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A swainsonine survey of North American *Astragalus* and *Oxytropis* taxa implicated as locoweeds

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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.

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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 (Fig. 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

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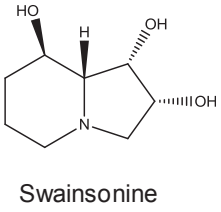

Fig. 1. Structure of the indolizidine alkaloid swainsonine.

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., 1999; 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, 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

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

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

[a] Taxonomic section according to Barneby (1964).