**A Swainsonine Survey of North American *Astragalus* and *Oxytropis* Taxa Implicated as
Locoweeds**

Daniel Cook^{1*}, Dale R. Gardner¹, Stephen T. Lee¹, James A. Pfister¹, Clinton A. Stonecipher¹,
and Stanley L. Welsh²

¹USDA/ARS Poisonous Plant Research Laboratory, 1150 East 1400 North, Logan, Utah
84341

²Brigham Young University, Provo, UT 84602

*Corresponding author (tel: (435) 752-2941; fax: (435) 753-5681; email:
daniel.cook@ars.usda.gov)

ABSTRACT

Swainsonine, an indolizidine alkaloid with significant physiological activity, is an α -mannosidase and mannosidase II inhibitor that causes lysosomal storage disease and alters glycoprotein processing. Swainsonine is found in a number of plant species worldwide, and causes severe toxicosis in livestock grazing these plants, leading to a chronic wasting disease characterized by weight loss, depression, altered behavior, decreased libido, infertility, and death. Swainsonine has been detected in 19 *Astragalus* and 2 *Oxytropis* species in North America by thin layer chromatography, gas chromatography–mass spectrometry, liquid chromatography-mass spectrometry and a jack bean α -mannosidase inhibition assay. In addition, 5 species in North America are presumed to contain swainsonine based upon reports from field cases. Many of these plant species have not been analyzed for swainsonine using modern instrumentation such as gas or liquid chromatography coupled with mass spectrometry. To provide clarification, 22 *Astragalus* species representing 93 taxa and 4 *Oxytropis* species representing 18 taxa were screened for swainsonine using both liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. Swainsonine was detected in 48 *Astragalus* taxa representing 13 species and 5 *Oxytropis* taxa representing 4 species. Forty of the fifty-three swainsonine-positive taxa had not been determined to contain swainsonine previously using liquid or gas chromatography coupled with mass spectrometry. The list of swainsonine-containing taxa reported here will serve as a reference for risk assessment and diagnostic purposes.

Keywords: *Astragalus*; *Oxytropis*; locoweed; swainsonine; herbarium

1. Introduction

Several species among two genera in the Fabaceae family, *Astragalus* and *Oxytropis*, are toxic to grazing livestock throughout North America, South America, and Asia (Marsh, 1909; Huang et al., 2003; Cook et al., 2014). Many species within these genera are non-toxic and are important forages; however, others are toxic to both livestock and wildlife. “Loco” is the Spanish word for crazy and has been used by the people of North America for over a century to describe the behavior and neurologic disease of animals poisoned by specific *Astragalus* and *Oxytropis* spp., while “locoweed” has been used to describe these plants that cause the poisoning. Locoism was first noted by De Soto and other Spanish explorers in the Southwestern United States in their horses. As the American frontier was settled, locoweeds along with other poisonous plants emerged as a serious livestock poisoning problem. Due to the significant number of livestock losses attributed to locoism throughout the west, C.D. Marsh of the USDA Bureau of Plant Investigations initiated studies and demonstrated through a series of experiments that *Astragalus* and *Oxytropis* species were the cause of locoism, and documented many of the clinical symptoms associated with this neurologic disease (Marsh, 1909; Marsh and Clawson, 1919; Burrows and Tyrl, 2001).

The clinical signs and pathology associated with this neurologic disease in animals is similar in *Astragalus* and *Oxytropis* species (Panter et al., 1999). Swainsonine (Figure 1), an indolizidine alkaloid, was first identified as the bioactive principle of a neurologic disease in *Swainsona canescens*, a legume native to Australia (Colegate et al., 1979). Swainsonine was later identified as the active principle in *A. lentiginosus* in the United States (Molyneux and James, 1982). Locoweeds are *Astragalus* and *Oxytropis* species that cause locoism and contain swainsonine. Swainsonine is an α -mannosidase and mannosidase II inhibitor that causes

lysosomal storage disease and alters glycoprotein processing (Colegate et al., 1979; Dorling et al., 1980; Tulsiani et al., 1988). Consumption of these plants by grazing livestock and wildlife leads to a chronic neurologic disease characterized by weight loss, depression, altered behavior, decreased libido, infertility, abortion, birth defects, and death (Panter et al., 1989; Cook et al., 2009a).

To date, the majority of plant species reported to contain swainsonine primarily result from episodes of livestock poisoning (Kingsbury, 1964; Cook et al., 2014). Many of these early reports of poisoning lacked experimental verification of the suspected plant as being the causal agent (Kingsbury, 1964). Subsequently, some initial swainsonine screening was performed on select species using thin-layer chromatography (Molyneux et al., 1991), and a jack bean (*Canavalia ensiformis*) α -mannosidase inhibition assay (Smith et al., 1992; Fox et al., 1998). Later, methods such as liquid and gas chromatography coupled with mass spectrometry were developed and utilized to identify swainsonine-containing *Astragalus* and *Oxytropis* species (Gardner et al., 2001). As a result, a list of plant species suspected of causing locoism coupled with suspected swainsonine-containing *Astragalus* and *Oxytropis* taxa of North America has been propagated throughout the literature (Table 1) (Ralphs et al., 2002; Cook et al., 2009a; Cook et al., 2014). Only eight of the 26 species implicated as locoweeds have been verified to contain swainsonine by liquid and/or gas chromatography coupled with mass spectrometry (Gardner et al., 2001; Ralphs et al., 2008).

Swainsonine is not a plant-derived secondary metabolite; but is produced by an endophyte associated with all swainsonine-containing *Astragalus* and *Oxytropis* species in North America and China investigated to date (Braun et al., 2003; Yu et al., 2010; Baucom et al., 2012; Gao et al., 2012). Initial reports described this endophyte as an *Alternaria* species (Braun et al.,

2003), then as an *Embellisia* species (Wang et al., 2006). Subsequently, it was described as a new genus, *Undifilum* (Pleosporales), that is phylogenetically related to the genera *Alternaria*, *Embellisia*, and *Ulocladium* (Pryor et al., 2009). More recent phylogenetic and morphological data resulted in the reclassification of the endophyte as *Alternaria* spp. section *Undifilum* (Woudenberg et al., 2013; Lawrence et al., 2016). Furthermore, a swainsonine-producing *Alternaria* spp. section *Undifilum* has also been identified in the Australian species *S. canescens* (Grum et al., 2013).

A systematic examination for swainsonine in these species would provide a definitive reference in regard to these species containing swainsonine and would be a valuable reference for land managers. Herbarium specimens provide an excellent resource to screen multiple specimens of several species in a relatively short period of time. Furthermore, a modified swainsonine extraction method was recently published that allows for the rapid extraction of hundreds of samples simultaneously (Gardner and Cook, 2011). Consequently, the objective of this study was to determine if the species listed in Table 1 contain swainsonine, and to determine the relative concentration of swainsonine using herbarium voucher specimens.

2. Materials and Methods

2.1. Plant Material

Samples from 22 *Astragalus* species representing 93 taxa (n=670 specimens) and from 4 *Oxytropis* species representing 18 taxa (n=211 specimens) were provided courtesy of the Stanley L. Welsh herbarium at Brigham Young University (BRY), the herbarium at the USDA ARS Poisonous Plant Research Laboratory (PPRL), and the herbarium at the Missouri Botanical Garden (MO). In general, vegetative tissues were sampled from the specimens. The number of samples from each taxon ranged from 1 to 83. Typically a minimum of 4 to 6 specimens were

sampled per taxon with exceptions based upon the availability of specimens and the geographic distribution of the species. Individual BRY, PPRL, and MO specimen accession numbers for the specimens sampled of the surveyed taxa are provided in Supplemental Table 1. Sampled plant material was analyzed for the presence of swainsonine.

2.2. *Swainsonine Analysis*

Swainsonine was extracted using a modification of the procedure described by Gardner and Cook (2011). Dried plant material (25 mg) was extracted in 2% acetic acid (1.5 mL) for 18 h with agitation. After extraction, samples were centrifuged and an aliquot (0.5 mL) from the extraction was diluted into 20 mM ammonium acetate (0.5 mL) in a 1 mL auto-sampler vial. Samples were analyzed by LC-MS/MS to quantitate swainsonine as previously described (Gardner et al., 2001). The detection limit of swainsonine was 0.001% of dry weight using this extraction procedure.

Any species that tested positive for swainsonine by the LC-MS/MS method was subsequently verified to contain swainsonine by GC-MS. In brief, a 0.1 mL aliquot of the acetic acid extract from a swainsonine positive species was added to a 8 mL screw cap glass vial and 2 mL of ammoniated methanol (1 to 10 dilution of methanol saturated with NH₃ with methanol) added. The solvent was removed by evaporation under a flow of nitrogen at 60°C. To the vial was then added 0.200 mL of pyridine and 0.050 mL of BSTFA silylation reagent (Supelco, Bellefonte, PA) and the vial capped and heated for 30 min at 60°C. After heating the samples were diluted with 1.0 mL of chloroform. For samples with a low concentration of swainsonine a 1 mL aliquot of the acetic acid extract was added to an SPE cartridge (Strata-XC 60 mg) (Phenomenex, Torrance, CA) that had been previously conditioned with methanol (2 mL) and water (2 mL). The SPE cartridge was washed with water (2 mL) and methanol (2 mL) and

swainsonine eluted with 3 mL of ammoniated methanol. The ammoniated methanol eluent was evaporated to dryness under a gentle stream of N₂ at 60°C. Pyridine (0.200 mL) and BSTFA silylation reagent (0.050 mL) were added and the sample heated (60 °C) for 30 min and then transferred to an autosample vial with a 300 µL glass insert. All samples were analyzed by GC-MS for swainsonine (TMS derivative) using the GC-MS conditions previously described (Gardner et al., 2001).

3. Results and Discussion

Swainsonine concentrations in locoweeds have been shown to vary among species, varieties, and populations often ranging from not being detected to 0.4% (Gardner et al. 2001; Ralphs et al. 2008). Previous to this report, swainsonine had been detected by LC-MS/MS in 12 *Astragalus* and 2 *Oxytropis* taxa including: *A. amphioxys*; *A. lentiginosus* vars. *araneosus*, *diphysus*, *lentiginosus*, and *wahweapensis*; *A. mollissimus* vars. *bigelovii*, *earlei*, *matthewsii*, *mollissimus*, and *thompsonae*; *A. pubentissimus*; *A. wootoni*; *O. lambertii*; and *O. sericea* (Gardner et al., 2001; Ralphs et al., 2008; Delaney et al., 2011). In this study, swainsonine was detected in 13 of the 14 above mentioned taxa where swainsonine had been detected previously, the exception being *A. mollissimus* var. *matthewsii* (Tables 2 and 3). It was not surprising that swainsonine was not detected in this taxon as the concentrations reported previously were approximately 0.001%, essentially at the detection threshold (Delaney et al., 2011). Among the 13 taxa, mean swainsonine concentrations in the herbarium voucher specimens were similar to the concentrations reported from field collections (Tables 2 and 3) (Ralphs et al., 2008). For example, mean concentrations in *A. pubentissimus* from field collections were 0.21% (Ralphs et al., 2008) while the mean concentration found in the herbarium voucher specimens was 0.16%. Likewise, mean concentrations in *A. mollissimus* var. *mollissimus* from field collections were

0.14% (Ralphs et al., 2008) while the concentration found in the herbarium voucher specimens was 0.11%. One exception to this observation was *A. lentiginosus* var. *diphysus* where mean concentrations from field collections were 0.23% (Ralphs et al., 2008) while the mean concentration found in herbarium voucher specimens was 0.05%. Differences in swainsonine concentrations between field collections (Ralphs et al., 2008) and the data reported may be due to differences in sites of collection and/or the time of year the specimens were collected, both of which have been shown to influence swainsonine concentrations (Achata Böttger et al., 2012; Cook et al., 2012; 2013)

Among the remaining 81 *Astragalus* taxa and 16 *Oxytropis* taxa surveyed, swainsonine was detected in 37 *Astragalus* taxa representing 10 species and 3 *Oxytropis* taxa representing 2 species (Tables 2 and 3). Swainsonine had been detected previously in many of these taxa using TLC and/or the jack bean α -mannosidase assay (Molyneux et al., 1991; Smith et al., 1992; Fox et al., 1998). Swainsonine concentrations ranged from 0.001% to 0.4% among specimens from the different taxa. The variation in swainsonine concentrations reported here is consistent with field observations where swainsonine concentrations segregate into two different chemotypes; one which has swainsonine concentrations greater than 0.01%, and the other which has concentrations much less than 0.01% (generally near 0.001% or not detected) (Cook et al., 2009b; 2011). Using qPCR, it was determined that these two chemotypes differ significantly in the amount of endophyte, further supporting the differences in swainsonine concentrations of the respective host plants (Cook et al., 2009b; 2011).

Swainsonine concentrations in locoweeds vary among varieties often ranging from not being detected to 0.4% (Gardner et al., 2001; Ralphs et al., 2008). For example, in *A. mollissimus* var. *thompsonae*, and *O. lambertii* vars. *articulata* and *lambertii*, mean swainsonine

concentrations were less than 0.001% in all populations, yet in other varieties within these species mean concentrations were greater than 0.01% (Gardner et al., 2001; Ralphs et al., 2008). Similar observations in swainsonine concentrations are present in the data reported among other species with multiple varieties such as *A. lentiginosus*, *O. besseyi*, and *O. campestris*. For example, in *O. besseyi* vars. *salmonensis* and *besseyi* as well as *O. campestris* var. *dispar* mean swainsonine concentrations were greater than 0.03% while in other varieties of these respective species, no swainsonine was detected (Table 3). Likewise, in *A. lentiginosus* vars. *fremontii* and *wahweapensis* mean swainsonine concentrations were greater than 0.1% while in other varieties such as *A. lentiginosus* vars. *chartaceus*, *platyphyllidius*, and *pohlui* no swainsonine was detected (Table 2).

Swainsonine concentrations vary considerably among the three varieties and populations of *O. lambertii* vars. *articulata*, *bigelovii*, and *lambertii*. In the 10 populations of *O. lambertii* var. *bigelovii* surveyed by Gardner et al., (2001), all the plants in 5 populations contained mean swainsonine concentrations less than 0.001% while in the other 5 populations mean swainsonine concentrations were greater than 0.01%. Additionally swainsonine concentrations were less than 0.001% in all the plants from the 6 populations of *O. lambertii* vars. *articulata* and *lambertii* (Gardner et al., 2001). Populations that contained swainsonine were found in parts of southern Utah, parts of Arizona, and Southwest New Mexico (Gardner et al., 2001). Eighty-three specimens from thirteen states representing the three different varieties of *O. lambertii* were surveyed in the current study. Swainsonine was detected in fifteen specimens, all of which had been collected in the same geographical region as the swainsonine-containing field collections of *O. lambertii* var. *bigelovii* with one exception, a specimen of *O. lambertii* var. *articulata* collected in Oklahoma. In summary, the swainsonine data in regard to the herbarium specimens

of *O. lambertii* was consistent with the previous report from field collections (Gardner et al., 2001).

Swainsonine concentrations have been shown to differ spatially among populations of *O. sericea*. Research has demonstrated that the endophyte genotype, not the plant genotype nor differences in endophyte amount, was responsible for differences in swainsonine concentration between the populations (Cook et al., 2013). Additionally, *Alternaria* spp. (section *Undifilum*) from different locoweed species have been shown to produce different amounts of swainsonine (Braun et al., 2003). Among select varieties within *A. lentiginosus* and *A. mollissimus*, swainsonine concentrations vary considerably. For example, mean swainsonine concentrations in *A. lentiginosus* vars. *floribundus*, *fremontii*, *kennedyi*, *latus*, *wahweapensis*, and *wilsonii* were greater than 0.12% while concentrations in *A. lentiginosus* vars. *borreganus*, *higginsii*, *maricopae*, *mokiensis*, *palans*, and *variabilis* were less than 0.04% (Table 2). Likewise mean swainsonine concentrations in *A. mollissimus* var. *earlei* were 0.14% while concentrations in *A. mollissimus* var. *bigelovii* were 0.06% (Table 2). It would be of interest to confirm if these differences are observed within field collections and if so to determine if the genotype of the endophyte or plant and/or the environment are responsible for these concentration differences.

Swainsonine was detected infrequently in three *Astragalus* taxa previously not reported to contain swainsonine by LC-MS/MS, *A. flavus* var. *flavus*, *A. missouriensis*, and *A. purshii* var. *lagopinus* (Table 2). Concentrations in *Astragalus missouriensis* were similar to certain varieties within species such as *A. mollissimus*, and *O. lambertii* where concentrations are not detected or are very low among individuals within select varieties. *Astragalus flavus* and *A. purshii* were unique in that swainsonine concentrations were greater than 0.01% but occurred infrequently. Two possibilities may explain this observation. First, *A. flavus* and *A. purshii* populations are

consistently infected with the endophyte but at low frequencies leading to infrequently detected concentrations of swainsonine; secondly, some populations are infected by the endophyte at some unknown frequency whereas other populations are not infected at all. Further research is needed to clarify endophyte-swainsonine relationships in both species.

Swainsonine was not detected in several species suspected of causing locoism and/or previously reported to contain swainsonine including *A. bisulcatus*, *A. didymocarpus*, *A. drummondii*, *A. emoryanus*, *A. humistratus*, *A. lonchocarpus*, *A. nothoxys*, *A. praelongus*, and *A. tephrodes* (Table 2). The reports implicating these plants in causing locoism and/or containing swainsonine do not report voucher specimens making it impossible to experimentally verify these reports (see Table 1 for references). Reasons for the lack of congruence between the initial reports and the data reported herein may be due to misidentification of the plant material representing the species of interest, lack of robustness in the method of detection, and/or confusion of the clinical signs in poisoned animals with other poisonous principles. For example, in the case of *A. emoryanus*, previously reported to contain swainsonine by TLC, enzyme, and MS, it is possible that the plant material collected was misidentified as *A. emoryanus* (Davis et al., 1984). The plant material in this report may be *A. mollissimus* var. *earlei* as it grows in the same geographic region as *A. emoryanus* and contains swainsonine. It is also possible that misidentification of the plant material used for analysis contributed to misinformation regarding the TLC data in the cases of *A. bisulcatus*, *A. didymocarpus*, and *A. praelongus* (Molyneux et al., 1991). Additionally, the lack of robustness or repeatability in the jack bean α -mannosidase assay or misidentification of the plant material used for the analysis may have led to false positive results in the case of *A. bisulcatus*, *A. drummondii*, *A. lonchocarpus*, *A. praelongus*, and *A. tephrodes* (Smith et al., 1992; Fox et al., 1998). Experience

in our laboratory has been that the jack bean assay is problematic in terms of repeatability (B. Stegelmeier, personal communication). Nonetheless the jack bean α -mannosidase assay gave results consistent with the current survey in the case of some *Astragalus* taxa including *A. allochrous*, *A. mollissimus*, and *A. thurberi* (Smith et al., 1992; Fox et al., 1998).

Some *Astragalus* spp. are known to contain nitrotoxins which can result in acute or chronic toxicity. Severely poisoned animals may have difficulty breathing, and loss of coordination in the hindquarters. Chronically poisoned animals may show depression, incoordination, weight loss and diarrhea. Sometimes they show a tendency to have their rear heels “click” together (called cracker heels) when they walk (Williams and Barneby, 1977). These clinical signs may often be confused with the clinical signs of animals poisoned by locoweeds resulting in a plant being mischaracterized as a locoweed. Three species, *A. emoryanus*, *A. nothoxys*, and *A. thurberi*, listed in Table 1 that were thought to be locoweeds do not contain swainsonine; however, all three are reported to contain nitrotoxins (Williams and Barneby, 1977).

The presence and lack or the relative amount of swainsonine in some taxa of select species may correlate with taxonomic characters in the taxa of interest. For example, the populations of *O. lambertii* var. *bigelovii* that contain swainsonine have unique morphological characters, although not to the extent of a formal taxonomic rank, that differ from other populations of the same variety that do not contain or contain very little swainsonine (Welsh, 2001). Likewise, *A. lentiginosus* vars. *chartaceus*, *platyphyllidius*, and *pohlui* do not contain swainsonine and have common morphological characters among the three that differ from other varieties of *A. lentiginosus* that contain swainsonine (Barneby, 1964; Welsh, 2007). Lastly, *A. mollissimus* vars. *matthewsii*, *mogollonicus*, and *thompsonae* have similar morphological

characters among the three that differ from some of the other varieties of *A. mollissimus* that contain swainsonine (Barneby, 1964; Welsh, 2007). Similar correlations in morphological characters may be present among other taxa that differ in their relative swainsonine content, though this remains to be investigated. This morphological and chemotaxonomic correlation may correlate to the phylogenetic relatedness of these select taxa. For example, it has been reported using restriction fragment length polymorphism of two chloroplast genes that *A. mollissimus* vars. *matthewsii* and *thompsonae* were more closely related than *A. mollissimus* var. *mogollonicus* which was found to be more closely related to *A. mollissimus* vars. *earlei* and *mollissimus* (Kulshreshtha et al., 2004).

Rupert Barneby's taxonomic treatment, *Atlas of North American Astragalus*, divided *Astragalus* species into different groups based upon morphological characters (Barneby, 1964). All of the swainsonine-containing *Astragalus* taxa identified in this survey belong to the Piptoloboid Phalanx with the exception of *A. flavus*. The Piptoloboid Phalanx is subsequently divided into sections and subsections. Four sections within the Piptoloboid Phalanx contain the majority of the swainsonine-containing taxa identified in this survey: 1) Section *Diphyssi* contains the 31 varieties of *A. lentiginosus* reported to contain swainsonine; 2) Section *Mollissimi* contains the six varieties of *A. mollissimus* reported to contain swainsonine; 3) Section *Inflatii* subsection *Aridi* contains four swainsonine containing species, *A. allochrous*, *A. pubentissimus*, *A. thurberi*, and *A. wootoni*; and 4) Section *Densifolii* contains two swainsonine containing species, *A. oxyphysus* and *A. pycnostachyus* (Table 1).

The *Astragalus* genus is extremely speciose and complex morphologically and phylogenetically (Barneby, 1964; Wojciechowski et al., 1999). Molecular phylogenetic data supports a well-defined clade composed of New World *Astragalus* spp., the Neo-Astragalus

(Wojciechowski et al., 1999). However, within the Neo-Astragalus there is generally poor resolution with only a few well defined clades representing species pairs (Wojciechowski et al., 1999). One exception is a clade composed of North American species representing sections *Densifolii*, *Inflati*, and *Trichopodi* with four swainsonine containing species, *A. allochrous*, *A. asymmetricus*, *A. oxyphysus*, and *A. thurberi*, in a single well-supported clade. This clade also contains *A. pehuenches*, a swainsonine-containing *Astragalus* species from South America (Molyneux and Gomez-Sosa, 1991). It is probable that this clade may contain morphologically similar species that belong to the *Densifolii* and *Inflati* sections that are reported to contain swainsonine including *A. pubentissimus*, *A. pycnostachyus*, and *A. wootoni*. This data in total suggests that there may be a morphological and/or phylogenetic signature for swainsonine-containing species. Sections *Densifolii*, *Inflati*, and *Trichopodi* are composed of several additional species that may contain swainsonine, and a survey of swainsonine in these additional species within these sections merits further investigation. Furthermore, this clade contains several South American *Astragalus* spp. in which the presence of swainsonine should be determined (Scherson et al., 2008). Further supporting the hypothesis that there may be a phylogenetic signature for swainsonine-containing species is the fact that *A. lentiginosus* and *A. purshii* cluster together in a well-supported clade and both contain swainsonine (Wojciechowski et al., 1999).

In summary, 22 *Astragalus* species representing 93 taxa and 4 *Oxytropis* species representing 18 taxa were screened for swainsonine. Swainsonine was detected in 48 *Astragalus* taxa representing 13 species and 5 *Oxytropis* taxa representing 4 species. Forty of the fifty-three swainsonine-positive taxa had not been determined to contain swainsonine previously using liquid or gas chromatography coupled with mass spectrometry. The data suggests there may be a

326 morphological and phylogenetic signature for *Astragalus* species that contain swainsonine.
327 Additionally, swainsonine was not detected in several species heretofore thought to contain
328 swainsonine or be termed locoweeds. This data will be a valuable tool for land managers and
329 highlights the utility of herbarium specimens in phytochemical studies.

330 **Acknowledgements**

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332 We thank Jessie Roper and Scott Larsen for their technical assistance. We thank the Stanley L.

333 Welsh herbarium at Brigham Young University (BRY), the herbarium at the USDA ARS

334 Poisonous Plant Research Laboratory (PPRL), and the herbarium at the Missouri Botanical

335 Garden (MO).

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484 Table 1. Summary of the *Astragalus* and *Oxytropis* Species Suspected of Causing Locoism
 485 and/or Reported to have Swainsonine as well as the Swainsonine Detection Methods.

Species	Taxonomic Section ^a	Swainsonine detection	References
<i>Astragalus allochrous</i>	Inflati	Enzyme	Smith et al., 1992; Fox et al., 1998
<i>Astragalus amphioxys</i>	Argophylli	LC/MS	Ralphs et al., 2008
<i>Astragalus asymmetricus</i>	Trichopodi	TLC	Molyneux et al., 1991
<i>Astragalus bisulcatus</i>	Bisulcati	TLC, Enzyme	Molyneux et al., 1991; Smith et al., 1992; Fox et al., 1998
<i>Astragalus didymocarpus</i>	Microlobium	TLC	Molyneux et al., 1991
<i>Astragalus drummondii</i>	Drummondiani	Enzyme	Smith et al., 1992; Fox et al., 1998
<i>Astragalus emoryanus</i>	Leptocarpi	TLC, Enzyme, MS	Davis et al., 1984; Molyneux et al., 1991
<i>Astragalus flavus</i>	Ocreati	TLC	Molyneux et al., 1991
<i>Astragalus humistratus</i>	Humistrati		Ralphs et al., 2002
<i>Astragalus lentiginosus</i>	Diphysi	TLC, LC/MS	Molyneux et al., 1991; Ralphs et al., 2008
<i>Astragalus lonchocarpus</i>	Lonchocarpi	Enzyme	Smith et al., 1992; Fox et al., 1998
<i>Astragalus missouriensis</i>	Argophylli	Enzyme	Smith et al., 1992; Fox et al., 1998
<i>Astragalus mollissimus</i>	Mollissimi	TLC, Enzyme, LC/MS	Molyneux et al., 1991; Smith et al., 1992; Fox et al., 1998; Ralphs et al., 2008
<i>Astragalus nothoxys</i>	Leptocarpi		Kingsbury, 1964
<i>Astragalus oxyphysus</i>	Densifolii	TLC	Molyneux et al., 1991
<i>Astragalus praelongus</i>	Preussiani	TLC / Enzyme	Molyneux et al., 1991; Smith et al., 1992; Fox et al., 1998
<i>Astragalus pubentissimus</i>	Inflati	LC/MS	Ralphs et al., 2008
<i>Astragalus purshii</i>	Argophylli		Ralphs et al., 2002
<i>Astragalus pycnostachyus</i>	Densifolii	TLC	Molyneux et al., 1991
<i>Astragalus tephrodes</i>	Argophylli	Enzyme	Smith et al., 1992; Fox et al., 1998
<i>Astragalus thurberi</i>	Inflati	Enzyme	Smith et al., 1992; Fox et al., 1998
<i>Astragalus wootoni</i>	Inflati	TLC, LC/MS	Molyneux et al., 1991; Ralphs et al., 2008
<i>Oxytropis besseyi</i>			Kingsbury, 1964
<i>Oxytropis campestris</i>			Kingsbury, 1964
<i>Oxytropis lambertii</i>		TLC, Enzyme, GC/MS, LC/MS	Molyneux et al., 1991; Fox et al., 1998; Gardner et al., 2001; Ralphs et al., 2008
<i>Oxytropis sericea</i>		TLC, Enzyme, LC/MS	Molyneux et al., 1991; Smith et al., 1992; Fox et al., 1998; Ralphs et al., 2008

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487 ^aTaxonomic section according to Barneby (1964).

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489 Table 2. *Astragalus* taxa surveyed for swainsonine, number of specimens surveyed, number of
490 specimens containing swainsonine, and swainsonine concentrations (mean % $\pm$ standard error).

Genus	Species	Variety	Number of Specimens Surveyed	Number of Specimens containing Swainsonine	Swainsonine (Mean % $\pm$ S.E.)
<i>Astragalus</i>	<i>allochrous</i>		8	8	0.18 $\pm$ 0.03
<i>Astragalus</i>	<i>amphioxys</i>	<i>amphioxys</i>	10	n.d.	
		<i>modestus</i>	8	n.d.	
		<i>musimonum</i>	2	1	0.22
		<i>vesperinus</i>	13	n.d.	
		*	17	n.d.	
<i>Astragalus</i>	<i>asymmetricus</i>		5	5	0.22 $\pm$ 0.07
<i>Astragalus</i>	<i>bisulcatus</i>	<i>bisulcatus</i>	10	n.d.	
		<i>haydenianus</i>	13	n.d.	
		<i>major</i>	13	n.d.	
		<i>nevadensis</i>	4	n.d.	
		*	5	n.d.	
<i>Astragalus</i>	<i>didymocarpus</i>	<i>didymocarpus</i>	5	n.d.	
		<i>dispermus</i>	6	n.d.	
<i>Astragalus</i>	<i>drummondii</i>		24	n.d.	
<i>Astragalus</i>	<i>emoryanus</i>		15	n.d.	
<i>Astragalus</i>	<i>flavus</i>	<i>argillosus</i>	6	n.d.	
		<i>flavus</i>	13	2	0.04 $\pm$ 0.04
		<i>higginsii</i>	5	n.d.	
		*	11	n.d.	
<i>Astragalus</i>	<i>humistratus</i>	<i>crispulus</i>	1	n.d.	
		<i>hosackiae</i>	3	n.d.	
		<i>humistratus</i>	5	n.d.	
		<i>humivagans</i>	10	n.d.	
		<i>sonorae</i>	3	n.d.	
		<i>tenerimus</i>	3	n.d.	
		*	6	n.d.	
<i>Astragalus</i>	<i>lentiginosus</i>	<i>ambiguus</i>	4	4	0.06 $\pm$ 0.02
		<i>araneosus</i>	17	13	0.06 $\pm$ 0.005
		<i>australis</i>	4	2	0.03 $\pm$ 0.005
		<i>borreganus</i>	4	3	0.01 $\pm$ 0.006
		<i>chartaceus</i>	3	n.d.	
		<i>coachellae</i>	4	2	0.01 $\pm$ 0.008
		<i>diphysus</i>	12	9	0.05 $\pm$ 0.02
		<i>floribundus</i>	4	4	0.12 $\pm$ 0.03
		<i>fremontii</i>	5	5	0.14 $\pm$ 0.03
		<i>higginsii</i>	4	4	0.01 $\pm$ 0.002
		<i>idriensis</i>	1	n.d.	
		<i>ineptus</i>	3	1	0.013
		<i>kennedyi</i>	5	5	0.13 $\pm$ 0.04
		<i>kernensis</i>	1	1	0.04
		<i>latus</i>	4	3	0.17 $\pm$ 0.04
		<i>lentiginosus</i>	5	1	0.16
		<i>macrolobus</i>	4	4	0.07 $\pm$ 0.03
		<i>maricopae</i>	4	4	0.04 $\pm$ 0.02
		<i>micans</i>	2	2	0.002 $\pm$ 0.0004
		<i>mokiensis</i>	6	6	0.04 $\pm$ 0.007
		<i>negundo</i>	1	n.d.	
		<i>nigricalcis</i>	4	3	0.002 $\pm$ 0.0004
		<i>oropedii</i>	4	3	0.07 $\pm$ 0.06
		<i>palans</i>	5	4	0.02 $\pm$ 0.003
		<i>piscinensis</i>	1	n.d.	
		<i>platyphyllidius</i>	7	n.d.	
		<i>pohlii</i>	4	n.d.	
		<i>salinus</i>	5	3	0.08 $\pm$ 0.03
		<i>scorpionis</i>	3	2	0.14 $\pm$ 0.009
		<i>semotus</i>	1	n.d.	
		<i>sesquimetralis</i>	1	n.d.	
		<i>stramineus</i>	7	3	0.04 $\pm$ 0.007
		<i>terabensis</i>	2	2	0.05 $\pm$ 0.006
		<i>trambullensis</i>	3	1	0.06
		<i>variabilis</i>	6	6	0.03 $\pm$ 0.01
		<i>vitreus</i>	5	5	0.09 $\pm$ 0.03
		<i>wahweapensis</i>	7	7	0.13 $\pm$.006
		<i>wilsonii</i>	4	3	0.14 $\pm$ 0.02
		<i>yucannus</i>	3	3	0.008 $\pm$ 0.003
<i>Astragalus</i>	<i>lonchocarpus</i>		17	n.d.	
<i>Astragalus</i>	<i>missouriensis</i>		33	1	0.002
<i>Astragalus</i>	<i>mollissimus</i>	<i>bigelovii</i>	6	6	0.06 $\pm$ 0.02
		<i>coryi</i>	2	2	0.14 $\pm$ 0.03
		<i>earlei</i>	6	5	0.15 $\pm$ 0.01
		<i>tolanus</i>	1	1	0.16
		<i>marcidus</i>	1	n.d.	
		<i>matthewsii</i>	5	n.d.	
		<i>mogollonicus</i>	2	n.d.	
		<i>mollissimus</i>	9	9	0.11 $\pm$ 0.03
		<i>thompsonae</i>	21	2	0.001 $\pm$ 0.0003
<i>Astragalus</i>	<i>nothoxys</i>		9	n.d.	
<i>Astragalus</i>	<i>oxyphysus</i>		7	7	0.10 $\pm$ 0.007
<i>Astragalus</i>	<i>praelongus</i>	<i>avonensis</i>	4	n.d.	
		<i>ellisiae</i>	14	n.d.	
		<i>lonchopus</i>	6	n.d.	
		<i>praelongus</i>	19	n.d.	
<i>Astragalus</i>	<i>pubentissimus</i>		15	14	0.16 $\pm$ 0.02
<i>Astragalus</i>	<i>purshii</i>	<i>concinus</i>	3	n.d.	
		<i>glaucescus</i>	13	n.d.	
		<i>lagopinus</i>	8	1	0.06
		<i>lectulus</i>	4	n.d.	
		<i>ophiogenes</i>	3	n.d.	
		<i>pumilio</i>	3	n.d.	
		<i>purshii</i>	26	n.d.	
		<i>tinctus</i>	7	n.d.	
<i>Astragalus</i>	<i>pyncostachyus</i>		5	5	0.20 $\pm$ 0.07
<i>Astragalus</i>	<i>tephrodes</i>	<i>brachylobus</i>	7	n.d.	
		<i>tephrodes</i>	6	n.d.	
<i>Astragalus</i>	<i>thurberi</i>		6	6	0.16 $\pm$ 0.03
<i>Astragalus</i>	<i>wootoni</i>		9	8	0.26 $\pm$ 0.02

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Table 3. *Oxytropis* taxa surveyed for swainsonine, number of specimens surveyed, number of specimens containing swainsonine, and swainsonine concentrations (mean % $\pm$ standard error).

			Number of	Number of	Swainsonine
Genus	Species	Variety	Specimens Surveyed	Specimens containing Swainsonine	(Mean % $\pm$ S.E.)
<i>Oxytropis</i>	<i>besseyi</i>	<i>argophylla</i>	3	n.d.	
		<i>besseyi</i>	4	2	0.03 $\pm$ 0.002
		<i>fallax</i>	2	n.d.	
		<i>obnapiformis</i>	4	n.d.	
		<i>salmonensis</i>	5	4	0.03 $\pm$ 0.005
<i>Oxytropis</i>	<i>campestris</i>	<i>ventosa</i>	4	n.d.	
		<i>chartacea</i>	1	n.d.	
		<i>columbiana</i>	4	n.d.	
		<i>cusickii</i>	10	n.d.	
		<i>davisii</i>	6	n.d.	
		<i>dispar</i>	4	4	0.09 $\pm$ 0.02
		<i>jordalii</i>	4	n.d.	
		<i>minor</i>	3	n.d.	
		<i>spicata</i>	15	n.d.	
		<i>varians</i>	5	n.d.	
		*	3	n.d.	
<i>Oxytropis</i>	<i>lambertii</i>	83	15	0.06 $\pm$ 0.009	
<i>Oxytropis</i>	<i>sericea</i>	46	39	0.08 $\pm$ 0.007	
<i>Oxytropis</i>	<i>sericea</i> $\times$ <i>campestris</i>	5	n.d.		

498 **FIGURE LEGENDS**

499

500 Figure 1. Structure of the indolizidine alkaloid swainsonine

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Figure 1.

Swainsonine