



Locoweed Endophytes: A Review

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Abstract

Locoweeds, which are a common name for poisonous species of the genera *Astragalus* and *Oxytropis*, are toxic leguminous plants through a symbiotic association with the fungal endophyte *Alternaria* section *Undifilum*. The fungus produces an indolizidine alkaloid, swainsonine, which causes a toxicosis of grazing animals, named locoism. By what means stress from environment or genetics of locoweed endophytes affect fungal growth and synthesis of swainsonine as well as how fungal endophyte synthesizes swainsonine in locoweeds, in addition to disruption of the toxic alkaloid swainsonine in locoweed plants, have not been well identified. To answer the above questions, further research is needed as locoweed plants are most palatable to grazing animals especially when locoweed plants are at flowering stage. This review outlines the present situation on locoweed plants in China, production of swainsonine, detection and interaction of locoweed endophytes as well as their transmission within locoweed plants.

Keywords: Locoweeds; Locoweed endophytes; Swainsonine

Introduction

Locoweeds are toxic legume plants of the genera *Astragalus* and *Oxytropis* [1,2], Fabaceae family, which live symbiotically with the fungal endosymbionts being part to *Alternaria* section *Undifilum* [3]. Locoweed toxicity is due to a mutualistic association with a locoweed fungal endophyte of *Alternaria oxytropis* that produce swainsonine

(Figure 1) [4-6]. The classification of locoweed endophytes has passed through different changes where at first they were described as species of *Alternaria* [4], followed by species of *Embellisia* [7] and then, they were described as a new genus, *Undifilum oxytropis*, phylogenetically related to additional *Pleosporaceae* genera as *Alternaria*, *Embellisia*, and *Ulocladium* [5]. After that, morphological and phylogenetic data brought about the reclassification of these endophytes as *Alternaria* section *Undifilum* [6,8]. Locoweed endophytes colonize the plant hosts and are vertically transmitted by means of seeds [9]. The locoweed endophytes, classified as *Alternaria oxytropis*, are asexual and produce an alkaloid toxin, swainsonine [3,10], which is an agent of a neurological disease (locoism) after grazing animals consume the plants [1,7,11]. The fungal endophyte *Alternaria* section *Undifilum* produces an indolizidine alkaloid, swainsonine, which in the Golgi apparatus inhibits α -mannosidase and mannosidase II [12-16]. It was confirmed that as the concentration of swainsonine increases, the amount of endophytes also increases in locoweed plants and vice-versa [12,17].

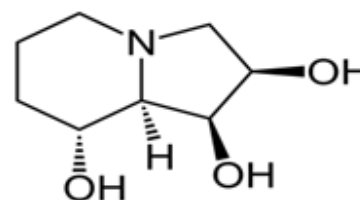


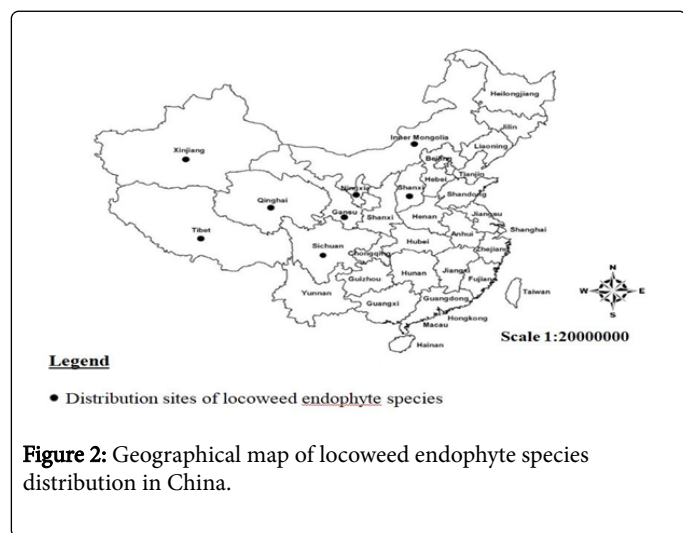
Figure 1: Structure of swainsonine [24-26].

It is in eight locoweed species that the isolation of endophytes was made for determining the presence of swainsonine-producing fungal endophyte for main locoweed species in China, and they are distributed in different parts of China (Table 1 and Figure 2) [18,19], while worldwide, fungal endophytes were isolated in sixteen locoweed species (Table 2). According to phylogenetic analyses, the fungal morphology and also based on adequate ITS sequences, these endophytes were reported as *Alternaria oxytropis* [18,19]. Fungal endophytes known as *Embellisia oxytropis* have been also isolated from some species of locoweed, *Oxytropis kansuensis* [7] and *Oxytropis glabra* [20,21] that grow far and wide on western rangelands of China. The morphological along with biological characteristics of these endophytes are related to that of swainsonine-producing endophytes isolated from locoweeds [18].

Locoweed species	Geographical distribution
<i>Astragalus variabilis</i>	Inner Mongolia, Gansu, Ningxia, Qinghai [45-47].
<i>Astragalus strictus</i>	Tibet [19,45].
<i>Oxytropis glacialis</i>	Tibet [19,45].
<i>Oxytropis kansuensis</i>	Gansu, Qinghai, Tibet, Sichuan [7,19,45,47]
<i>Oxytropis ochrocephala</i>	Inner Mongolia, Gansu, Ningxia, Qinghai, Tibet, Sichuan [19,45,48].
<i>Oxytropis sericopetala</i>	Tibet [19,45].

<i>Oxytropis glabra</i>	Inner Mongolia, Gansu, Xinjiang, Tibet, Shanxi, Ningxia [19,20,45,47,49]
<i>Oxytropis falcata</i>	Qinghai, Gansu, Sichuan, Inner Mongolia [45,47].

Table 1: Isolation and geographical distribution of locoweed endophyte species in China.

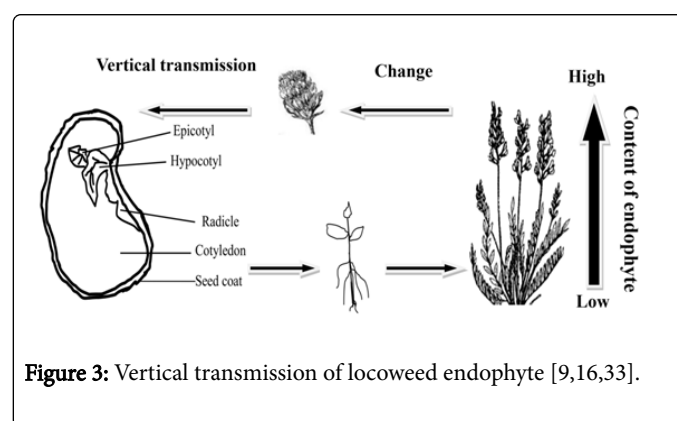


Locoweed species	Discovered place	Detection method	References
<i>Astragalus mollissimus</i>	USA	PCR	[50]
<i>A. lentiginosus</i>	USA	PCR	[51]
<i>A. agrestis</i>	USA	PCR	[51]
<i>A. amphioxys</i>	USA	PCR	[52]
<i>A. pubentissimus</i>	USA	PCR	[52]
<i>A. wootoni</i>	USA	PCR	[53]
<i>A. variabilis</i>	China	PCR	[45-47]
<i>A. strictus</i>	China	PCR	[19,45]
<i>Oxytropis sericea</i>	USA	PCR	[50]
<i>O. lambertii</i>	USA	PCR	[50]
<i>O. kansuensis</i>	China	PCR	[7]
<i>O. glabra</i>	China	PCR	[45,49,54]
<i>O. ochrocephala</i>	China	PCR	[19,45]
<i>O. glacialis</i>	China	PCR	[19,45]
<i>O. sericopetala</i>	China	PCR	[19,45]
<i>O. falcata</i>	China	PCR	[45,47]

Table 2: Worldwide species of locoweeds containing endophytic fungi.

The locoweeds from North America, China, and in addition to *Swainsona* from Australia, were revealed to hold an endophyte,

Alternaria section *Undifilum* that produces swainsonine [22,23]. All toxic plants that hold swainsonine have shown to have a swainsonine producing fungal endophyte, although not each plant in a population is toxic or contains the endophyte [22]. The entire locoweed plant parts contain endophytes, but they are highest in the crown (Figure 3) and they are hard to isolate from the roots. Amounts of endophyte alter seasonally in the upper parts of plants until the plants are mature [23].



Transmission Paths of the Locoweed Endophytes

Grazing livestock and wildlife that consume *Astragalus* and *Oxytropis* plants get a neurologic toxicosis “locoism” distinguished by altered behaviour, weight loss, depression, decreased libido, infertility and abortion, as well as defects of birth along with death [14,24-28]. It was confirmed that the main path of locoweed endophyte transmission is through invaded seeds into the next generation throughout vertical transmission in locoweed plants [9,16]. As a result, the infection of new seedlings is thought to be at the stage of germination and the fungal endophytes move into all parts of the plant but are considered minor in roots [9,16,29]. When the seed coats are removed, the results showed that there is no detectable swainsonine in plants and hence the transmission of the endophyte through the vertical process can be blocked [30]. In a vertically transmitted process, locoweed endophytes are typically moved clonally and are transmitted by fungal hyphae. The mycelium of the fungus grows into the sheath, stem, leaf tissues, and lastly go in the flowering stem, seeds and then is through the seed that the endophyte is passed to the next generation of plants [31,32], and the propagation of locoweed fungal endophyte is closely linked with its seeds distribution [21,29]. However, locoweed endophytes have not been found in the root of field-collected plants [9,29].

Cultural Characteristics of Locoweed Fungal Endophytes

In a number of locoweeds species in China, fungal endophytes are recognized to fit in the similar taxonomy species of fungus, except, given that these fungal endophytes are isolated in their respective hosts and vertically transmitted to the seeds of the next plant generation, it is likely that the endophytic fungus of the different species of locoweed

have developed distinctive biological traits. According to [33-36], fungal endophytes are generally defined as species that exist inside the tissues of living plants for some of their whole life cycle. The plant entry of the fungal endophytes can be through root hairs or stomata in leaves and then disseminate systemically throughout the plant. Some fungal endophytes are capable of colonizing roots, aerial parts of plants, the vascular system, and seeds [37], and may support the growth of host plants, improve nutrient supply and protect plants from both biotic as well as abiotic stresses [38-40]. However, some endophytes are pathogenic to their host, while others interact as a commensally [41,42]. As reported by [29,43,44], locoweeds are toxic plants and their toxic component is an alkaloid, which can be divided into two classes: quinolizidine alkaloids and indolizidine alkaloids that are known as swainsonine and swainsonine N-oxide, and it is swainsonine alone that causes the symptoms of the locoweed endophyte poisoning [43].

Functions of Locoweed Endophytes

It is thought that locoweed endophytes might play a role in the health and productivity of plants. Locoweed endophytes are a likely natural source of biological control agents against plant pathogens. Some fungal endophytes identified have been found to have a virtuous impact for health and productivity of plant by promoting plant growth and suppressing pathogens, for others the role is less clear [45-56]. Species of fungal endophyte depend on the plant host for nutrition, reproduction and guardianship from abiotic and biotic stress. Profits to the host plant for grass endophytes comprise tolerance to pathogens, enhanced competitive abilities, and resistance to insect herbivory in addition to animals [57].

The Interaction between Endophytic Fungi and Locoweed Plants

Interactions of fungal endophytes with locoweed plants offer the nutrients of endophytes, hormones and also a number of other signals in inducing their ability to produce swainsonine. Locoweed plants, as the host of the endophytes, have a straight influence on the swainsonine production [58]. Locoweed and fungal endophytes have likely been associated for a long time. First, locoweeds provide fungi the minerals and photosynthetic products, a fixed internal environment for its growth and reproduction and play a role in its spread through the seeds. The metabolites produced by endophytic fungi induce the development and growth of locoweeds, and improve the resistance of locoweed plants to the biotic stress along with abiotic stress, then produce toxic metabolite swainsonine for plants protection from the feeding of animals [10,56].

Detection of the endophyte *Alternaria* section *Undifilum* in tissues of locoweed plants

Locoweed plants are perennial legumes characterized by a hard seed coat that allows the seeds for living in rough conditions and stay viable for several years [59,60]. The endophyte *Alternaria* section *Undifilum* is a seed-borne fungus that passes between generations through the seed coats of locoweeds; plants grown from seeds lacking seed coats are endophyte-free, while plants grown from whole seeds comprise the endophyte and swainsonine [9]. By means of microscopy, culturing, as well as polymerase chain reaction (PCR), *Alternaria oxytropis* can be detected in locoweeds, and among them, PCR is the most sensitive [9,52,61]. In the amount of *Alternaria* section *Undifilum*, the

difference was thought to be coupled with the highly variable concentrations of swainsonine in *Astragalus* and *Oxytropis* [12,17]. In the locoweed seeds, endophytes were detected inside the coat but not the embryo. The fungus can be cultured from seed and the endophytes could be maternally transmitted [9].

Fungal endophytes and their interactions with host plants

Fungal endophytes with the interaction of host plants are defined by a barely tuned equilibrium between plant defences and fungal virulence, where the endophytic fungus produces secondary metabolites [62,63]. As reported by Wilson, grass fungal endophyte and plant profit for both sides based on their symbiotic relationship: for the grass fungal endophyte, it is advantageous because of the plant nutrients availability [64] and for the plant; it can be advantageous owing to the defence from pathogens, nutrient enhancement uptake, and promotion of plant growth [65] and stress tolerance [66] (Figure 4). Vitamins [67] or levels of phytohormone can also be produced by grass fungal endophytes within the plant [68].

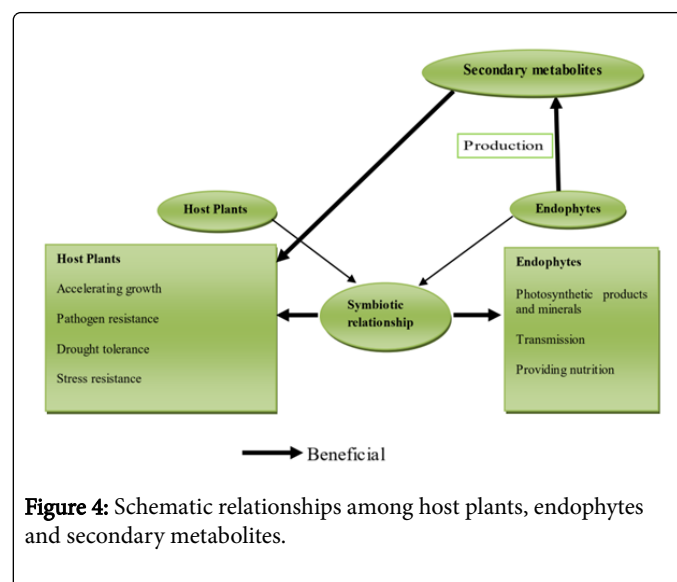


Figure 4: Schematic relationships among host plants, endophytes and secondary metabolites.

Morphology of locoweed fungal endophytes

The majority of locoweed fungal endophytes show thread-like hyphae of 2–10 µm in diameter and reach up to various centimetres in length. The growth of hyphae at their tips and new hyphae are normally formed by the new emergence of tips along existing hyphae [3]. When hyphae come into contact, they fuse then this process is called hyphal fusion. Processes of these growths lead to the mycelium development, an interconnected network of hyphae [69].

Most of locoweed fungal endophytes produce conidia in culture [5,70]; conversely, the conidia have not been found in infected plants [71]. The fungus grows very slowly, less than 0.2 mm/day [5,10]. Conidia of locoweed endophytes produce germ tubes in process of germination. The shape of conidia produced from locoweed fungal endophytes is ellipsoid, with a small number of ovate spores, and produce a sole wavy hypha at the time of germination [5]. Most conidia have thin septa which are undifferentiated from conidial walls, a common characteristic in conidia of *Embellisia* species [5,70].

Endophyte *Embellisia* spp. and locoweed endophytes relationships

The pathogenic fungus *Embellisia astragali* [72], recently known as *Alternaria gansuense* [73], because of its molecular and morphological characteristics [5,70], is similar to locoweed endophytes *Alternaria cinerea* and *Alternaria fulva*. Their hosts belong to the genus *Astragalus* [70] and are found in similar tissues of its host where the fungus resides, within the seeds, stems and leaves. Furthermore, the growth of all these fungi on artificial media is slow. But, some differences exist in the features of conidia and conidiophores between locoweed endophytes and *Embellisia astragali* [73]. *Embellisia astragali* known as *Alternaria gansuense* is found in roots and is pathogenic to its host [4]. *Alternaria* section *Embellisia* is known based on a combination of aspects including predominantly transversely septate conidia with distinctly pigmented and thickened septa, variously swollen, curved or sigmoid conidia, umbilicate sites of conidium production at conidiophore geniculations and intrahyphal proliferating chlamydospores in culture [74,75].

Production of the alkaloid swainsonine

Fungi belonging to *Alternaria* section *Undifilum* found in locoweeds [14], an endophyte infecting *Swainsona canescens* [24,76], the insect pathogens *Metarhizium anisopliae* [77-79], the clover pathogen, *Slafractonia leguminicola* that previously was recognized as *Rhizoctonia leguminicola* [80,81] and a recently discovered fungus belonging to the order of Chaetothyriales found in morning glory are known to produce the alkaloid swainsonine [13,14,44]. Swainsonine may play a role in mutualistic symbiosis and plant diseases in addition to animal toxicoses. This indolizidine alkaloid is under consideration as a constituent of chemotherapeutic treatments for some cancers [82, 83].

The variety of locoweed fungal endophytes that produce swainsonine suggests numerous ecological roles for the alkaloid among symbionts and parasites of plants as well as animals. Concerning toxic locoweed plants, livestock develops a preference in spite of the disturbing cytotoxic and neurological effects caused by the alkaloid swainsonine [14].

Conclusion

The toxicity of locoweed plants is due to endophytic fungi. Locoweed plants are resistant to cold temperatures, drought as well as to poor and saline soil conditions. For these reasons, locoweeds have a fine impact on the regeneration of rangelands with prevention of windstorms, sand fixation, soil conservation and moisture, enhancing the fertility of the soil and can fit the harsh environment of western China. Even though locoweed plants may have a beneficial impact on the ecosystem of rangeland, they cause poisoning and death to the grazing animals through the production of alkaloid swainsonine and in consequence, applies a great negative influence on the production of livestock and the income of herdsmen in western China. For this reason, additional research on locoweed plants, locoweed endophytes and swainsonine is needed as, although animals are poisoned by eating locoweeds, locoweed plants could have a great impact in the ecological communities, and thus, they should be considered as “better than nothing”.

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References

- Marsh CD (1909) The loco-weed disease of the plains: US Department of Agriculture, Bureau of Animal Industry, USA.
- Patterson PE(1982) Loco, la yerba mala Locoweed, cattle, poisoning. *Rangelands Arch* 4: 147-148.
- Noor A, Nava A, Cooke P (2018) Creamer R Evidence of non-pathogenic relationship of *Alternaria* section *Undifilum* endophytes within three host locoweed plants species. *Bot* 96: 187-200.
- Braun K, Romero J, Liddell C, Creamer R (2003) Production of swainsonine by fungal endophytes of locoweed. *Mycol Res* 107: 980-988.
- Pryor BM, Creamer R, Shoemaker RA, McLain-Romero J, Hambleton S (2009) *Undifilum*, a new genus for endophytic *Embellisia oxytropis* and parasitic *Helminthosporium bornmuelleri* on legumes. *Bot* 87: 178-194.
- Woudenberg J, Groenewald JZ, Binder M, Crous PW (2013) *Alternaria* redefined. *Stud Mycol* 75: 171-212.
- Wang Q, Nagao H, Li YuLing, HongSheng W, Kakishima M (2006) *Embellisia oxytropis*, a new species isolated from *Oxytropis kansuensis* in China. *Mycotaxon* 95: 255-260.
- Lawrence DP, Rotondo F, Gannibal PB (2016) Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. *Mycol Prog* 15: 1-8.
- Oldrup E, McLain-Romero J, Padilla A, Moya A, Gardner D, et al. (2010) Localization of endophytic *Undifilum* fungi in locoweed seed and influence of environmental parameters on a locoweed *in vitro* culture system. *Bot* 88: 512-521.
- Creamer R, Baucom D (2013) Fungal endophytes of locoweeds: a commensal relationship. *J Plant Physiol Pathol* 1: 1-6.
- James LF, Hartley W, Van Kampen K (1981) Syndromes of *Astragalus* poisoning in livestock. *J Am Vet Med Assoc* 178: 146-150.
- Achata BJ, Creamer R, Gardner D (2012) Seasonal Changes in *Undifilum* Colonization and Swainsonine Content of Locoweeds. *J Chem Ecol* 38: 486-495.
- Cook D, Beaulieu WT, Mott IW, Riet-Correa F, Gardner DR, et al. (2013) Production of the alkaloid swainsonine by a fungal endosymbiont of the Ascomycete order Chaetothyriales in the host *Ipomoea carnea*. *J Agric Food Chem* 61: 3797-3803.
- Cook D, Donzelli BGG, Creamer R, Baucom DL, Gardner DL, et al. (2017) Swainsonine biosynthesis genes in diverse symbiotic and pathogenic fungi. *G3 (Bethesda)* 7: 1791-1797.
- Dorling PR, Huxtable CR, Colegate S (1980) Inhibition of lysosomal α -mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *Biochem J* 191: 649-651.
- Ralphs MH, Cook D, Gardner DR, Grum DS (2011) Transmission of the locoweed endophyte to the next generation of plants. *Fungal Ecol* 4: 251-255.

17. Cook D, Gardner DR, Ralphs MH, Pfister JA, Welch KD, et al. (2009) Swainsonine concentrations and endophyte amounts of *Undifilum oxytropis* in different plant parts of *Oxytropis sericea*. *J Chem Ecol* 35: 1272-1278.
18. Yu Y, Zhao Q, Wang J, Wang J, Wang Y, et al. (2010) Swainsonine-producing fungal endophytes from major locoweed species in China. *Toxicon* 56: 330-338.
19. Zhao M, Gao X, Han B (2011) Locoweed poisoning in the native grasslands of China. *Int J Pois Plant Res* 1: 41-46.
20. Lu P (2006) Factors related to swainsonine in three *Oxytropis* species in Inner Mongolia: Ph. D. Thesis, Inner Mongolia Agricultural University, Huhehaote, Inner Mongolia.
21. Lu P, Child D, Meng-Li Z, Gardener DR, Gui-Fen L, et al. (2009) Culture and identification of endophytic fungi from *Oxytropis glabra* DC. *Acta Ecologica Sinica* 29: 53-58.
22. Cook D, Shi L, Gardner DR, Pfister JA, Grum D, et al. (2012) Influence of Phenological Stage on Swainsonine and Endophyte Concentrations in *Oxytropis sericea*. *J Chem Ecol* 38: 195-203.
23. Lu H, Quan H, Zhou Q, Ren Z, Xue R, et al. (2017) Endogenous fungi isolated from three locoweed species from rangeland in western China. *Afr J Microbiol Res* 11: 155-170.
24. Colegate SM, Dorling PR, Huxtable CR (1979) A spectroscopic investigation of swainsonine: an α -mannosidase inhibitor isolated from *Swainsona canescens*. *Aus J Chem* 32: 2257-2264.
25. Molyneux RJ, James LF (1982) Loco intoxication: indolizidine alkaloids of spotted locoweed (*Astragalus lentiginosus*). *Sci* 216: 190-191.
26. Stegelmeier B, James LF, Panter KE, Ralphs MH, Gardner DR, et al. (1999) The pathogenesis and toxicokinetics of locoweed (*Astragalus* and *Oxytropis* spp.) poisoning in livestock. *J Nat Toxins* 8: 35-45.
27. Cook D, Gardner DR, Pfister JA (2014) Swainsonine-containing plants and their relationship to endophytic fungi. *J Agric Food Chem* 62: 7326-7334.
28. Cook D, Ralphs MH, Welch KD, Stegelmeier BL (2009) Locoweed poisoning in livestock. *Rangelands* 31: 16-21.
29. Wu C, Han T, Lu H, Zhao B (2016) The toxicology mechanism of endophytic fungus and swainsonine in locoweed. *Environ Toxicol Pharmacol* 47: 38-46.
30. Grum DS, Cook D, Gardner DR, Roper JM, Pfister JA, et al. (2012) Influence of seed endophyte amounts on swainsonine concentrations in *Astragalus* and *Oxytropis* locoweeds. *J Agric Food Chem* 60: 8083-8089.
31. Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. *Appl Microbiol Biotechnol* 90: 1829-1845.
32. Tadych M, Bergen MS, White JF (2014) *Epichloë* spp. associated with grasses: new insights on life cycles, dissemination and evolution. *Mycologia* 106: 181-201.
33. Hodgson S, Cates C, Hodgson J, Morley NJ, Sutton BC, et al. (2014) Vertical transmission of fungal endophytes is widespread in forbs. *Ecol Evol* 4:1199-1208.
34. Rodriguez R, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. *New Phytol* 182: 314-330.
35. Wilson D (1993) Fungal endophytes: out of sight but should not be out of mind. *Oikos* 68: 379-384.
36. Yan JF, Broughton S, Yang SL, Gange AC (2015) Do endophytic fungi grow through their hosts systemically?. *Fungal Ecol* 13: 53-59.
37. Bernardi WJ, Garcia A, Filho CJ, Prioli AJ, Pamphile JA (2010) Evaluation of foliar fungal endophyte diversity and colonization of medicinal plant *Luehea divaricata* (Martius et Zuccarini). *Biol Res* 43: 375-384.
38. Knoth JL, Kim SH, Ettl GJ, Doty SL (2014) Biological nitrogen fixation and biomass accumulation within poplar clones as a result of inoculations with diazotrophic endophyte consortia. *New Phytol* 201: 599-609.
39. Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. *J Exp Bot* 59: 1109-1114.
40. Schulz B, Boyle C (2005) The endophytic continuum. *Mycol Res* 109: 661-686.
41. Redman RS, Dunigan DD, Rodriguez RJ (2001) Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? *New Phytol* 151: 705-716.
42. Schulz BJ, Römmer AK, Dammann U, Strack D (1999) The endophyte-host interaction: a balanced antagonism?. *Mycol Res* 103:1275-1283.
43. Li Qf, et al. (2005) Detection of alkaloid and isolation of swainsonine from *Oxytropis glacialis*. *Acta Veterinaria et Zootechnica Sinica* 36: 1339.
44. Lu H, Quan H, Ren Z, Wang S, Xue R, et al. (2016) The genome of *Undifilum oxytropis* provides insights into swainsonine biosynthesis and locoism. *Scienti Rep* 6: 30760.
45. Lu H, Cao DD, Hao L, Zaho BY (2014) Characterisation of locoweeds and their effect on livestock production in the western rangelands of China. *Rangeland J* 36: 121-131.
46. Lu P, ZHAO M, HAN G, LI X, WANG Y (2006) The Locoweed in Inner Mongolia and Research Progress. *Chin J Grassland* 1: 014.
47. Zhao BY, Tong DW, Ge PB, Mo CH (2003) Locoweed harm investigation in the West grassland of China. *Chin J Grassland* 25: 65-68.
48. He W, Zhuang H, Fu Y, Guo L, Guo B, et al. (2015) De novo transcriptome assembly of a Chinese Locoweed (*Oxytropis ochrocephala*) species provides insights into genes associated with drought, salinity, and cold tolerance. *Front Plant Sci* 6: 1086.
49. Yu R, Li X, Song L, yang B, Zhu T, et al. (1991) [Toxic principles of *Oxytropis glabra* DC] 16: 160-163.
50. Braun K (1999) Fungal endophyte infection and swainsonine toxicity in locoweed, New Mexico State University, USA.
51. McLain-Romero J, Padilla A, Creamer R (2004) Microscopic localization of *Embellisia* in seed and seedlings of southern specklepod locoweed (*Astragalus lentiginosus*). *Phytopathology*.
52. Ralphs MH, Creamer R, Baucom D, Gardner DR, Welsh SL, et al. (2008) Relationship between the endophyte *Embellisia* spp. and the toxic alkaloid swainsonine in major locoweed species (*Astragalus* and *Oxytropis*). *J Chem Ecol* 34: 32-38.
53. Barillas JRV, Paschke MW, Ralphs MH, Child RD (2007) White locoweed toxicity is facilitated by a fungal endophyte and nitrogen-fixing bacteria. *Ecology* 88: 1850-1856.
54. http://en.cnki.com.cn/Article_en/CJFDTotal-STXB200901008.htm
55. Delaney KJ, Klypina N, Maruthavanan J, Lange C, Sterling TM (2011) Locoweed dose responses to nitrogen: Positive for biomass

- and primary physiology, but inconsistent for an alkaloid. *Am J Bot* 98: 1956-1965.
56. Klypina N, Pinch M, Schutte BJ, Maruthavanan J, Sterling TM (2017) Water-deficit stress tolerance differs between two locoweed genera (*Astragalus* and *Oxytropis*) with fungal endophytes. *Weed Sci* 65: 626-638.
 57. Saikkonen K, Young CA, Helander M, Schardl CL (2016) Endophytic Epichloë species and their grass hosts: from evolution to applications. *Plant Mol Biol* 90: 665-675.
 58. Cook D, Grum DS, Gardner DR, Welch KD, Pfister JA (2013) Influence of endophyte genotype on swainsonine concentrations in *Oxytropis sericea*. *Toxicon* 61: 105-111.
 59. James LF (1999) *Astragalus* and *Oxytropis* poison livestock with different toxins: Locoweed research, New Mexico Coop. Ext Ser Res Rep 730: 10-11.
 60. Ralphs MH, Graham D, Molyneux RJ, James LF (1993) Seasonal grazing of locoweeds by cattle in northeastern New Mexico. *J Range Manag* 46: 416-420.
 61. Cook D, Gardner DR, Welch KD, Roper JM, Ralphs MH, et al. (2009) Quantitative PCR method to measure the fungal endophyte in locoweeds. *J Agri Food Chem* 57: 6050-6054.
 62. Panaccione DG, Beaulieu WT, Cook D (2014) Bioactive alkaloids in vertically transmitted fungal endophytes. *Func Ecol* 28: 299-314.
 63. Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycol Res* 106: 996-1004.
 64. Wilson D (1995) Endophyte : The evolution of a term, and clarification of its use and definition. *Oikos* 73: 74-276.
 65. Alvin A, KI Miller, BA Neilan (2014) Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. *Microbiol Res* 169: 483-495.
 66. Larran S, Simón MR, Moreno MV, Santamarina Siurana MP, Perelló A (2016) Endophytes from wheat as biocontrol agents against tan spot disease. *Biolo Cont* 92: 17-23.
 67. Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. *Nat Prod Rep* 23: 753-771.
 68. Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda Mdel C, Glick BR (2016) Plant growth-promoting bacterial endophytes. *Microbiol Res* 183: 92-99.
 69. Miles PG, Chang ST (2004) *Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact*, CRC press, USA.
 70. Baucom DL, Romero M, Belfon R, Creamer R (2012) Two new species of *Undifilum*, fungal endophytes of *Astragalus* (locoweeds) in the United States. *Botany* 90: 866-875.
 71. Reyna R, Cooke P, Grum D, Cook D, Creamer R (2012) Detection and localization of the endophyte *Undifilum oxytropis* in locoweed tissues. *Botany* 90: 1229-1236.
 72. Li Y (2011) Pathogenic *Embellisia astragali* on *Astragalus adsurgens* is very closely related to locoweed endophyte. *Phytopathology* 101: S102-S103.
 73. Liu J, Li Y, Creamer R (2016) A Re-examination of the Taxonomic Status of *Embellisia astragali*. *Curr Microbiol* 72: 404-409.
 74. Li Y, Nan Z (2007) A new species, *Embellisia astragali* sp. nov., causing standing milk-vetch disease in China. *Mycologia* 99: 406-411.
 75. Li YZ, Nan ZB (2009) Nutritional study on *Embellisia astragali*, a fungal pathogen of milk vetch (*Astragalus adsurgens*). *Antonie Van Leeuwenhoek* 95: 275-284.
 76. Grum DS, Cook D, Baucom D, Mott IW, Gardner DR, et al. (2013) Production of the alkaloid swainsonine by a fungal endophyte in the host *Swainsona canescens*. *J Nat Prod* 76: 1984-1988.
 77. Hino M, Nakayama O, Tsurumi Y, Adachi K, Shibata T, et al. (1985) Studies of an immunomodulator, swainsonine. *J Antibiot (Tokyo)* 38: 926-935.
 78. Molyneux RJ, McKenzie RA, O'Sullivan BM, Elbein AD (1995) Identification of the glycosidase inhibitors swainsonine and calystegine B2 in weir vine and correlation with toxicity. *J Nat Prod* 58: 878-886.
 79. Patrick M, Adlard M, Keshavarz T (1993) Production of an indolizidine alkaloid, swainsonine by the filamentous fungus, *Metarhizium anisopliae*. *Biotechnol Lett* 15: 997-1000.
 80. Alhawatema MS, Sanogo S, Baucom DL, Creamer R (2015) A search for the phylogenetic relationship of the ascomycete *Rhizoctonia leguminicola* using genetic analysis. *Mycopathologia* 179: 381-389.
 81. Schneider MJ, Uncemach FS, Broquist HP, Harris TM (1983) (1S, 2R, 8R, 8aR)-1, 2, 8-trihydroxyoctahydroindolizine (swainsonine), an α -mannosidase inhibitor from *Rhizoctonia leguminicola*. *Tetrahedron* 39: 29-32.
 82. Li Z, Huang Y, Dong F, Li W, Ding L, et al. (2012) Swainsonine promotes apoptosis in human oesophageal squamous cell carcinoma cells *in vitro* and *in vivo* through activation of mitochondrial pathway. *J Biosci* 37: 1005-1016.
 83. Santos FM, Latorre AO, Hueza IM, Sanches DS, Lippi LL, et al. (2011) Increased antitumor efficacy by the combined administration of swainsonine and cisplatin *in vivo*. *Phytomedicine* 18: 1096-1101.