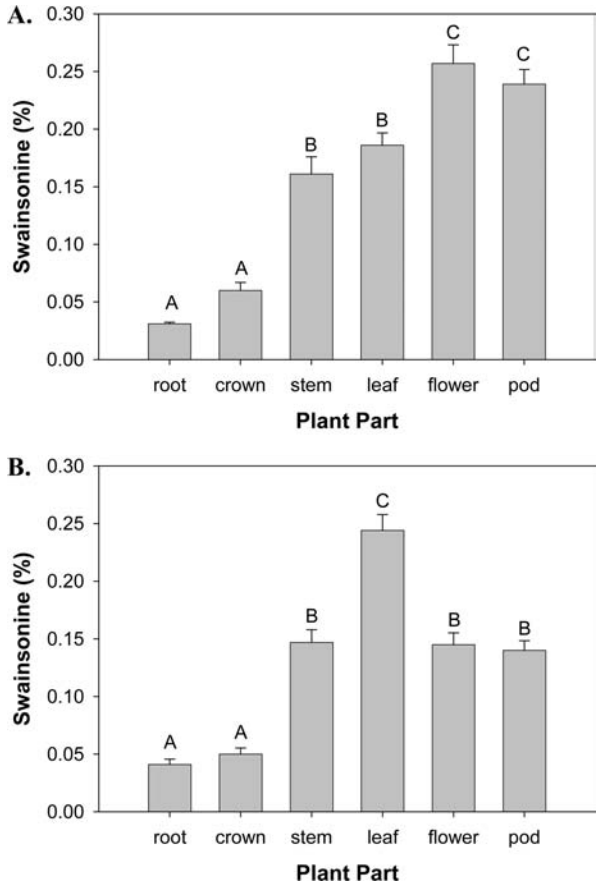

Figure 1. Swainsonine concentrations (%) in plant parts: (A) *Astragalus mollissimus*; (B) *Astragalus lentiginosus*. Mean swainsonine concentrations ± standard error of parts (root, crown, stem, leaf, flower, and pod) from 12 individual plants are shown. Different letters above each bar represent significance at $P < 0.05$.

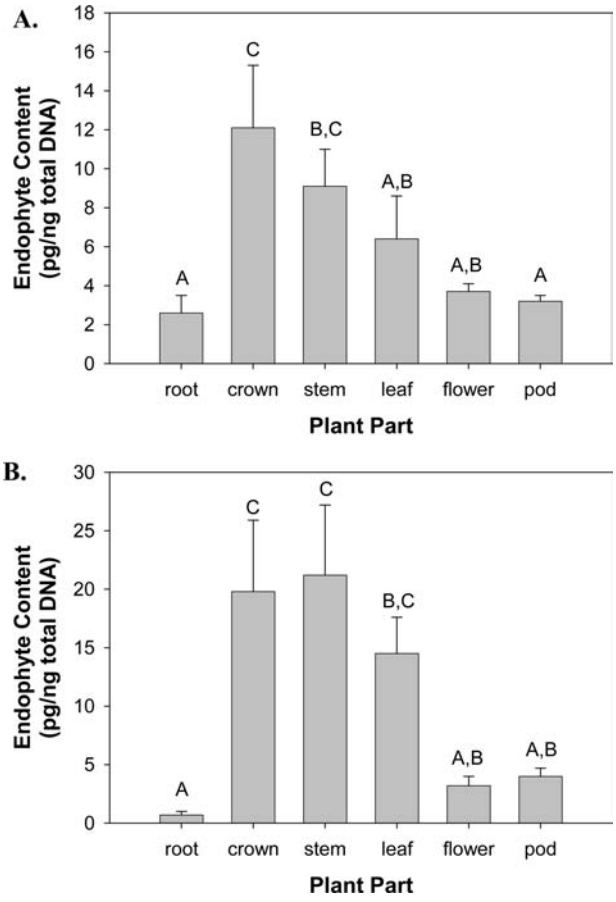

Figure 2. Endophyte amounts (pg/ng total DNA) in plant parts: (A) *Astragalus mollissimus*; (B) *Astragalus lentiginosus*. Mean endophyte amounts ± standard error of parts (root, crown, stem, leaf, flower, and pod) from 12 individual plants are shown. Different letters above each bar represent significance at $P < 0.05$.

Swainsonine concentrations of chemotype 2 plants averaged 0.002% in the flowers of three plants of *A. mollissimus*; no swainsonine was detected in any of the other plant parts (root, crown, stems, leaves, and pods). In *A. lentiginosus* a single chemotype 2 plant contained swainsonine concentrations of 0.001% in the crown, stems, and leaves and 0.002% in the root and pod. Swainsonine was not detected in the flowers of this plant.

The swainsonine concentrations of chemotype 1 and 2 plants were compared for all plant parts. There was a plant part × chemotype interaction ($P<0.001$) for swainsonine concentration in *A. mollissimus*. Chemotype 1 plants had greater swainsonine concentrations in all aboveground parts ($P < 0.001$) and the crown ($P<0.01$) compared to chemotype 2 plants. No statistical comparison was made between these groups for *A. lentiginosus* because only a single chemotype 2 plant was identified. However, swainsonine concentrations of chemotype 1 plants of *A. lentiginosus* were much greater (10× or greater) in all plant parts when compared to this single chemotype 2 plant.

Endophyte Content in Chemotypes and Plant Parts. A qPCR method recently developed[12] for the quantitation of *U. oxytropis* allowed determination of endophyte amounts in each of the tissues. Endophyte amounts were investigated in the plant parts of the 12 preselected chemotype 1 plants and the chemotype 2 plants of both species. The endophyte was detected in all plant parts of chemotype 1 and 2 plants of both *Astragalus* species as they were in *O. sericea*.[15] Mean endophyte amounts of *A. mollissimus* chemotype 1 plants ranged from 2.6 pg/ng in roots to 12.1 pg/ng in the crown (Figure 2A). Mean endophyte amounts of *A. lentiginosus* chemotype 1 plants ranged from 0.7 pg/ng in roots to 21.2 pg/ng in the stems (Figure 2B). Mean endophyte amounts were at or below the limit of quantitation (0.2 pg/ng) in all plant parts of *A. mollissimus* chemotype 2 plants. In *A. lentiginosus* the single chemotype 2 plant contained 0.6 pg/ng endophyte in the leaves, and in all other parts endophyte amounts were at or below the limit of quantitation.

The endophyte amounts of chemotype 1 and 2 plants were compared for all plant parts. There was a chemotype interaction ($P < 0.001$) for *A. mollissimus* endophyte amounts. As expected, chemotype 1 plants contained more endophyte than did chemotype 2 plants. No other significant interactions were observed; however, chemotype 1 plants had greater mean amounts of endophyte (10× or greater) in all plant parts. No statistical comparison was made between chemotypes of *A. lentiginosus* because there was only a single chemotype 2 plant identified. However, endophyte amounts in chemotype 1 plants of *A. lentiginosus* were much greater (10× or greater) in all plant parts but the root (3× greater) when compared to the single chemotype 2 plant. In conclusion, endophyte amounts were approximately 10-fold greater in most plant parts of chemotype 1 plants when compared to chemotype 2 plants. The endophyte *U. oxytropis* has

been shown to produce swainsonine; therefore, these quantitative differences in endophyte amount may result in the differences in swainsonine concentrations between these two groups of plants. We hypothesize that these observations made for the *Astragalus* populations studied here and the *O. sericea* population in our previous work[15] can be applied to other populations of *Astragalus* and *Oxytropis* locoweeds in the western United States and possibly to locoweeds worldwide.[11,14,19]

Endophyte amounts differed between plant parts in chemotype 1 plants and in some instances did not reflect the concentrations of swainsonine in the corresponding tissues (Figures 1 and 2). For instance, in *A. mollissimus*, the crown contained an amount of endophyte comparable to that of some aboveground tissues of the plant (= 12.1 pg/ng), but did not contain a comparable amount of swainsonine (= 0.06%), whereas the flowers contained relatively low amounts of endophyte (= 3.7 pg/ng) but had relatively high amounts of swainsonine (= 0.26%). Similar observations were made for *A. lentiginosus* and *O. sericea*.[15,18] These discrepancies may be explained by swainsonine acting as a potential mobile secondary compound[20] and, if produced in the crown, is translocated into the aboveground parts. It is also possible that the endophyte in the crown tissue is not producing swainsonine. However, irrespective of the low swainsonine concentration in the crown, the large amount of endophyte in the crown, as reported here and previously in *O. sericea*,[15] may serve as a reservoir for the endophyte for subsequent growth in the following year as has been shown in the endophyte-containing grasses.[21]

Endophyte distribution in plant parts of *Astragalus* was similar to those reported in *O. sericea*;[15] however, some differences were observed. For instance, *O. sericea* and the two *Astragalus* species investigated here all contained very low amounts of endophyte in the root and relatively high amounts of endophyte in the crown. However, they differed in the relative amounts of endophyte in some plant parts and the distribution between plant parts. For example, in *O. sericea* all aboveground parts contained endophyte amounts similar to or greater than the crown, whereas in *A. mollissimus* and *A. lentiginosus* endophyte amounts were greatest in the crown and decreased to significantly smaller amounts in the flowers and pods. We hypothesize that the differences in endophyte distribution between plant parts in *Oxytropis* and *Astragalus* are due to the different growth habits of the plant and which plant part gives rise to other plant parts. In grasses it has been shown that endophyte amounts follow a gradient with the greatest amounts in the basal regions (crown) and greatly reduced amounts at the apical ends (tips of leaf blade).[21,22] We speculate that a similar gradient may be found within *Astragalus* as shown by the distribution of the endophyte between plant parts.

Swainsonine and Endophyte Content in Stalks. Previous research has suggested that swainsonine and endophyte amounts are not uniform within different stalks of the same plant of *O. sericea*.[15] To determine if the distributions of swainsonine and endophyte were uniform within individual plants of *Astragalus* spp., 5 stalks per plant from 10 different plants of *A. mollissimus* and *A. lentiginosus* were analyzed for swainsonine and endophyte amounts. Additionally, five aliquots from a control sample, representing a composite of a single *A. lentiginosus* plant, were extracted to determine the range, mean, and relative standard deviation associated with the method of extraction and analysis for swainsonine and endophyte. The control sample had a mean swainsonine concentration of 0.19 ± 0.009% (RSD = 5%) (Table 1). The control sample had a mean endophyte amount of 5.2 ± 1.0 pg/ng (RSD = 19%).

Swainsonine was detected in all 10 plants of *A. mollissimus* and 9 of 10 plants of *A. lentiginosus* (Table 1). Swainsonine was detected in each stalk (n = 5 stalks/plant) of both species in plants containing swainsonine at >0.01% (Table 1). On the basis of the results of the control sample analysis, an RSD for swainsonine concentration of >5% for the stalks representing individual plants would suggest that swainsonine is not uniformly distributed throughout the plant. Greater than 85% of the plants analyzed of *A. mollissimus* (9 of 10) and *A. lentiginosus* (7 of 8) showed that the concentrations of swainsonine were not equally distributed (Table 1).

U. oxytropis was detected in all plants of both species. The endophyte was detected in every stalk from all 10 plants of both species (Table 1). Plants 9 and 10 of *A. lentiginosus* were plants with low concentrations of swainsonine or no detectable swainsonine, and they contained endophyte amounts near or below the limit of quantitation of 0.2 pg/ng total DNA. Plants 9 and 10 of *A. lentiginosus* represent two additional chemotype 2 plants. Mean endophyte content in aboveground parts of *A. mollissimus* and *A. lentiginosus* containing swainsonine was approximately 9.8 and 4.9 pg/ng total DNA, respectively, or a relative endophyte biomass of 0.98 and 0.49% with a range of 0.2−3.3% between the two species (Table 1). The relative endophyte biomass reported here for *A. mollissimus* and *A. lentiginosus* was similar to the relative endophyte biomass in *O. sericea* (0.5−4.0%) and an *Epichloe*/*Neotyphodium* symbiotic association with perennial ryegrass, estimated to be between 0.3 and 1.9%.[23] On the basis of the results of the control sample analyses, an RSD for endophyte amount of >19% for the stalks representing individual plants would suggest that the endophyte is not uniformly distributed throughout the plant. The RSD was >19% in all of the plants analyzed of both species. Thus, like swainsonine, the endophyte was not equally distributed in the analyzed plants (Table 1). Lastly, these results have important implications in regard to sampling techniques for future studies, indicating that sampling different stalks from the same plant over a growing season may lead to erroneous conclusions in measurements of endophyte and swainsonine content. We recommend that for any sampling regimen, the whole plant or all aboveground parts be harvested.[15]

ITS Sequence. Previous research has suggested that endophyte genetics may explain the low, or lack of, alkaloid production in Arizona fescue (*Festuca arizonica*) and other native grass species.[24] Consequently, we investigated if differences occur in the ITS sequences of the endophyte *U. oxytropis* in plants of *A. mollissimus*, *A. lentiginosus*, or *O. sericea*, where little or no swainsonine is detected (chemotype 2) compared to plants with quantitatively higher amounts of swainsonine (chemotype 1). The ITS region was amplified from 6−10 plants of *A. mollissimus* var. *mollissimus*, *A. lentiginosus* var. *wahweapensis*, and *O. sericea* representing chemotype 1 and 2 plants. The ITS region was distinct between each of the three species. Sequence comparisons between chemotype 1 and 2 plants were only made within the same species. The data are summarized in Table 2. Two sequence variants of *Undifilum* were present among the six *A. mollissimus* plants analyzed. One variant was identical to the GenBank sequence FJ486217, whereas the other was identical to GenBank sequence HM588119; the two sequences differed at a single position, 340, where T (FJ486217) is substituted with a C (HM588119). One plant contained only one sequence variant corresponding to GenBank sequence FJ486217, whereas the other five plants contained both sequence variants. Two sequence types of *Undifilum* were identified among the 10 *A. lentiginosus*

Table 1. Swainsonine and Endophyte Distribution within Stalks of *A. mollissimus* and *A. lentiginosus* Plants

	swainsonine (%)				endophyte (pg/ng total DNA)			
plant	mean	range	SD	RSD[a] (%)	mean	range	SD	RSD[a] (%)
				A. mollissimus				
1	0.182	0.164−0.214	0.020	11	7.9	4.7−13.9	3.9	49
2	0.168	0.092−0.263	0.063	38	33.4	8.3−67.0	25.8	77
3	0.168	0.161−0.180	0.008	5	7.5	2.0−12.3	3.9	52
4	0.123	0.078−0.169	0.033	27	5.3	3.4−7.5	2.0	38
5	0.122	0.093−0.152	0.025	20	4.5	3.0−7.5	1.9	42
6	0.120	0.089−0.174	0.033	28	11.7	8.1−17.9	3.7	32
7	0.118	0.087−0.147	0.027	23	14.1	9.5−21.7	4.7	33
8	0.113	0.059−0.157	0.036	32	2.6	0.5−4.7	1.9	73
9	0.112	0.101−0.125	0.010	9	6.9	3.5−16.7	3.2	46
10	0.094	0.056−0.115	0.023	24	3.7	1.7−5.6	1.9	51
mean (1−10)	0.132			23	9.8			50
control[b]	0.193	0.186−0.209	0.009	5	5.2	4.0−6.2	1.0	19
				A. lentiginosus				
1	0.229	0.207−0.258	0.021	9	6.1	2.3−8.0	2.2	36
2	0.220	0.188−0.235	0.018	8	3.7	2.6−6.1	1.4	38
3	0.209	0.205−0.212	0.002	1	2.2	1.1−3.4	0.9	41
4	0.189	0.170−0.210	0.019	10	4.7	2.4−6.6	1.7	36
5	0.176	0.157−0.189	0.013	7	10.3	5.7−14.7	4.1	40
6	0.170	0.150−0.192	0.018	11	2.6	2.0−3.7	0.9	35
7	0.167	0.128−0.195	0.025	15	6.4	4.1−11.7	3.2	50
8	0.127	0.089−0.166	0.035	28	3.0	1.4−4.4	1.3	43
9	0	0.0−0.001			0.2			
10	0				0.2			
mean (1−8)	0.186			11	4.9			40
control[b]	0.193	0.186−0.209	0.009	5	5.2	4.0−6.2	1.0	19

[a] RSD (%), relative standard deviation was calculated by dividing the standard deviation by the mean and multiplying by 100. [b] The control, representing a composite of a single plant, represents five aliquots that were extracted to determine the range, mean, standard deviation, and relative standard deviation associated with the method of extraction and analysis for swainsonine concentration and endophyte amount.

Table 2. Sequence Variants of *Undifilum* in *Astragalus* and *Oxytropis* Species

		nucleotide position[a] (bp)								
species	haplotye	184	190	198	340	428	502	chemotype 1	chemotype 2	Genbank
A. mollissimus	1	−	−	T	T	C	C		1	FJ486217
	2	−	−	T	C	C	C			HM588119
	1/2	−	−	T	T/C	C	C	3	2	
A. lentiginosus	1	A	−	G	C	C	C	2	3	FJ486218
	2	−	−	T	C	T	T	1	1	HQ690248
	1/2	A/−	−	G/T	C	C/T	C/T	2	1	
O. sericea	1	−	T	T	C	C	C	4	4	HM588134

[a] Nucleotide position 184 is characterized by a repeat of three to four A's and position 190 is characterized by a repeat of four to five T's.

plants analyzed. Type 1, representing five plants, was identical to GenBank sequence FJ486218. Type 2 (HQ690248), representing two plants, differed at four nucleotide positions compared to type 1 of *A. lentiginosus*. The three remaining *A. lentiginosus* plants contained both sequence types. One sequence variant of *Undifilum* was identified among the eight *O. sericea* plants analyzed, which was identical to the GenBank sequences HM588134 and HM588135, both from *O. sericea* and EU817504 from *Oxytropis glabra*. Interestingly, the ITS sequences of *U. oxytropis* from *O. sericea* and *O. glabra* are identical between the two plant

species, although one originates in North America and the other in Asia. In conclusion, *Undifilum* ITS sequence variants were present between the plant species, suggesting that there may be different species of *Undifilum* corresponding to each of three species of locoweeds surveyed. *Undifilum* ITS sequence variants are present within both *Astragalus* species but not *O. sericea*. Lastly, the data suggest that differences in the ITS sequence do not explain differences in swainsonine concentrations between the two chemotypes of the same species.

The perennial ryegrass/fescue endophyte relationship is agronomically and ecologically important in shaping ecosystems.[25,26] Likewise, the locoweed endophyte relationship is economically important in rangeland grazing systems and, as a legume, locoweeds may play an important role in shaping ecosystems. The locoweed endophyte shares some similarities with the perennial ryegrass and fescue endophyte relationship, but there are also some significant differences between the two systems. For example, in the perennial ryegrass and fescue endophyte relationship, plants are either endophyte positive or endophyte negative. Furthermore, grass plants that are endophyte positive may or may not contain alkaloids depending upon the system being studied. However, in the locoweed endophyte system, in the species *A. mollissimus*, *A. lentiginosus*, and *O. sericea*[15] surveyed thus far from field collections, all plants contain the endophyte, even though the endophyte can be cultured only from chemotype 1 plants containing higher swainsonine concentrations.[11] Additionally, these plants can be classified into two groups based upon swainsonine concentration and endophyte amounts as described in this work and previously.[15]

The difference in accumulation of swainsonine between the two chemotypes of *A. mollissimus* and *A. lentiginosus* suggests that a difference in relative toxicity exists between each group. A conservative threshold concentration for swainsonine toxicity of 0.001% was suggested previously.[27] Swainsonine dosed at or above 0.2 mg/kg of body weight/day for at least 21 days produced irreversible neurological disease.[28] Much less is known about lower doses over longer periods of time; however, it has been suggested that chronic low doses may lead to weight loss and biochemical lesions.[28] Swainsonine concentrations of 0.002%, the mean concentration found in flowers of chemotype 2 *A. mollissimus* plants, would produce a dose of 0.2 mg/kg of body weight/day only when locoweed consumption was 50% of a grazing animals' diet at an intake of 2% of their body weight. Therefore, it is unlikely that plants containing swainsonine concentrations of 0.002% would pose a significant risk of causing irreversible neurological disease; however, grazing animals may incur other reversible negative consequences (e.g., abortion, weight loss) due to long-term doses of <0.002%.

In conclusion, *A. mollissimus* and *A. lentiginosus* plants can be classified into two chemotypes that differ in their swainsonine accumulation, and these differences are associated with different endophyte amounts between the two groups. Currently we cannot explain how plants with low or nondetectable swainsonine concentrations may have arisen; however, some possibilities merit consideration. First, plants with low or nondetectable swainsonine concentrations may have arisen due to imperfect transmission of a critical amount of the endophyte to the seed or seedling[29] that is required for the plant to be colonized with amounts of endophyte similar to chemotype 1 plants. Second, low-swainsonine (chemotype 2) plants may suppress endophyte growth due to a plant genotype by endophyte interaction.[30] Third, the genotype of the endophyte may yet be different between plants that differ in their swainsonine accumulation, although on the basis of the current data we may suggest otherwise. These hypotheses, as well as others, merit further investigation to describe the locoweed endophyte relationship.

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