Seasonal Variation in Swainsonine and Endophyte Concentrations of *Undifilum oxytropis* in Different Plant Parts of *Oxytropis sericea*

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by

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MASTER OF SCIENCE

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Abstract

Locoweeds (*Astragalus* and *Oxytropis* spp.) are widely distributed in the rangelands of the western United States. The primary toxin present in locoweeds is identified as the alkaloid swainsonine. The consumption of locoweeds by domesticated cattle, sheep, and horses leads to significant economic losses due to locoism, a neurological disease caused by swainsonine. The fungal-endophyte *Undifilum oxytropis*, found in locoweed plant species of *Astragalus* and *Oxytropis* spp., is thought to be responsible for the synthesis of swainsonine. It has been shown that the swainsonine alkaloid and fungal endophyte are not uniformly distributed within the different parts of *Oxytropis sericea*. To understand better how swainsonine and endophyte are influenced by the phenology of the plant, both swainsonine and endophyte concentrations were measured in different plant parts (leaf, flower, and crown). These concentrations were determined at five developmental stages (vegetative, early flower, early pod, full pod, and pod shattered) of *O. sericea* at four different geographic locations. The results showed that the overall swainsonine concentration of *O. sericea* increased significantly from early flower stage to full pod stage, while endophyte concentration significantly increased from early flower stage to pod shattered stage. There were location× stage× part interactions in swainsonine concentration, but not in endophyte concentration ($P < 0.0001$ and $P = 0.1147$, respectively). Swainsonine and endophyte concentrations differed among locations ($P < 0.0001$ and $P < 0.0001$, respectively) but did not mirror each other. While endophyte and swainsonine concentrations were correlated (all $P < 0.05$ except for Harstel, CO), other factors may influence the products of swainsonine in these plants, such as environment, endophyte and plant genotypes.

Key words: *O. sericea; Swainsonine; Endophyte; locoweeds; poison.*

Acknowledgment

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Table of Contents

List of Tables

List of Figures

1. Introduction

1.1 Locoweeds ecology

Locoweeds (*Astragalus* and *Oxytropis* spp.) are common poisonous plants in the western US that frequently cause poisoning in livestock. *Astragalus* and *Oxytropis* spp. are legumes that are widely distributed in western North America (Cook et al., 2009c). The *Astragalus* genus contains 354 known species in North America and among these there are 20 *Astragalus* species that were noted for containing swainsonine: *Astragalus allochrous, A. asymmetricus, A. bisulcatus, A. didymo carpus, A. drummondii, A. emoryanus, A. humistratus, A. lentiginosus, A. lonchocarpus, A. missouriensis, A. mollissimus, A. nothoxys, A. oxyphysus, A. praelongus, A. pubentissimus, A. purshii, A. pycnostachyus, A. tephrodes, A. thurberi and A. wootonii*. The *Oxytropis* genus contains 22 species in North America and Canada (Welsh et al., 2007). There are four species of toxic *Oxytropis* that contain swainsonine: *O. besseyi, O. campestris, O. lambertii,* and *O. sericea* (Ralphs et al., 1993).

The locoweed species are perennial plants; they are abundant during wet years and die-off in drought years (Ralphs et al., 1998). For example, a large number of locoweeds died off in Colorado in 1998 and early 1999 because of the drought (Ralphs et al., 2002). *O. sericea* (Fig.1) is a stemless plant with aboveground parts (scape, leaf, flower, and pod) arising from the crown. Their seeds can remain viable for multiple years which allow them to germinate during ecological advantageous conditions (Cook et al., 2009c).

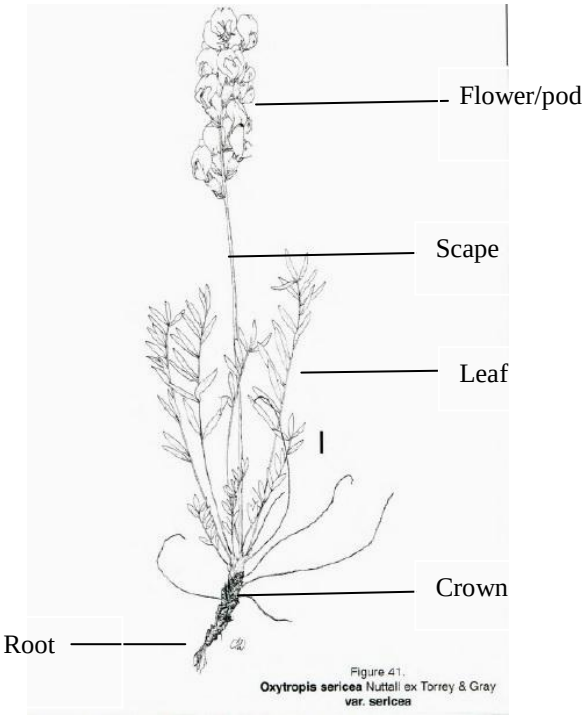

Fig. 1. *Oxytropis sericea*

1.2 Economic impact of locoweeds

The toxicity of locoweeds has a major economic impact on the livestock industry in the western United States. The overall economic losses due to the effects of locoweeds on livestock may reach several million dollars per year (James and Nielson, 1988). Economic losses from locoweeds include the reduction in livestock value, decreased production, and increased management costs (Torell et al., 2000). For example, a study from a Wyoming ranch showed that 250 calves were affected by locoweed poisoning every year and these calves lost on average of 22.5 kg, which lost about $15,000 per year (James and Nielson, 1988). According to a survey conducted from 1990 to 1996 on average beef prices, moderately locoweed-poisoned steers lost $75 per head, and severely poisoned steers lost $282 per head. Producers reported reduced sale

prices from 10% to 85% for affected animals with visible poisoning symptoms (Torell et al., 2000).

1.3 Swainsonine in locoweeds

The primary toxin present in locoweeds is swainsonine, a trihydroxy indolizidine alkaloid (Fig. 2). Colegate (1979) first isolated swainsonine from a member of the Australian legume genus *Swainsona*, *S. canesscens*. Swainsonine is an α-mannosidase inhibitor which inhibits mannosidase enzymes from cleaving sugar molecules in oligosaccharides (Broquist, 1986). The major pathological consequence of mannosidase inhibition is the accumulation of complex carbohydrates and glycoproteins in cells which causes the cell vacuolization and eventual cell death (Dorling et al., 1980). Swainsonine also inhibits α-mannosidase II which alters glycoprotein synthesis, affecting hormones, membrane receptors and enzymes, causing damage to cell-to-cell communication and cell movement (Pfister et al., 2001).

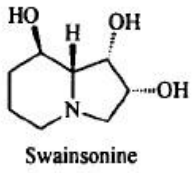

Fig. 2. The structure of swainsonine in locowe

Swainsonine and its N-oxide were first isolated from *A. lentiginosus* and identified as the causative agents of locoism in livestock. James et al. (1989) found swainsonine in other locoweed species (*i.e. O. lambertii* and *O. sericea*) which were identified as poisonous. The term "loco" is from the Spanish word, meaning crazy. Poisoned animals show symptoms of neurological disturbances, such as depression, slow staggering gait, and aggression. Locoweed toxicity also

causes reproductive problems, such as loss of libido, embryonic death, fetal death, and abortion (Colegate et al., 1979). It has been reported that about 5% of newborn calves born from locoweed fed cows have some degree of malformation and 50% of these calves usually die from toxicity (Ralphs et al., 1998).

A swainsonine dose of 0.2 mg/kg for at least 21 days can cause irreversible neurological disease (Stegelmeier et al., 1999) and it can be produced with the swainsonine concentration in locoweeds of 0.002% based on 50% locoweed consumption with intake at 2% body weight (Cook et al., 2011). The toxic threshold of swainsonine concentration for animals is recognized as approximately 0.001 % (Ralphs et al., 2008). However, prolonged locoweed exposure at low doses can result in production losses and functional damage for animals (Stegelmeier et al., 1999), thus, swainsonine concentrations < 0.002% may be potentially toxic (Cook et al., 2011).

1.4 Variation in Swainsonine concentration

Swainsonine concentrations differ between locoweed species and populations. *Astragalus* species have higher swainsonine concentrations than *Oxytropis* species (Ralphs et al., 2008); however, *Oxytropis* populations cause more persistent poisoning problems in livestock because their populations are more stable and have broader distributions (Cook et al., 2009c). Cook et al. (2009a) found two groups of *O. sericea*: one that contained swainsonine and the others contained no detectable swainsonine concentrations or near the detection threshold. Similar results were also found in *A. mollissimus* and *A. lentiginosus* (Cook et al., 2011). In addition, in the same species of *O. lambertii*, the plant samples found to contain higher swainsonine concentrations were at the most southern and western part of its distribution and identified as belonging to *var .bigelovii*, whereas, *var. articulata* and *var. lambertii* and some *var. bigeloyii* were identified with swainsonine concentrations <0.001% (Gardner et al., 2001, 2004).

Swainsonine concentration is not uniformly distributed in plant parts of locoweeds, including *O. sericea, A. mollissimus* and *A. lentiginosus*. It was reported that above ground tissues (leaf, scape, and flower/pod) had approximately ten times greater swainsonine concentrations than below ground tissues (crown and root; Cook et al., 2009a, 2011). Romero et al. (2002) separated seeds from toxic locoweeds into seed coats and embryos and found seed coats contained swainsonine and fungal endophyte, but not the embryos. Liquid chromatography mass spectrometry (LC-MS) has been used as a final toxicity determination technique for quantifying the concentration of swainsonine in locoweeds with the detection threshold 0.001% of plant dry weight (Gardner et al., 2001).

1.5 Fungal endophyte in locoweeds

Fungal endophytes are commonly diverse in plants. They are traditionally considered as plant mutualists and often do not cause any apparent symptoms to plants (Wilson, 1995). Fungal endophytes usually inhabit above-ground plant tissues (leaves, stems, bark, petioles, and reproductive structures) and root tissues. As mutualists, fungal endophytes can produce mycotoxins, such as alkaloids. The alkaloid production of fungal endophyte also depends on other factors, such as environment, nutrient levels, and host genotypes (Faeth, 2002a). For example, Hunt and Newman (2005) found that increased nitrogen reduced the concentration of alkaloids in agronomic perennial ryegrass (*Lolium perenne L.*). Faeth et al. (2002b) found that peramine concentrations in Arizona fescue (*Festuca*) plants infected by the same endophyte haplotype were significantly different between plant genotypes.

Fungal endophytes in locoweeds were first identified as a species of *Alternaria* (Braun et al., 1997) and then *Embellisia* (Braun et al., 2003). In recent years, Pryor et al. (2009) identified the fugal endophyte in locoweeds as *Undifilum oxytropis* based on morphological and molecular

analyses. The fungal endophyte grown in locoweeds did not cause any obvious symptoms to the locoweeds (Braun et al., 2003). Fungal endophytes isolated from leaves, stems, seeds, and flowers of the toxic locoweed species, *A. mollissinmus*, *O. lambertii*, and *O. sericea*, were proved to produce swainsonine (Braun et al., 2003). Furthermore, Ralphs et al. (2008) found that fungal endophytes can be cultured in most cases from locoweed plants that contained swainsonine, but not from plants where little or no swainsonine was detected. Gardner et al. (2004) found that low concentrations of swainsonine (<0.01%) were correlated with endophyte absence and high concentrations of swainsonine were correlated with endophyte presence by comparing 16 populations of *O. lambertii*.

Recently, the quantitative PCR (qPCR) method was developed to quantify the fungal endophyte concentrations in locoweeds. The endophyte, which cannot be isolated from locoweeds, can be detected by qPCR methods (Cook et al., 2009b). By using the qPCR method, Cook et al. (2009b) found that locoweeds with higher swainsonine concentrations had relatively higher endophyte concentrations, and plants containing only trace amounts of swainsonine had very low levels of the endophyte. Cook et al. (2009a) also found that endophyte concentrations were not uniformly distributed in *O. sericea* and did not always correspond to the swainsonine concentration in the same plant part. Similar results were also found in *A. mollissimus* and *A. lentiginosus* (Cook et al., 2011).

Few studies have been conducted to determine the seasonal effects on swainsonine and endophyte concentrations in *O. sericea* during the growing season. The objectives of this study were to 1) determine the variation in swainsonine and fungal endophyte concentrations of *O. sericea* over the growing season; 2) determine the swainsonine and endophyte concentrations in different plant parts of *O. sericea* from different growth stages; 3) describe the variation in

swainsonine and fungal endophyte concentrations between locations and over years. Previous studies showed that alkaloid concentration in native grasses infected by a fungal endophyte generally increased through the growing season (Leuchtmannj et al., 2000), thus, we hypothesized for objective 1 that swainsonine and endophyte concentrations in *O. sericea* would increase as plants grow through the growing season. In regards to objective 2, because above ground tissues had approximately 10 times greater swainsonine concentrations than below ground tissues in *O. sericea* (Cook et al., 2009a) at a single developmental stage; we assumed that swainsonine and endophyte concentrations would be higher in above ground tissues than crowns within all developmental stages. We hypothesized for objective 3 that swainsonine and endophyte concentrations in *O. sericea* would differ among locations and years for the reasons that environmental stress and genetics may influence swainsonine production and endophyte growth in locoweeds.

2. Materials and Methods

2.1 Plant materials

The locoweed samples of *O. sericea* were provided by the United States Department of Agriculture Agricultural Research Service Poisonous Plant Laboratory. Plant samples (n = 25/stage) were collected at two sites at Colorado in 2010 and two sites at Utah in 2009 and 2010. The collection sites and corresponding phenological development stages of *O. sericea* are listed in Table 1. Each plant was divided into three parts: leaf (leaflets), crown (the woody tissue underground until tap root) and flower (scapes with flowers/pods).

Another fifteen plants of *O. sericea* were collected at full pod stage, nine from Cuchara, CO and six from Harstel, CO. Leaves from these plant samples were separated into groups of old

and young leaves for measuring swainsonine concentrations. In addition, another twelve plant samples of *O. sericea* were collected at full pod stage from Cuchara, CO. Flower parts were separated into seeds and scapes for the swainsonine analysis as a part of objective 1 and 2. For objective 3, we compared the swainsonine and endophyte concentrations at Rafter River Mountain between 2009 and 2010. In 2009, plant samples were collected from vegetative stage, early pod stage, and full pod stage; thus, we only compared swainsonine and endophyte concentrations at these three corresponding stages between 2009 and 2010 at Rafter River Mountain, UT. Each plant part was bagged and frozen on dry ice. Upon returning to the laboratory, each part was ground through a 2-mm screen using a Wiley mill.

Table 1. The collection sites and corresponding phonological development stages of *O. sericea* in 2009 and 2010.

Sites	**GPS**	**Growing stages/Collection dates**				
		Vegetative	Early flower	Early pod	Full pod	Pod shattered
Hanksville, UT	N 38°4'15.6" W 110° 48' 35.6"	5/19/2010	6/15/2010	7/19/2010	8/18/2010	10/4/2010
Cuchara, CO	N 37°19'15.7" W 105° 04'23.8"	6/4/2010	6/26/2010	7/29/2010	9/1/2010	10/18/2010
Harstel, CO	N 38°57'58.3" W 105° 43' 32.2"	6/4/2010	6/26/2010	7/29/2010	9/1/2010	10/18/2010
Raft River Mountain, UT	N 41°55.910' W 113° 28.174'	5/17/2010	6/16/2010	7/14/2010	8/16/2010	9/15/2010
Raft River Mountain, UT	N 41°55.817' W113°28.059'	5/21/2009	—	7/13/2009	8/12/2009	—

2.2 Swainsonine Analysis

Plant samples of *O. sericea* were analyzed for swainsonine using the methods described by Gardner et al. (2010). Each plant sample (50 mg of dry, ground plant material) was weighed

into 1.5 ml screw-cap microtubes and extracted with 2% acetic acid (1.5 ml) by continuous mixing in a shaker/incubator for 18 h (overnight). The samples were vibrated again the following day and centrifuged for 5 minutes to separate acetic acid extracts and plant materials. First, 50 µl of each extracted solutions were moved to autosampler vials after adding 950 µl Ammonium Acetate (20nM). The samples were mixed and capped. Two control samples were set at the front and end to ensure the consistency of the swainsonine quantitation. The final extract was analyzed by liquid chromatography mass spectrometry (LC-MS) at USDA/ARS Poisonous Plant Laboratory. The LC-MS system includes a Betasil C18 reverse-phase HPLC column (100 × 2 mm, Thermo Fisher), a guard column of the identical phase, a Survey or MS Pump Plus, a Surveyor Auto Sampler Plus and a LCQ Advantage Max mass spectrometer (Thermo Electron Corp, San Jose, CA, USA). A MS^2 mode was used with atmospheric pressure chemical ionization (APCI) for ionization and the mass spectrometer. The swainsonine concentration (µg/ml) was calculated based on an external calibration standard and converted to percent dry mass of the original plant material. The detection limit of swainsonine is 0.001% of dry weight.

2.3 DNA extraction

The swainsonine analysis results showed that not all 25 plant samples were detected with swainsonine. A previous study showed that plant parts with no detectable swainsonine concentrations contained very small amounts of endophyte (Cook et al., 2009a). Thus, we selected 12 plant samples with detectable swainsonine at each stage for DNA extraction and endophyte analysis. However, there were not always 12 plant samples detected with swainsonine at some stages and locations. In those cases, we selected all the plant samples detected with swainsonine for DNA extraction and endophyte analysis under this situation. Therefore, there were unbalanced sample sizes for swainsonine analysis and endophyte analysis.

DNA was extracted from the selected ground plant material (~25mg, n ≤ 12/stage) by using the DNEasy Plant Mini Kit (Qiagen Inc., Valencia, CA), following the manufacturer's instructions. However, when extracting DNA from flower parts at pod shattered stage, the concentration of total DNA was as low below 10 ng/µl. Thus, we weighed more plant samples for flower parts at pod shattered stage to achieve the required DNA concentration (10 ng/µl) for qPCR procedure. DNA was quantified with the ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and each sample was diluted to a concentration of 10ng/µl for further fungal endophyte quantification (Cook et al., 2009b).

2.4 PCR Primer Design

PCR amplification was performed using OR1a (5' GTC AAA AGT TGA AAA TGT GGC TTG G 3') and ITS 5 (5' GGA AGT AAA AGT CGT AAC AAG G) (White et al., 1990) to amplify the internal transcribed spacer region (ITS). These PCR primers have been demonstrated to verify the presence of the endophyte in locoweed of *Oxytropis* and *Astragalus* species (Ralphs et al., 2008; Cook et al., 2009b). Primers were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA, USA).

2.5 Quantitation of the fungal endophyte

The quantitation of the fungal endophyte was performed by methods described by Cook et al. (2009b). A standard curve (30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001 ng of fungal DNA) was prepared from DNA extracted from the pure fungal endophyte, *Undifilum oxytropis*, which was confirmed to be positive amplified as PCR product of *Oxytropis* and *Astragalus* species. The equation of the standard curve was $y = -3.54x + 17.9$, $R^2 = 0.996$. The amplification efficiency was 91.6% and the detection limit was 0.001ng. This method has been successfully used to

quantify the fungal endophyte in *O. sericea*, *A. mollissimus*, and *A. lentiginosus* (Cook et al., 2009b).

We ran three replicate 25 µl reactions for fungal endophyte quantitation. Each reaction contained 12.5 µL of the QuantiFast SYBR Green PCR Kit master mix (Qiagen Inc., Valencia, CA), 50 ng (5 µl of l0ng/µl stock) of total DNA and 500 nM each of the forward and reverse primers. Three positive controls were set by using 50 ng of *Undifilum Oxytropis* DNA instead of sample DNA stock. Three negative controls were set by using water in order to make sure that the reagents were free from contaminating template DNA. The cycle threshold (Ct) value was read from the qPCR detector. We used the equation of $y = -3.54x + 17.9$ and standard curve to calculate the endophyte content in each sample. Each reaction contained 50 ng of total DNA, thus endophyte concentration was divided by 50 to convert to picogram/nanogram of total DNA. The final concentration of endophyte was determined by taking the mean of triplicate samples.

We used the Bio-Rad CHROMO4 quantitative PCR detector for fungal endophyte DNA amplification and fluorescence detection. The samples were heated to 95°C for 7 min, and kept 15 s for 40 cycles, followed by 58°C for 30 s and then 72°C for 40s. There was a plate reading after each cycle. The melting profile was made to check the specificity and purity of each reaction. The procedure was to hold for 2 s at each temperature point when the temperature was raised from 55°C to 90°C in 2°C increments. A plate read was followed at each temperature point. This procedure has been successfully used in quantifying the endophyte concentration in *Oxytropis* and *Astragalus* species (Ralphs et al., 2008).

2.6 Statistical analysis

Endophyte concentration (pg/ng of total DNA) was transformed to natural log of the original concentration for comparison. At each location, swainsonine concentrations and log transformed endophyte concentrations in each plant part from five stages were examined by using a general linear model of SAS with least squares means for unbalanced sample sizes (SAS Institute, Gary, NC, USA). The Duncan's Multiple Range Test method was used for mean comparisons among stages at a 5% significance level. In the analysis, locations, parts, and their interactions were treated as random effects and stages were treated as fixed effects using mixed procedure of SAS (SAS Institute, Gary, NC, USA). Swainsonine concentrations and log transformed endophyte concentrations were compared by stages, locations, parts, and years by using the GLM procedure with least squares means for unbalanced sample sizes.

3. Results

Swainsonine was not detected in all 25 samples of *O. sericea* from each stage at each location. However, in a plant sample, if one part contained swainsonine, it was detected in other parts; and if not, swainsonine was not detected in other parts. Endophyte concentrations were detected in all selected plant samples ($n \leq 12$/stage/location) of *O. sericea*.

3.1 Seasonal variation of swainsonine concentration and endophyte concentration

Swainsonine concentrations of *O. sericea* were significantly different among stages ($F_{4, 621} = 102.65$, $P < 0.0001$). Mean swainsonine concentration in *O. sericea* significantly increased from early flower stage (0.042%) to full pod stage (0.126%) and decreased at pod shattered stage (0.080%; Fig. 3). Endophyte concentration of *O. sericea* also significantly differed among stages ($F_{4, 595} = 148.73$, $P < 0.0001$). Mean endophyte concentration in *O. sericea* followed the similar trend of seasonal swainsonine concentration which increased from vegetative stage (1.2 pg/ng) to

full pod stage (12.0 pg/ng; Fig. 4). It significantly increased to maxima (18.8 pg/ng) at pod shattered stage instead of decreasing as swainsonine concentration.

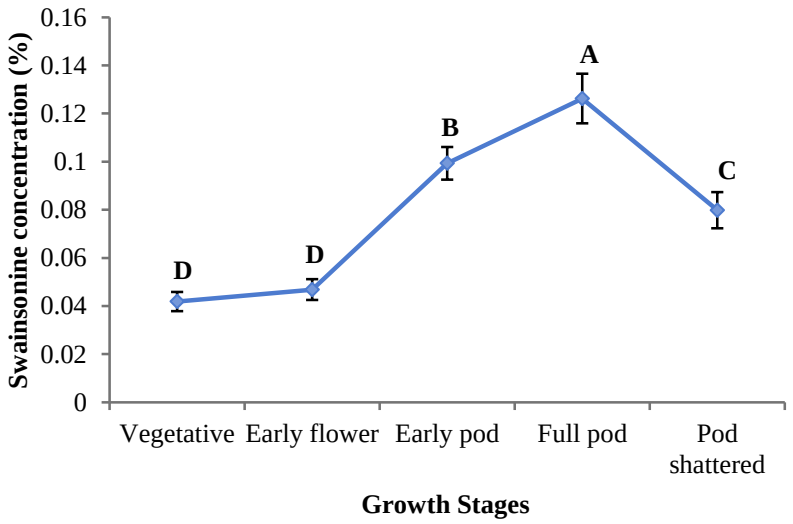

Fig. 3. Swainsonine concentration (%) differentially accumulated at different growth stages of *O. sericea* in 2010. Mean swainsonine concentration ± the standard error of stage (vegetative, early flower, early pod, full pod, and pod shattered stage). Different letters above each bar represent significance at $P < 0.05$.

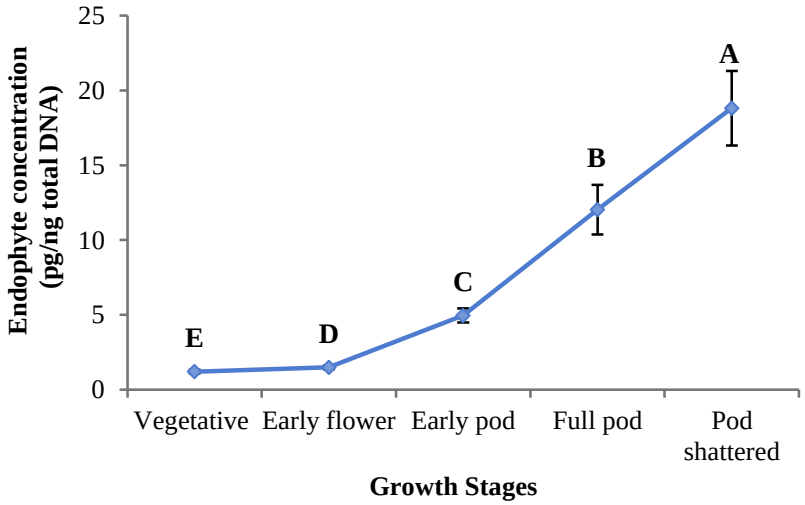

Fig. 4. Endophyte concentration (pg/ng total DNA) differentially accumulated at different growth stage of *O. sericea* in 2010. Mean endophyte concentration ± the standard error of stage (vegetative, early flower, early pod, full pod, and pod shattered stage). Different letters above each bar represent significance at $P < 0.05$.

3.2 Swainsonine concentration and endophyte concentration in plant parts

Swainsonine concentration of *O. sericea* was significantly different among plant parts ($F_{2, 621} = 588.74$, $P < 0.0001$). Leaves contained the highest swainsonine concentration (0.127%), followed by flowers (0.107%; Fig. 5) and then crowns (0.012%). Endophyte concentrations of *O. sericea* were also significantly different among plant parts ($F_{2,595} = 165.61$, $P < 0.0001$). Mean endophyte concentration was the highest in flowers (18.1 pg/ng), followed by leaves (5.3 pg/ng). Crowns contained the lowest endophyte concentration (1.4 pg/ng; Fig. 6).

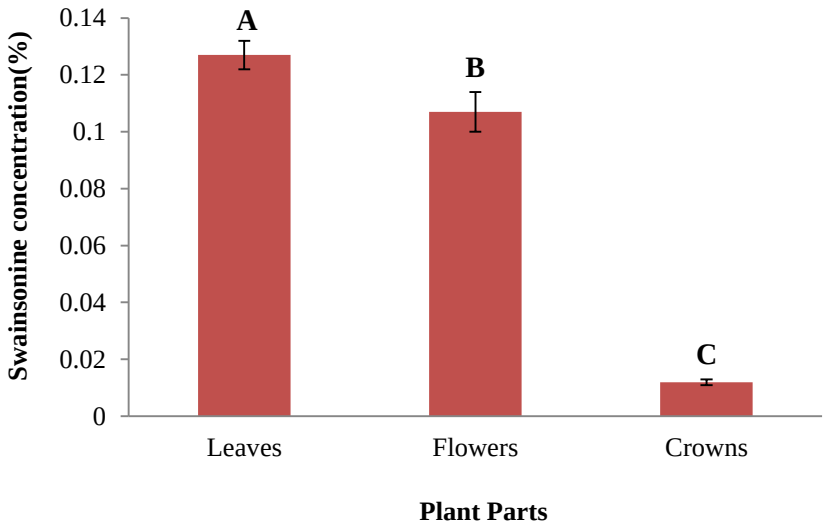

Fig. 5. Swainsonine concentration (%) differentially accumulated within plant parts in *O. sericea* in 2010. Mean swainsonine concentration ± the standard error of parts (leaves, flowers, and crowns). Different letters above each bar represent significance at $P < 0.05$.

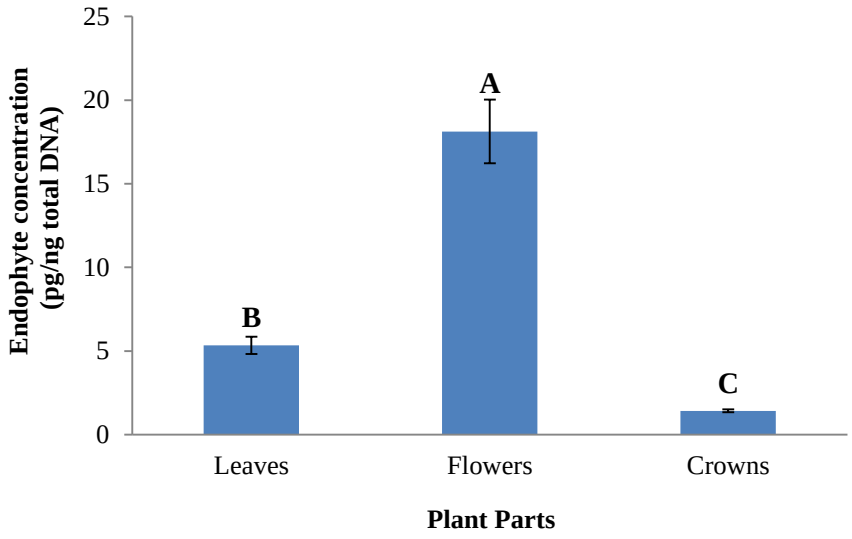

Fig. 6. Endophyte concentration (pg/ng total DNA) differentially accumulated in different plant parts of *O. sericea* in 2010. Mean endophyte concentration ± the standard error of parts (leaves, flowers, and crowns). Different letters above each bar represent significance at $P < 0.05$.

3.3 Variation of swainsonine concentration and endophyte concentration among locations and years

There was a significant variation of swainsonine concentration among locations in 2010 ($F_{3,621} = 129.66$, $P < 0.0001$). The mean swainsonine concentration in *O. sericea* sampled at four locations varied from 0.048% at Cuchara, CO to 0.129 % at Raft River Mountain, UT (Fig. 7). There was no significant difference in swainsonine concentration between Hanksville, UT and Harstel, CO (0.073% and 0.076 %, respectively). Endophyte concentrations were also significantly different among locations ($F_{3,595} = 23.69$, $P < 0.0001$). The maximum and minimum endophyte concentrations were at Raft River Mountain, UT and Hanksville, UT (12.0 pg/ng and 4.3 pg/ng, respectively; Fig. 8).

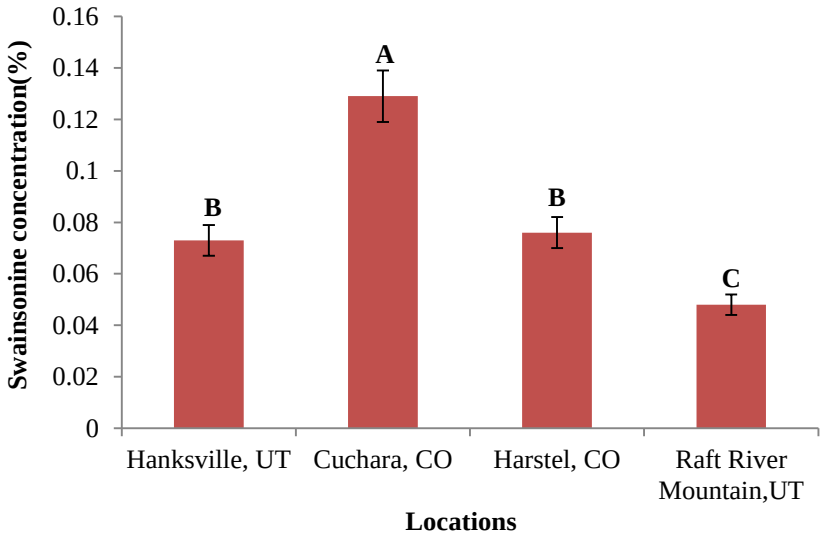

Fig. 7. Swainsonine concentration (%) in *O. sericea* at different locations in 2010. Mean swainsonine concentration ± the standard error of locations (Hanksville, Cuchara, Harstel, and Raft River Mountain). Different letters above each bar represent significance at $P < 0.05$.

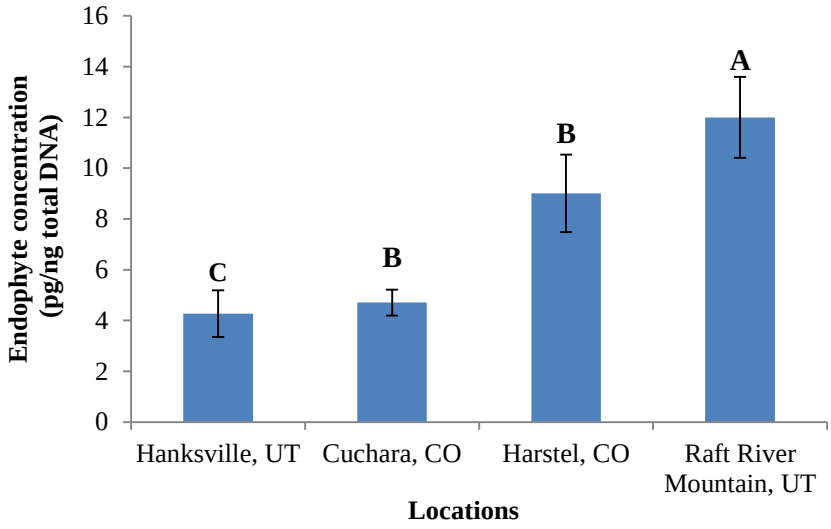

Fig. 8. Endophyte concentration (pg/ng total DNA) in *O. sericea* at different locations in 2010. Mean endophyte concentration ± the standard error of locations (Hanksville, Cuchara, Harstel, and Raft River Mountain). Different letters above each bar represent significance at $P < 0.05$.

The corresponding stages sampled at Raft River Mountain, UT from 2009 and 2010 were vegetative stage, early pod stage, and full pod stage. Mean swainsonine concentration at Raft

River Mountain varied between 2009 and 2010 (0.044% and 0.062%, respectively, $P = 0.0122$; Table 2); however, there was no significant difference in mean endophyte concentrations between 2009 and 2010 (10.6 pg/ng and 8.9 pg/ng, respectively, $P = 0.4302$). Similar seasonal trends of swainsonine and endophyte concentrations were observed between 2009 and 2010 (Fig. 15, 16, 17 and 18). Mean swainsonine concentrations of *O. sericea* in 2009 and 2010 at Raft River Mountain increased as plants matured, reaching the peak at full pod stage. Mean endophyte concentrations in these two years also increased during the growing season.

Table 2. Swainsonine concentration (%) and endophyte concentration (pg/ng total DNA) of *O. sericea* at Raft River Mountain, UT (2009 and 2010). Within the same column, values followed by different letters are statistically different ($P < 0.05$).

	Swainsonine concentration	**Endophyte concentration**
Raft River Mountain, UT 2009	0.044% ± 0.004[b]	10.567 ± 1.725[a]
Raft River Mountain, UT 2010	0.062% ± 0.006[a]	8.879 ± 1.253[a]

3.4 Seasonal variation of swainsonine and endophyte concentrations in plant parts at each location

Swainsonine concentration of *O. sericea* differed in plant parts at different stages at each location (location × stage × part interaction, $F_{21,621} = 11.41$, $P < 0.0001$). However, there was no location × stage × part interaction in endophyte concentration of *O. sericea* ($F_{21,595} = 1.39$, $P = 0.1147$). Therefore, we presented the seasonal variation of swainsonine and endophyte concentrations in plant parts at each location.

3.4.1 *O. sericea* at Hanksville, UT

At Hanksville, UT, the swainsonine concentration in plant parts was different among stages and varied by parts (stage × part interaction, $F_{8,621} = 55.25$, $P < 0.0001$; Table 3). For example, swainsonine concentration in leaves increased at early pod stage (0.143 %, $P = 0.0003$) and remained constant during following two stages (Fig. 9). Flowers reached the peak of swainsonine concentration (0.205%) at full pod stage and significantly decreased at pod shattered stage (0.090 %, $P < 0.0001$). Swainsonine concentration in crowns changed during the growing season ($P = 0.0008$), ranging from 0.007% at full pod stage to 0.016% at vegetative stage.

Endophyte concentration of *O. sericea* also differed in plant parts among stages (stage × part interaction, $F_{8,595} = 28.54$, $P < 0.0001$; Table 4). Endophyte concentration in leaves followed the same trend as swainsonine concentration, ranging from 0.6 pg/ng at early flower stage to 4.0 pg/ng at full pod stage ($P < 0.0001$). Flowers increased in endophyte concentration throughout the season from 0.6 pg/ng at early flower stage to 36.6 pg/ng at pod shattered stage ($P < 0.0001$; Fig.10). Crowns were variable in endophyte concentration ($P = 0.0129$), ranging from 0.9 pg/ng to 1.8 pg/ng throughout the season.

Table 3. Swainsonine concentration (%) in plant parts of *O. sericea* at different stages at Hanksville, UT. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	0.066 ± 0.005 ^{b}	0.062 ± 0.008 ^{b}	0.143 ± 0.021 ^{a}	0.141 ± 0.021^{a}	0.133 ± 0.020 ^{a}
Flowers	—	0.026 ± 0.004 ^{c}	0.117 ± 0.016 ^{b}	0.205 ± 0.016^{a}	0.090 ± 0.010 ^{b}
Crowns	0.016 ± 0.002 ^{a}	0.011 ± 0.001 ^{ab}	0.015 ± 0.002 ^{a}	0.007 ± 0.001 ^{b}	0.010 ± 0.002^{b}

Table 4. Endophyte concentration (pg/ng) in plant parts of *O. sericea* at different stages at Hanksville, UT. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	0.956 ± 0.500^{b}	0.555 ± 0.382^{b}	3.648 ± 5.009^{a}	4.023 ± 3.012^{a}	3.509 ± 1.092^{a}
Flowers	—	0.570 ± 0.414^{d}	3.581 ± 4.407^{c}	12.301 ± 18.260^{b}	36.592 ± 30.509^{a}
Crowns	0.875 ± 0.788^{bc}	0.550 ± 0.293^{c}	1.779 ± 1.695^{a}	1.247 ± 1.134^{abc}	1.246 ± 0.637^{ab}

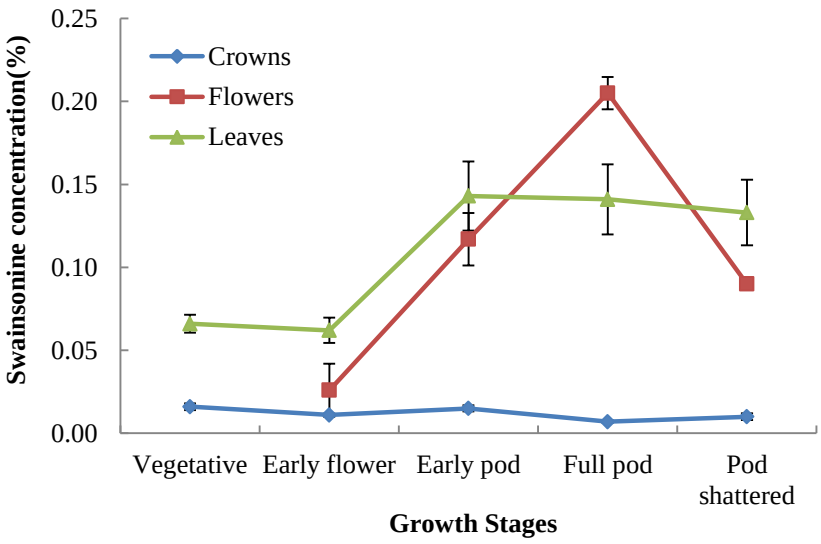

Fig. 9. Swainsonine concentration in plant parts of *O. sericea* at Hanksville, UT.

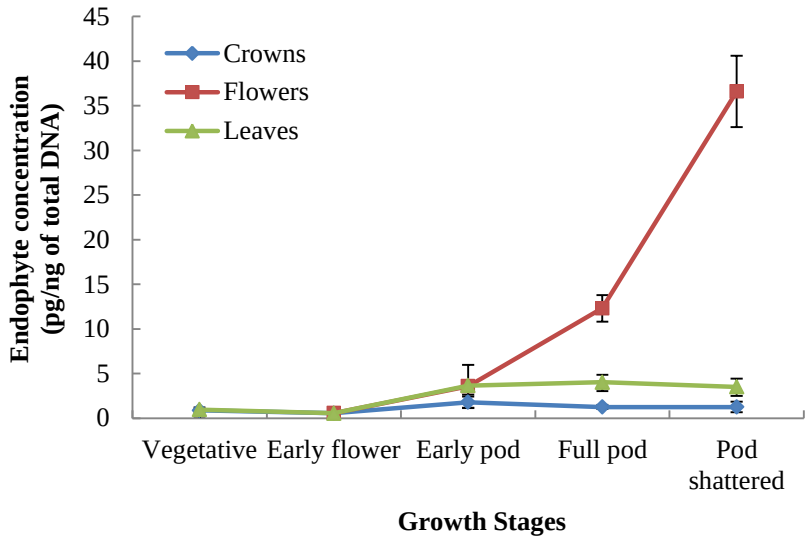

Fig. 10. Endophyte concentration in plant parts of *O. sericea* at Hanksville, UT. Y axis units: pg/ng total DNA.

3.4.2 *O. sericea* at Cuchara, CO

Mean swainsonine concentrations in plant parts varied from 0.010% in crowns at the early pod stage to 0.302% in leaves at the pod shattered stage (stage × part interaction, $F_{8,621} = 55.25$, $P < 0.0001$; Table 5). Swainsonine concentrations in leaves significantly increased from early pod stage to full pod stage (0.186% and 0.262%, respectively, $P < 0.0001$; Fig. 11). Flowers increased in swainsonine concentrations to full pod stage and significantly decreased at pod shattered stage (0.359% and 0.085%, respectively, $P < 0.0001$). Swainsonine concentrations in crowns were variable throughout the five growing stages ($P = 0.0023$), ranging from 0.010% at early pod stage to 0.022% at early flower stage.

Mean endophyte concentrations also significantly differed in plant parts among stages (stage × part interaction, $F_{8,595} = 28.54$, $P < 0.0001$; Table 5). Endophyte concentrations in leaves significantly increased from vegetative stage to full pod stage (1.0 pg/ng and 6.1 pg/ng,

respectively, $P < 0.0001$). Flowers increased in endophyte concentration to the maxima at pod shattered stage, ranging from 2.3 pg/ng to 18.8 pg/ng ($P < 0.0001$). Endophyte concentration in crowns was different throughout the season ($P = 0.0053$), but no certain trend was observed.

Table 5. Swainsonine concentration (%) in plant parts of *O. sericea* at different stages at Cuchara, CO. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	0.112 ± 0.010^{c}	0.175 ± 0.011^{b}	0.186 ± 0.018^{b}	0.262 ± 0.022^{a}	0.302 ± 0.022^{a}
Flowers	—	0.065 ± 0.006^{c}	0.174 ± 0.015^{b}	0.359 ± 0.027^{a}	0.085 ± 0.014^{c}
Crowns	0.021 ± 0.002^{a}	0.022 ± 0.002^{a}	0.010 ± 0.001^{b}	0.014 ± 0.003^{b}	0.016 ± 0.002^{ab}

Table 6. Endophyte concentration (pg/ng) in plant parts of *O. sericea* at different stages at Cuchara, CO. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	0.978 ± 0.240^{c}	2.419 ± 0.241^{b}	4.402 ± 0.607^{ab}	6.144 ± 0.822^{a}	4.329 ± 0.917^{ab}
Flowers	—	2.251 ± 0.573^{c}	8.575 ± 2.404^{b}	11.645 ± 1.481^{ab}	18.814 ± 4.002^{a}
Crowns	0.696 ± 0.149^{b}	1.172 ± 0.227^{ab}	2.203 ± 0.642^{a}	1.693 ± 0.231^{a}	1.605 ± 0.591^{a}

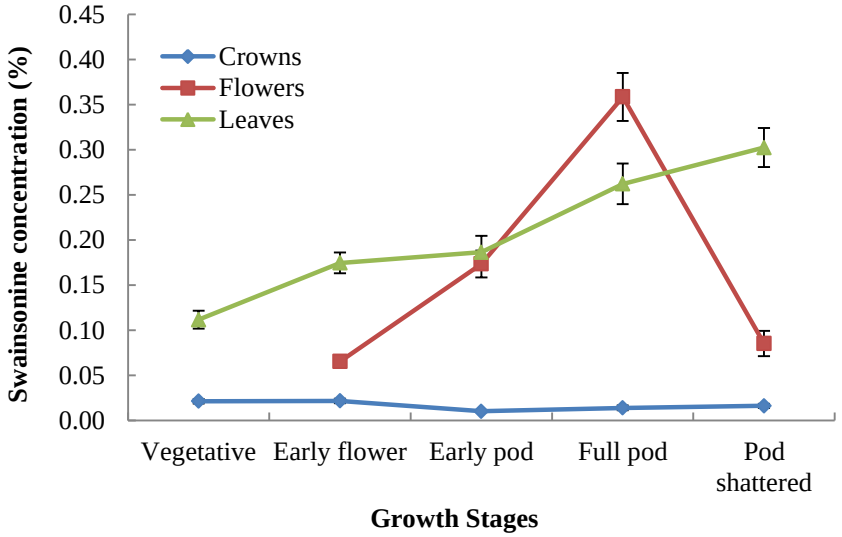

Fig. 11. Swainsonine concentration in plant parts of *O. sericea* at Cuchara, CO.

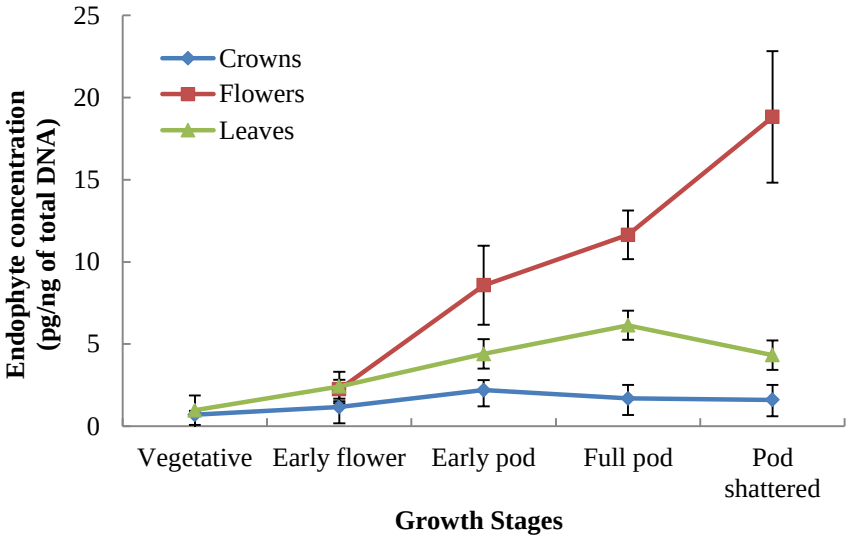

Fig. 12. Endophyte concentration in *O. sericea* at Cuchara, CO. Y axis units: pg/ng total DNA

3.4.3 *O. sericea* at Harstel, CO

The *O. sericea* sampled at Harstel, CO were more mature than the plants at other locations at each stage. Most flower parts sampled were aborted and did not have pods attached at early pod stage. Therefore, flower parts at early pod stage were mostly green scapes with little

flowers. At full pod stage, the collected flower parts were all senescent scapes. Thus, swainsonine and endophyte concentrations of *O. sericea* at Harstel were different than at other sites.

Mean swainsonine concentration differed significantly in plant parts at different stages (stage × part interaction, $F_{8,621} = 55.25$, $P < 0.0001$; Table 7). Leaves significantly increased in swainsonine concentration from early flower stage to early pod stage (0.114% and 0.161%, respectively, $P = 0.0006$; Fig. 13). Swainsonine concentrations reached the peak at early pod stage and significantly decreased at full pod stage (0.217% and 0.068%, respectively, $P < 0.0001$). Similarly with other locations, crowns were changed in swainsonine concentration during the growing season from 0.014% to 0.020% ($P = 0.0135$).

Endophyte concentration significantly differed in plant parts among stages (stage × part interaction, $F_{8,595} = 28.54$, $P < 0.0001$; Table 8). Leaves had the same endophyte concentration trend as swainsonine concentration all through the season, increasing from early flower stage to early pod stage (1.5 pg/ng and 3.8 pg/ng, respectively, $P = 0.0002$) and remained constant at full pod and pod shattered stage (3.9 pg/ng and 4.0 pg/ng, respectively; Fig. 14). Flowers significantly increased in endophyte concentration throughout the season from vegetative stage to pod shattered stage (0.5 pg/ng and 52.0 pg/ng, respectively; $P < 0.0001$). Endophyte concentration in crowns was variable, ranging from 1.1 pg/ng to 2.8 pg/ng ($P = 0.0167$).

Table 7. Swainsonine concentration (%) in plant parts of *O. sericea* at different stages at Harstel, CO. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	0.089 ± 0.005^{b}	0.114 ± 0.016^{b}	0.161 ± 0.018^{a}	0.132 ± 0.018^{ab}	0.174 ± 0.017^{a}
Flowers	0.009 ± 0.001^{c}	0.043 ± 0.004^{bc}	0.217 ± 0.020^{a}	0.068 ± 0.012^{b}	0.050 ± 0.010^{b}
Crowns	0.020 ± 0.003^{a}	0.014 ± 0.003^{ab}	0.011 ± 0.001^{b}	0.010 ± 0.001^{b}	0.014 ± 0.002^{ab}

Table 8. Endophyte concentration (%) in plant parts of *O. sericea* at different stages at Harstel, CO. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	1.497 ± 0.252^{b}	1.522 ± 0.273^{b}	3.829 ± 0.839^{a}	3.860 ± 0.815^{a}	3.951 ± 0.654^{a}
Flowers	0.531 ± 0.097^{d}	1.742 ± 0.270^{c}	10.219 ± 3.357^{b}	47.588 ± 10.635^{a}	52.011 ± 9.514^{a}
Crowns	1.508 ± 0.281^{b}	1.076 ± 0.175^{b}	1.956 ± 0.336^{ab}	1.234 ± 0.197^{b}	2.795 ± 0.772^{a}

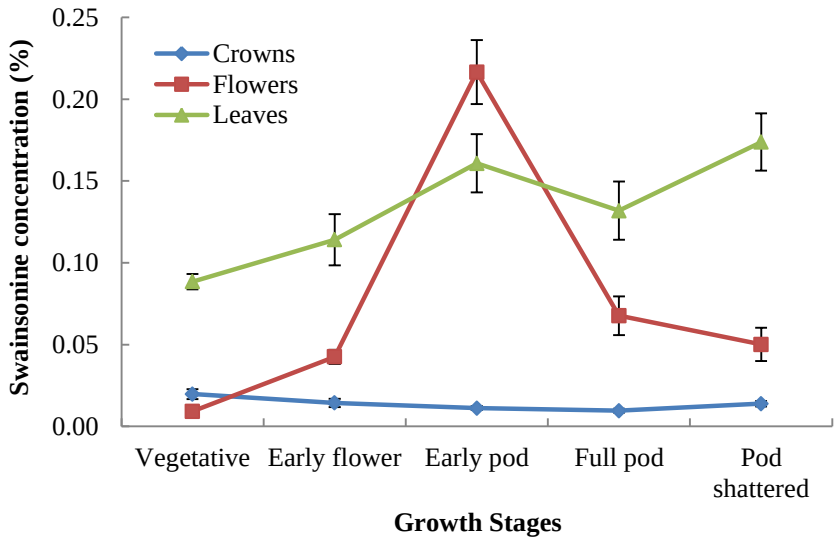

Fig. 13. Swainsonine concentration in plant parts of *O. sericea* at Harstel, CO

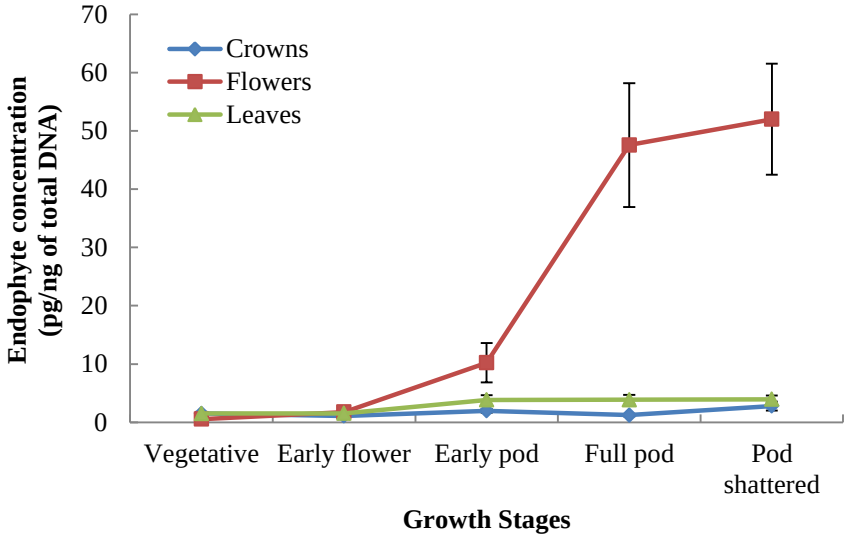

Fig. 14. Endophyte concentration in plant parts of *O. sericea* at Harstel, CO. Y axis units: pg/ng total DNA

3.4.4 *O. sericea* at Raft River Mountain, UT in 2010

Swainsonine concentration was significantly different in plant parts among stages (stage × part interaction, $F_{8,621} = 55.25$, $P < 0.0001$; Table 9). Leaves and flowers both increased in swainsonine concentration to the maximum at full pod stage (0.128% and 0.173%, respectively; $P < 0.0001$) and significantly decreased at pod shattered stage (0.074% and 0.054%, respectively; $P < 0.0001$; Fig.15 and16). Swainsonine concentrations in crowns remained constant throughout the season ($P = 0.6899$).

Endophyte concentration was highly variable in plant parts among stages (stage × part interaction, $F_{8,595} = 28.54$, $P < 0.0001$; Table 10), ranging from 0.4 pg/ng in crowns at vegetative stage to 62.9 pg/ng in flowers at pod shattered stage. Endophyte concentrations in leaves reached the maximum at pod shattered stage (25.4 pg/ng, $P < 0.0001$). Endophyte concentration in flowers increased from early flower stage to pod shattered stage (1.0 pg/ng and 62.9 pg/ng ,

respectively, $P < 0.0001$). Crowns had variable endophyte concentrations during the growing season ranging from 0.4 pg/ng to 2.5 pg/ng ($P < 0.0001$).

Table 9. Swainsonine concentration (%) in plant parts of *O. sericea* at different stages at Raft River Mountain, UT 2010. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	0.024 ± 0.003^{c}	0.026 ± 0.002^{c}	0.082 ± 0.005^{b}	0.128 ± 0.012^{a}	0.074 ± 0.007^{b}
Flowers	—	0.007 ± 0.001^{d}	0.091 ± 0.008^{b}	0.173 ± 0.015^{a}	0.054 ± 0.005^{c}
Crowns	0.005 ± 0.001^{a}	0.005 ± 0.001^{a}	0.006 ± 0.001^{a}	0.006 ± 0.001^{a}	0.005 ± 0.001^{a}

Table 10. Endophyte concentration (%) in plant parts of *O. sericea* at different stages at Raft River Mountain, UT 2010. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early flower	Early pod	Full pod	Pod shattered
Leaves	2.410 ± 0.583^{d}	4.166 ± 0.594^{c}	7.806 ± 1.235^{b}	20.620 ± 3.549^{a}	25.391 ± 4.787^{a}
Flowers	—	1.009 ± 0.170^{d}	9.733 ± 1.038^{c}	30.862 ± 4.913^{b}	62.951 ± 10.576^{a}
Crowns	0.440 ± 0.127^{c}	0.782 ± 0.235^{bc}	0.760 ± 0.205^{c}	1.819 ± 0.492^{ab}	2.449 ± 0.447^{a}

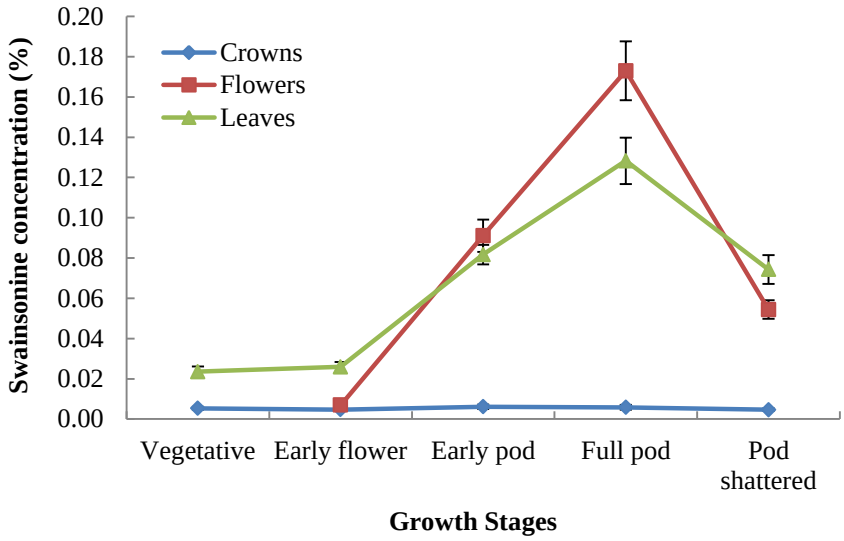

Fig. 15. Swainsonine concentration in plant parts of *O. sericea* at Raft River Mountain, UT 2010.

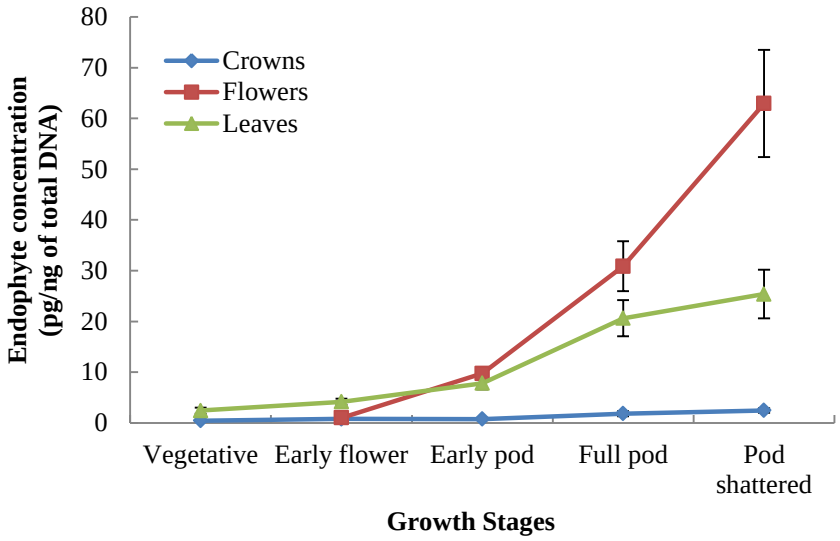

Fig. 16. Endophyte concentration in plant parts of *O. sericea* at Raft River Mountain, UT 2010. Y axis units: pg/ng total DNA

3.4.5 *O. sericea* at Raft River Mountain, UT in 2009

Similar trends of swainsonine and endophyte concentrations in flowers and leaves were observed between 2009 and 2010 (Fig. 15, 16, 17 and 18). Swainsonine concentrations significantly differed in plant parts among three developmental stages (stage × part interaction, $F_{3, 160}$ =15.84, $P < 0.0001$; Table 11). Swainsonine concentrations in leaves gradually increased from vegetative stage to full pod stage (0.017% and 0.074%, respectively, $P < 0.0001$; Fig. 17). Swainsonine concentrations in flowers reached the peak at full pod stage (0.139 %, $P < 0.0001$). Crowns contained variable swainsonine concentrations among growing stages ($P = 0.0026$), ranging from 0.003% to 0.005%.

Endophyte concentration was not significantly different in plant parts among stages (stage × part interaction, $F_{3, 88} = 0.46$, $P = 0.7113$; Table 12). Endophyte concentrations in leaves significantly rose from early pod stage to full pod stage (4.6 pg/ng and 13.3 pg/ng, respectively; $P = 0.0032$; Fig.17). Flowers had a large range of swainsonine concentration, increasing from early pod stage to full pod stage (8.0 pg/ng and 41.9 pg/ng, respectively; $P = 0.0001$). In crowns, endophyte concentrations significantly increased from early pod stage to full pod stage (1.9 pg/ng and 7.1 pg/ng, respectively; $P = 0.0128$).

Table 11. Swainsonine concentration (%) in plant parts of *O. sericea* at different stages at Raft River Mountain, UT 2009. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early pod	Full pod
Leaves	0.017 ± 0.002[c]	0.043 ± 0.002[b]	0.074 ± 0.009[a]
Flowers	—	0.057 ± 0.002[b]	0.139 ± 0.000[a]
Crowns	0.005 ± 0.001[a]	0.003 ± 0.000[b]	0.005 ± 0.000[a]

Table 12. Endophyte concentration (%) in plant parts of *O. sericea* at different stages at Raft River Mountain, UT 2009. Within the same row, values followed by different letters are statistically different ($P < 0.05$).

Plant parts	Vegetative	Early pod	Full pod
Leaves	5.366 ± 1.507[b]	4.568 ± 0.741[b]	13.308 ± 2.674[a]
Flowers	—	7.968 ± 1.697[b]	41.890 ± 8.857[a]
Crowns	2.419 ± 0.673[b]	1.931 ± 0.324[b]	7.088 ± 1.925[a]

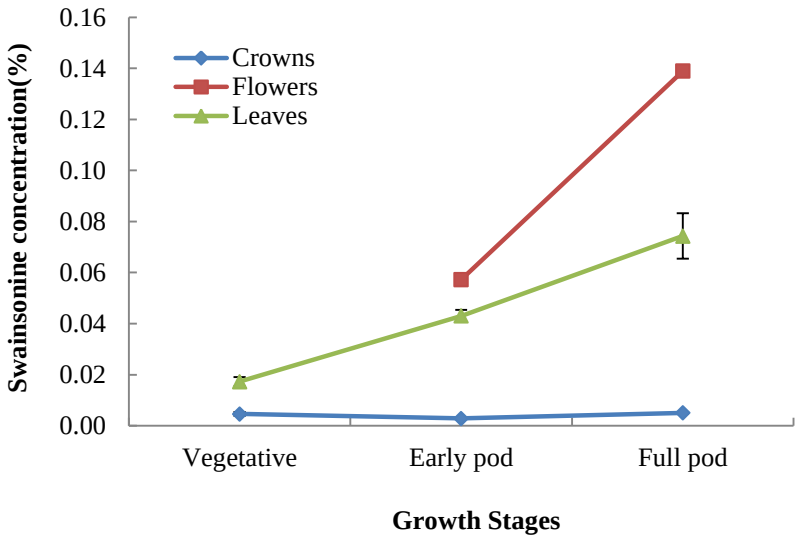

Fig. 17. Swainsonine concentration in plant parts of *O. sericea* at Raft River Mountain, UT 2009.

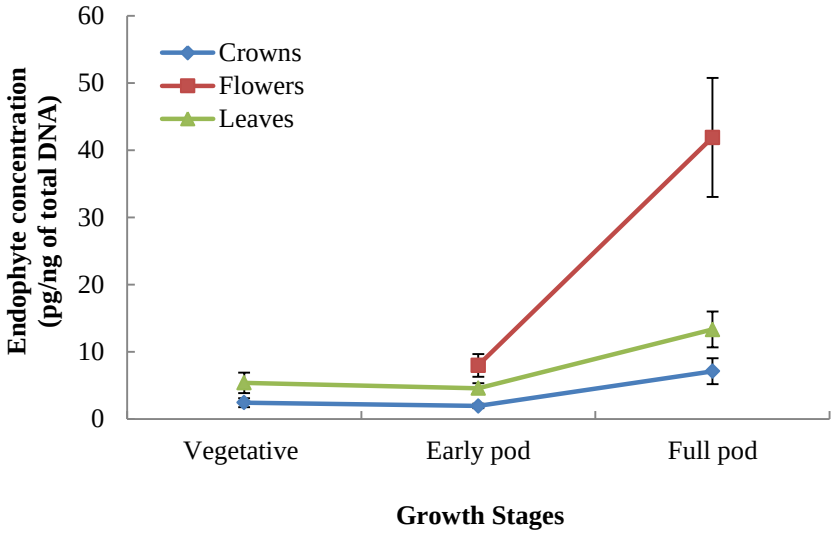

Fig. 18. Endophyte concentration in plant parts of *O. sericea* at Raft River Mountain, UT 2009. Y axis units: pg/ng total DNA

3.5 Relationship between endophyte and swainsonine in *O. sericea*

A plot of swainsonine concentrations against endophyte concentrations of *O. sericea* was made at each location, regardless of growing stages and plant parts (Plots not shown). The results showed that swainsonine concentrations were significantly correlated with endophyte concentrations at each location (all $P < 0.05$) except for Harstel, CO ($P = 0.9381$). However, R^2 values were low, indicating that swainsonine concentrations cannot be predicted by endophyte concentrations (Table 13).

Table 13. Results from GLM procedure of the relationship between swainsonine concentration (%) and endophyte concentration (pg/ng total DNA) in *O. sericea* regardless of growing stages and plant parts at each location with R^2 and *P*-values.

	Hanksville, UT	Cuchara, CO	Harstel, CO	Raft River Mountain, UT 2010	Raft River Mountain, UT 2009
$\mathbf{R^2}$	0.0837	0.0873	0.0000	0.1763	0.4860
P	0.0006	0.0001	0.9381	<0.0001	<0.0001

4. Discussion

Seasonal variation of swainsonine concentration in *O. sericea*

This study demonstrated the seasonal variation of swainsonine and fungal endophyte concentrations in *O. sericea*. On average, mean swainsonine concentrations increased from early flower stage to full pod stage and decreased at pod shattered stage (Fig. 3). However, swainsonine concentrations varied in plant parts at different stages and locations. For example, swainsonine concentrations in crowns were lower and slightly variable throughout the season at all locations, whereas, leaves and flowers significantly increased in swainsonine concentrations to full pod stage in most cases. Swainsonine concentration in leaves remained stable at pod shattered stage, whereas it decreased in flower parts at the same stage. The experiment of comparison of swainsonine concentration in old leaves and young leaves showed that old leaves contained more swainsonine concentration (0.309%) than young leaves (0.134%). This result indicated that leaves accumulated swainsonine as the plants grew. The fungal endophyte has been shown to be responsible for producing swainsonine in locoweeds (Braun et al., 2003; Ralphs et al., 2008), therefore, it was most likely that the fungal endophyte in leaves had more time to colonize as

plants grew in the growing season, and accumulated swainsonine. However, the endophyte concentrations in old and young leaves were not determined in this study. Thus, it is necessary to quantify the endophyte concentration in old and young leaves to further test the hypothesis.

Mean swainsonine concentration significantly decreased at pod shattered stage at all four locations. This is most likely due to the decreased swainsonine concentration in flowers at pod shattered stage. We assumed that the decreased swainsonine concentration in flower parts at pod shattered stage was because of the loss of seeds. However, we found that seeds and scapes contained similar swainsonine concentrations (0.35% and 0.41%, respectively) at full pod stage. Therefore, it is possible that because of plants were senesced at pod shattered stage, endophytes stopped colonizing and swainsonine concentration decreased in flower parts which were all senesced scapes.

We also found that swainsonine concentration in crowns was the lowest (Fig. 5) and variable throughout the season, but there was no consistent trend observed (Fig. 9, 11, 13, 15, and 17). Crowns contained comparable level of swainsonine compared to leaves at vegetative stage and flowers at early flower stage. However, swainsonine concentration in leaves and flowers significantly increased as plants grew while it changed slightly in crowns at following three stages (early pod stage, full pod stage and pod shattered stage). The fungal endophyte, *U. oxytropis*, has been shown to produce swainsonine, thus, the low level of swainsonine concentration in crowns can be explained by the low endophyte concentrations throughout the season. However, swainsonine concentrations did not mirror endophyte concentrations in crowns. This may be explained by the fact that swainsonine translates faster as a potential mobile secondary compound (Dreyer et al., 1985) or the endophyte in crowns produce swainsonine and is translocated into aboveground parts (Cook et al., 2011).

Seasonal variation of endophyte concentration in *O. sericea*

Mean endophyte concentrations of *O. sericea* followed the same trend as seasonal swainsonine concentrations, increasing from early flower stage to full pod stage. However, it kept increasing at pod shattered stage, whereas swainsonine concentrations decreased at this stage (Fig. 3, 4). The increased endophyte concentration at pod shattered stage was most likely due to a significantly higher amount of endophyte in flower parts. As mentioned above, we weighed more plant samples of flower parts at pod shattered stage because of their low total DNA concentration (below 10 ng/μl). At pod shattered stage, flower parts were mostly senesced scapes with little pods which caused the extreme low concentration of the plant DNA. Due to the low plant DNA concentration, the portion of *O. sericea* DNA was much lower than endophyte DNA in total DNA. Therefore, when calculating the endophyte content as picogram per nanogram of total DNA, the relatively higher portion of endophyte DNA in total DNA led to higher calculated results of endophyte concentration.

Unlike flowers and leaves, the endophyte concentration in crowns increased as plants grew, but there was no certain trend showing a change in endophyte concentrations in crowns during the growing season (Fig. 9, 10, 11, 12, 13, 14, 15, 16, 17, and 18). Endophytes in crowns colonized slowly. It is possible that endophytes in crowns just serve as a reservoir for the subsequent growth in the following year (Cook et al., 2009a).

Variation of swainsonine and endophyte concentration in plant parts of *O. sericea*

It has been shown that above ground tissues (leaf, scape, and flower/pod) had approximately 10 times greater swainsonine concentrations than below ground tissues (root and crown) at a single developmental stage (Cook et al., 2009a). The results of this study also showed

that leaf and flower contained more swainsonine concentration than crowns (Fig. 5). However, swainsonine concentration of *O. sericea* differed in plant parts at different stages at each location. For example, leaves had higher swainsonine concentrations than flowers at early flower stage and pod shattered stage at each location, where flowers contained higher swainsonine concentrations at full pod stage at all locations but Harstel, CO.

Endophyte concentrations differed in plant parts. However, the endophyte concentration did not mirror the swainsonine concentration in plant parts. For example, flowers contained the highest endophyte concentration, but contained less swainsonine concentration than leaves. The highest endophyte concentration in flower parts most likely caused by the artificial factor when calculating the endophyte concentrations in flowers at pod shattered stage as mentioned above (Fig. 6). Furthermore, different tissues had different colonization rates of fungal endophytes. For example, the fungal endophyte colonized the fastest in flowers when pods ripening and the lowest in crowns throughout the season.

Variation of swainsonine concentration and endophyte concentration among locations and years

Mean swainsonine and endophyte concentrations differed among locations in 2010, but did not mirror each other at the corresponding locations. For example, plant samples contained the highest swainsonine concentration (0.129%) at Cuchara, but had a medium amount of endophyte (4.7 pg/ng) compared to other locations, whereas plant samples from Raft River Mountain contained the lowest swainsonine concentrations (0.048%) but had the highest endophyte concentrations (12.0 pg/ng; Fig.7 and 8). The results from this study showed that swainsonine concentrations were significantly correlated with endophyte concentrations ($P < 0.05$) but cannot be predicted by endophyte concentrations. This suggested that there may be

other factors influencing swainsonine concentrations in locoweeds besides endophyte abundance, such as environmental stress, plants genotype and endophyte genotype. For example, locoweeds which experienced below-normal winter and spring precipitation contain more swainsonine (Purvines and Ralphs, 1999). Oldrup et al. (2010) also showed that water stress-inducting condition increased swainsonine content in *O. sericea*. The dinitrogen fixation can increase the swainsonine concentration in *O. sericea* infected with fungal endophyte (Barillas et al., 2007). Other environmental stresses, such as temperature and ph changes also appear to affect swainsonine concentrations in locoweeds (Bottger, 2009; Oldrup et al., 2010). However, environmental parameters were not collected from these four locations. Further study can be conducted to collect the environment information at these locations through the growing season to investigate the effect of environmental stress on swainsonine production and endophyte growth in *O. sericea*.

It has been known that plant genotypes influenced endophyte production of alkaloid in acremonium-infected tall fescue (Agee et al., 1994; Roylance et al., 1994). Faeth et al. (2002b) also found that peramine levels in Arizona fescue plants infected by the same endophyte haplotype were significantly different between plant genotypes. Therefore, it is possible that the *O. sericea* genotypes from Cuchara and Raft River Mountain were different, and thus affected endophyte producing swainsonine. Further research is necessary to identify the *O. sericea* and endophyte genotypes from each location to better determine the locoweed-endophyte relationship.

O. sericea at Raft River Mountain contained similar mean endophyte concentrations but different mean swainsonine concentration between 2009 and 2010 (Table. 2). The overall level of swainsonine concentration in a locoweed population between years can be influenced by the

differences in swainsonine levels among individual plant samples (Gardner et al., 2001). The phenology differences between years can also influence the overall swainsonine concentration. However, the seasonal variation trends of swainsonine and endophyte concentrations were similar between 2009 and 2010 at Raft River Mountain, indicating the same relationship between endophyte and swainsonine in plants between years.

O. sericea is one of the first species growing in early spring on many western rangelands. Locoweed poisoning generally occurs in early spring when warm-season grasses started rapid growth and locoweed matured (Ralphs et al., 1994). This study showed that swainsonine concentrations in *O. sericea* were higher than the toxic threshold 0.001% (Ralphs et al., 2008) throughout the growing season. This indicated that plants of *O. sericea* at these locations were poisonous at all stages of growth. The management strategies of grazing at these infested areas should be dependent on both swainsonine levels in locoweeds and locoweeds density. For example, the swainsonine concentration of *O. sericea* at Hanksville, UT was similar with the one at Harstel, CO (0.073% and 0.076 %, respectively). However, 48% plants samples were detected with swainsonine above 0.001% at Hanksville, UT whereas 72% at Harstel, CO, indicating that the risk of poisoning at Harstel, CO is higher than at Hanksville, UT. Thus, caution should be used based on the density of locoweeds every year and the level of swainsonine concentration in local plants, and ranchers should avoid grazing highly infected areas. Additionally, the swainsonine concentration in *O. sericea* remained high (0.080%) at pod shattered stage when plants senesced. Therefore, rangers should avoid grazing the senesced stalks since they are still toxic to livestock.

Summary

The swainsonine and endophyte *Undifilum* concentrations in *O. sericea* were successfully investigated in different plant parts during the growing season. Swainsonine concentrations in *O. sericea* generally increased from the early flower stage to full pod stage as plants grew and decreased at pod shattered stage as plants senesced. This seasonal trend was associated with the endophyte colonization in locoweeds. Moreover, leaves and flowers had faster endophyte colonization rates and contained more swainsonine concentrations than crowns. In addition, though swainsonine level was related with the endophyte concentration, it cannot be predicted only by endophyte concentration during the growing season. This result leads us to hypothesize that environmental stress, plants, and fungal endophyte genotype may also influence the endophyte growth and swainsonine production. Further study is necessary to determine the effect of environmental stress and plants and endophyte genotypes on swainsonine - endophyte relationship in locoweeds.

Reference

Agee, N. S., and Hill, C. S. 1994. Ergovaline variability in Acremonium infected tall fescue due to environment and plant genotype. *Crop Science*. 34: 221-226.

Barillas, J. R., Paschke, B.M., and Ralphs, M. H. 2007. White locoweed toxicity is fascinated by a fungal endophyte and nitrogen-fixing bacteria. *Ecology*. 88: 1850-1856.

Bottger, J. A. 2009. Variation in *Undifilum* DNA and swainsonine content in locoweed. M.S. Thesis. New Mexico State University.

Braun, K., Cook, T., and Liddell, C. M. 1997. Locoism of cattle caused by a species of *Alternaria*? *Phytopathology*. 87: S11–S12.

Braun, K. J., Romero, C. L., and Creamer, R. 2003. Production of swainsonine by fungal endophytes of locoweed. *Mycological Research*. 107: 980-988.

Broquist, H. P. 1986. Livestock toxicosis, slobers, locoism, and the indolizidine alkaloids. *Nutrition Reviews.* 44: 317-323.

Colegate, S. M., Peter, R. D., and Huxtable, C. R. 1979. A spectroscopic investigation of swainsonine: an α-mannosidase inhibitor isolated from swainsona canescens. *Australian Journal of Chemistry*. 32: 2257-2264

Cook, B. D., Gardner, D. R., Grum, D., Pfister, J. A., Ralphs, M. H., Welch, K. D., and Green B. T. 2011. Swainsonine and endophyte relationships in *Astragalus mollissimus* and *Astragalus lentiginosus. Journal of agricultural and food chemistry.* 59: 1281-1287.

Cook, B. D., Gardner, D. R, Ralphs, M. H., Pfister, J. A., Welch, K. D., and Green, B. T., 2009a. Swainsoninine concentrations and endophyte amounts of *Undifilum oxytropis* in different plant parts of *Oxytropis sericea*. *Journal of chemical ecology.* 35: 1272-1278.

Cook, B. D., Gardner, D. R., Welch, K. D., Roper, J. M., Ralphs, M. H., and Green, B. T. 2009b. Quantitative PCR method to measure the fungal endophyte in locoweeds. *Journal of Agricultural and Food Chemistry.* 57: 6050-6054.

Cook, B. D., Ralphs, M. H., Welch, K. D., and Stegelmeier, B. L. 2009c. Locoweed poisoning in livestock. *Rangelands*. 31:16-21.

Dorling, P. R., Huxtable, C. R., and Colegate, S. M. 1980. Inhibition of lysosomal alpha-mannosidase by swainsonine, an indolizidine alkaloid isolated from Swainsona canescens. *The Biochemical journal.* 191: 649-651.

Dreyer, D. L., Jones, K. C., and Molyneux, R. 1985. Feeding deterrence of some pyrrolizidine, indolizidine, and quinolizidine alkaloids towards pea aphid (*Acyrthosiphon pisum*) and evidence for phloem transport of indolizidine alkaloid swainsonine. *Journal of chemical ecology*. 11: 1045–1051.

Faeth, S. H. 2002a. Fungal endophytes: Common host plant symbionts but uncommon mutualists. *Integrative and Comparative Biology.* 42: 360-368.

Faeth, S. H., Bush, L. P., and Sullivan, T. J. 2002b. Peramine alkaloid variation in Neotyphodium-infected Arizona fescue: effects of endophyte and host genotype and environment. *Journal of chemical ecology*. 28: 1511-1526.

Gardner, D. R., and Cook, B. D. 2010. A comparison of alternative sample preparation procedures for the analysis of swainsonine using LC-MS/MS. *Phytochemical analysis.* 22: 124-127.

Gardner, D. R., Molyneux, R. J., and Ralphs, M. H. 2001. Analysis of swainsonine: extraction methods, detection, and measurement in populations of locoweeds (*Oxytropis* spp.). *Journal of agricultural and food chemistry.* 49: 4573-4580.

Gardner, D. R., J. Romero, M. H. Ralphs, and R. Creamer. 2004. Correlation of an endophytic fungus (*Alternaria* spp.) with the presence of swainsonine in lambert locoweed (*Oxytropis lambertii*). Pages 32–38 in Poisonous plants and related toxins. T. Acamovic, C. S. Stewart, and T. W. Pennycott, ed. Oxford University Press, Cary, NC.

Hunt, M. G., and Newman, J. A. 2005. Reduced herbivore resistance from a novel grass-endophyte association. *Journal of Applied Ecology*. 42: 762-769.

James, L. F., and Nielson, D. B. 1988. Locoweed assessment of the problem on Western US rangelands. Pages 171–180 in The ecology and economic impact of poisonous plants on livestock production. L. F. James, M. H. Ralphs, and D. B. Nielsen, ed. Westview Press, Boulder, CO.

James, L.F., Elbein, A.D., Molyneux, R. J., and Warren, C.D. ed. 1989. Swainsonine and related glycosidase inhibitors. Iowa State University Press, Ames, Iowa.

Leuchtmannj, A.D., Schmidt, and Bush, L. P. 2000. Different levels of protective alkaloids in grasses with stroma-forming and seed-transmitted *Epichloe*/*Neotyphodium* endophytes. *Journal of Chemical Ecology*. 26: 1025-1036.

Oldrup, E., McLain-Romero, J., Padilla, A., Moya, A., Gardner, D. R., and Creamer, R. 2010. Localization of endophytic *Undifilum* fungi in locoweed seed and influence of environmental parameters on a locoweed in vitro culture system. *Botany*. 88: 512-521.

Pfister, J. A., Panter, K. E., Gardner, D. R., Stegelmeier, B. L., Ralphs, M. H., Molyneux. R. J., and Lee, S. T. 2001. Alkaloids as anti-quality factors in plants on western U.S. rangelands. *Journal of Range Management*. 54: 447-461.

Pryor, B.M., Creamer, R., Shoemaker, R. A., McLain-Romero, J., and Hambleton, S. 2009. *Undifilum*, a new genus for endophytic *Embellisia oxytropis* and parasitic *Helminthosporium bornmuelleri* on legumes. *Botany*. 87:178-194.

Purvines, J., and Ralphs, M. H. 1999. Drought-stressed locoweed contains more swainsonine. Pages 34-35 in Locoweed research. T.M. Sterling and D.C. Thompson, ed. New Mexico Coop. Ext. Ser. Res. Rep.

Ralphs, M. H., Creamer, R., Baucom, D., Gardner, D. R., Welsh, S. L., Graham, J. D., Hart, C., Cook, B. D., and Stegelmeier, B. L. 2008. Relationship between the endophyte *Embellisia* spp. and the toxic alkaloid swainsonine in major locoweed species (*Astragalus* and *Oxytropis*). *Journal of Chemical Ecology*. 34: 32-38.

Ralphs, M. H., Gardner, D. R., Graham, D., Greathouse, G., and Knight, A. P. 2002. Influence of clipping and precipitation on locoweed vigor, mortality and toxicity. *Journal of Range Management*. 55: 394-399.

Ralphs, M. H., Graham, D., and James, L. F. 1994. Cattle grazing white locoweed in New Mexico: Influence of grazing pressure and phonological growth stage. *Journal of Range Management*. 47: 270-274.

Ralphs, M. H., Graham, D., Molyneux, R. J., and James, L. F. 1993. Seasonal grazing of locoweeds by cattle in northeastern New Mexico. *Journal of Range Management*. 46: 416-420.

Ralphs, M. H., Pfister, J. A., Welsh, S. L., Graham, J. D., Purvines, J., Jensen, D. T., and James, L. F. 1998. Outbreaks occur in wet years and die-off in drought. *Rangelands*. 25: 14-18.

Romero, J., Creamer, R., Ralphs, M. H., and Gardner, D. R. 2002. Association of a fungal endophyte with seed tissue and locoweed toxicity. *Phytopathology*. 92: S70.

Roylance, J. T., Hill, N. S., and Agee, C. S. 1994. Ergovaline and peramine production in endophyte-infected tall fescue: independent regulation and effects of plant and endophyte genotype. *Journal of Chemical Ecology*. 20: 2171-2183.

Stegelmeier, B. L., James, F. L., Panter, K. E., Gardner, D. R., Pfister, J. A., Ralphs, M. H., Molyneux, R. J. 1999. Dose response of sheep poisoned with locoweed (*Oxytropis sericea*). *Journal of Veterinary Diagnostic Investigation.* 11: 448–456.

Torell, L. A., Owen, L. P., Kirk, C., McDaniel, K. C., and Graham, D. 2000. Perceptions and economic losses from locoweed in northeastern New Mexico. *Journal of Range Management*. 53: 376-383.

Welsh, S. L., Ralphs, M. H., Panter, K. E., Pfister, J. A., and James, L. F. 2007. Locoweeds of North America; taxonomy and toxicity. Pages 20–29 in Poisonous Plants: Global Research and Solutions. K. E. Panter, and J. A. Pfister, ed., CABI. Wallingford, UK.

Wilson, D. 1995. Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos*. 73: 274-276.

White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. Sninsky and T. J. White, ed. Academic Press, New York, NY.