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Fungicide treatment and clipping of *Oxytropis sericea* does not disrupt swainsonine concentrations

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ABSTRACT

Swainsonine, an indolizidine alkaloid, 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 is produced by associated endophytic fungi. Prolonged consumption of swainsonine-containing plants by livestock causes a condition characterized by weight loss, depression, altered behavior, decreased libido, infertility, and death. In contrast, *Astragalus* and *Oxytropis* that do not contain swainsonine may present a valuable food source for grazing livestock in regions where palatable forage is scarce. This study tested the hypothesis that swainsonine concentrations may be reduced by fungicide treatment or by clipping, thus reducing plant toxicity. Additionally we hypothesized that clipping plants may provide a mechanism for horizontal transmission of the endophyte. To this end, four different fungicides were applied to render the endophyte non-viable, and plant vegetative tissues were periodically clipped. Treatment of *Oxytropis sericea* with any of four different fungicides did not alter swainsonine concentrations in plants at any of three harvest times. Additionally, we found that individual or multiple clippings had no effect on swainsonine concentrations; plants that contained swainsonine maintained concentrations, and plants low or absent in swainsonine also remained as such at each harvest. These results suggest that there is no evidence of horizontal transmission of the endophyte among individual plants due to clipping.

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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” has been used 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. Consumption of these plants by grazing livestock and wildlife leads to a chronic neurologic disease (i.e., locoism) characterized by weight loss, depression, altered behavior, decreased libido, infertility, abortion, birth defects, and death (Panter et al., 1999).

Swainsonine (Fig. 1), an indolizidine alkaloid, was first identified as the bioactive principle in *Swainsona canescens*, a legume native to Australia that causes a neurologic disease (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, subsequently leading to neurologic and other disease conditions (Colegate et al., 1979; Dorling et al., 1980; Tulsiani et al., 1988).

Swainsonine is produced by an endophyte associated with all swainsonine-containing *Astragalus*, *Oxytropis*, and *Swainsona* species investigated to date (Braun et al., 2003; Yu et al., 2010; Baucom et al., 2012; Grum et al., 2013). Initial reports described this endophyte as an *Embellisia* species (Wang et al., 2006). Subsequently, it was described as the genus *Undifilum* (Pleosporales) that is phylogenetically related to the genera *Alternaria*, *Embellisia*, and *Ulocladium* (Pryor et al., 2009), and more recently they have been

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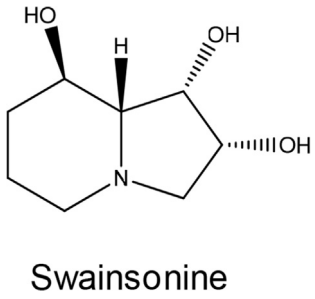

Fig. 1. Structure of the indolizidine alkaloid swainsonine.

reclassified as *Alternaria* spp. Section *Undifilum* (Woudenberg et al., 2013; Lawrence et al., 2016).

Swainsonine concentrations differ among individuals within toxic populations of *Astragalus* and *Oxytropis* spp. (Gardner et al., 2001; Ralphs et al., 2008). Plants can be generally grouped into two groups, namely chemotype 1 plants, which have swainsonine concentrations higher than 0.01%, and chemotype 2 plants, which have concentrations much lower than 0.01% (generally near 0.001% or not detected). These two chemotypes differ significantly in the amount of endophyte in that chemotype 1 plants have more endophyte than chemotype 2 plants (Cook et al., 2009, 2011). Additionally, plants derived from embryos where the seed coat was removed contain little or no swainsonine (Oldrup et al., 2010; Grum et al., 2012).

Contrasting results have been reported about the application of fungicides to seeds and/or leaves of swainsonine-containing plants. Application of pyraclostrobin to seeds of swainsonine-containing *Astragalus* and *Oxytropis* spp. as well as *Ipomoea carnea* resulted in plants that lacked swainsonine (Grum et al., 2012; Cook et al., 2013). In contrast, Barillas et al. (2007) reported that application of thiophanate ethyl, a nucleic acid synthesis inhibitor, (Cleary's 3336®) to seeds and leaves of *O. sericea* resulted in decreased swainsonine concentrations in plants derived from fungicide-treated seeds and no effect on swainsonine concentrations in plants where leaves were fungicide-treated. Differences in these results may be due to the different modes of action of the respective fungicides and/or method of treatment.

Plant defense theory suggests defoliation may alter the production of secondary compounds in some plants (Tallamy and Raupp, 1991). Defoliation has been shown to reduce alkaloid concentrations in plant species such as tall fescue (Belesky and Hill, 1997) and lupine (Johnson et al., 1987). Removal of plant tissue by herbivores, and changes in plant nitrogen status due to changes in soil or external nitrogen inputs may alter concentrations of nitrogenous toxins such as swainsonine. Swainsonine has been shown to respond positively in *O. sericea* associated with *Rhizobium* (Barillas et al., 2007) but no changes were observed in swainsonine concentrations in several locoweed species in response to deficient and supplemental nitrogen (Delaney et al., 2011).

Clavicipitaceous endophytes associated with the Poaceae have been shown to be vertically transmitted (i.e., parent to offspring), horizontally transmitted (i.e., individual to individual), or transmitted by both mechanisms (Panaccione et al., 2014). Recently, Wiewióra et al. (2015) reported horizontal transmission of *Neotyphodium lolii* in perennial ryegrass in response to mowing. To date, endophytes associated with locoweeds have only been shown to be transmitted vertically (Oldrup et al., 2010; Ralphs et al., 2011).

We hypothesized that fungicide application and clipping vegetative plants may alter swainsonine concentrations thus rendering the plant less toxic. Additionally we hypothesized that clipping plants may provide a mechanism for horizontal transmission of the endophyte. If fungicide application and/or clipping are successful in reducing swainsonine concentration these tools may be used to provide a valuable food source for grazing livestock in regions where palatable forage is scarce. To test these hypotheses, the objectives of this study were to determine 1) if fungicide application to vegetative plants would reduce the viability of the endophyte as shown by reduced swainsonine concentrations, 2) if swainsonine concentrations are altered as a function of plant clipping, and 3) if there is any evidence of horizontal transmission of the endophyte, thus swainsonine concentrations, between chemotype 1 and chemotype 2 plants due to clipping.

2. Materials and methods

2.1. Plant materials

Seed was collected from *Oxytropis sericea* plants from the poisonous plant garden at the USDA ARS Poisonous Plant Research Laboratory. The plants in the garden were derived from *O. sericea* seeds collected from Park Valley, UT (N 41° 54′ 15.4", W 113° 20′ 54.9"). Plants derived from the above mentioned seeds were grown in the greenhouse with a 16 h photoperiod and day/night temperatures of 25°C/20 °C. Plants were grown in cone-tainers (Stuewe and Sons Inc., Tangent, Oregon) with a 1:1:1 sand, top soil and potting soil mixture. Seeds were planted on March 1, 2015 and the resulting plants were sprayed with one of four fungicides on May 13, 2015. Subsequently plants were clipped on May 27, 2015, allowed to regrow, clipped again on July 19, 2015, allowed to regrow, and clipped on August 20, 2015. Plants were clipped and allowed to regrow several times as we suspected that if the fungicide treatments were effective, plants at harvest 1 would contain equivalent concentrations of swainsonine, plants at harvest 2 would contain significantly reduced amounts of swainsonine, and plants at harvest 3 would not contain swainsonine. Plants were clipped when the plants generally had 5-8 expanded leaves and only one leaf remained on each plant after clipping. The clipped vegetative material was frozen, and then subsequently freeze-dried and ground.

2.2. Fungicide treatments

Plants (25 per treatment) were treated with four different fungicides at two rates (i.e., recommended and 2× recommended rate) plus a control treatment (n = 9 treatments). Treatments included: 1) pyraclostrobin (23.6% active ingredient, 2.09 lb ai/gal) (Headline®), a respiration inhibitor (FRAC group 11), applied at 12 and 24 fl oz per acre, 2) metconazole (8.6% active ingredient, 0.75 lb ai/gal) (Caramba®), a sterol synthesis inhibitor (FRAC group 3), applied at 17 and 34 fl oz per acre, 3) a premixture of pyraclostrobin and metconazole (12 and 7.4% active ingredient, 1.083 and 0.67 lb ai/gal, respectively) (Twinline®), a sterol synthesis and respiration inhibitor (FRAC group 3 and 11), at 9 and 18 fl oz per acre, and 4) a premixture of pyraclostrobin and fluxapyroxad (21.26 and 21.26% active ingredient, 2.09 and 2.09 lb ai/gal, respectively) (Merivon®), respiration inhibitors (FRAC group 7 and 11), applied at 6.7 and 13.4 fl oz per acre (http://www.frac.info/docs/default-source/publications/frac-mode-of-action-poster/frac-moa-poster-2016.pdf?sfvrsn=2). In terms of mobility, FRAC group 3 fungicides are xylem mobile, FRAC group 7 fungicides are locally systemic, FRAC group 11 fungicides are xylem mobile as well as being locally systemic. All fungicide treatments included a non-ionic surfactant (Activator 90®) sprayed at 0.25% v/v. Treatment applications were performed using an enclosed cabinet track sprayer calibrated to deliver 20 gallons per acre of spray solution through a single even-flat-fan nozzle (TeeJet, XR8002E®) at 30 psi.