Review Article

Fungal Endophytes of Locoweeds: A Commensal Relationship?

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Abstract

Fungi can be found in an endophytic symbiosis with most plants. Determining the nature of the symbiosis has proven difficult, especially with the Undifilum endophyte of locoweeds. Undifilum is a vertically-transmitted, asexual, systemic endophyte that produces a toxin that affects vertebrates that feed on locoweeds. Other fungal endophytes that are vertically transmitted and produce toxins, such as the endophytes of agronomic grasses, appear to be in a mutualistic relationship. Similarly the fungal endophytes of some trees appear to function as mutualists with their hosts. In contrast, fungal endophytes of wild grasses and the Undifilum endophyte of locoweeds appear to act more like commensals than mutualists, offering few benefits to their hosts under certain environmental conditions. Any benefits that the locoweed endophytes might confer onto their hosts are as yet unknown.

Introduction

Fungal plant relationships are ubiquitous, but the nature of the interactions are not well defined. This is particularly true for the recently characterized intimate fungal association of the toxic locoweed plant and its fungal endophyte partner. Locoweeds are toxic legumes of Oxytropis or Astragalus species that are found primarily within the western United States [1] and north central China [2]. The toxicity is due to the presence of the indolizidine alkaloid swainsonine that affects mammalian nervous systems resulting in localism [3]. Localism symptoms include a lack of muscular coordination and an inability to eat or drink resulting in cellular vacuolization and death [4-6]. Livestock poisoning from locoweeds causes major economic losses in the western United States [7]. While the magnitude of economic loss depends on the degree of intoxication, losses from locoweeds due to poor animal health, low reproductive performance, increased death and reduced weight can cause reductions in cattle production [8]. In New Mexico in 1985, over 10% of the cattle/calf and 40% of the cow stocker operations reported losses of over $20 million from localism. Effective management of locoweeds and localism has been difficult to implement and costly. Management recommendations for locoweeds include restricting access to pastures for grazing in the spring when locoweeds are prevalent and grasses are still dormant, supplementing cattle feed so that they do not graze, spraying herbicides during flowering of locoweeds, using insects for biological control of locoweed, and modifying the behaviour of cattle or horses so that they do not want to graze locoweeds [9].

Many fungi exist in an endophytic symbiosis with a host plant. Wilson [10] used the term endophyte to refer to an organism that lives within plant tissues without causing disease symptoms. Bacon and White [11] defined a fungal endophyte as one that lives in biological association with a living host. Maheshwari [12] suggested that to be classified as an endophyte, the fungal mycelium should be present within the plant, but cautioned that facultative endophytes might become biotrophic under certain environmental conditions.

Fungal endophytes of plants are generally classified as pathogens, commensals, or mutualists. Mutualism is a symbiosis where both plant and fungus benefit, and commensalism can be defined as a symbiosis where one species (the fungus) benefits, while the host is not affected. In contrast, fungal pathogens cause harm to their plant hosts. The term ‘reciprocal parasitism’ is preferred by some ecologists over that of mutualism, especially for mycorrhizal symbionts, since some mycorrhizal endophytes appear to be capable of being both a pathogen and a mutualist depending on the environmental conditions and health of the plant [13]. The symbiosis would be parasitic if the costs of supporting a symbiont were higher and mutualistic if the benefits from the symbiont presence were higher. Schultz and coworkers [14] suggested that fungal endophytes have a balanced antagonism with their hosts, while fungal pathogens have an imbalanced interaction that end in disease.

Rodriguez and coworkers [15] classified endophytes into four groups based on their transmission and ecological interactions. Class 1 endophytes are those that infect a narrow host range, are generally vertically transmitted, and produce mycotoxins. Grass endophytes Epichloë festucae and Neotyphodium sp. fit within that grouping. Class 2 endophytes have a broad host range and are vertically and horizontally transmitted. Class 2 endophytes include Phoma, Colletotrichum sp., Fusarium sp., and Curvulicola sp. Class 3 and Class 4 endophytes infect broad host ranges, are horizontally transmitted, and infect shoots and roots, respectively. All four classes of endophytes are reported to enhance the growth of the host and confer benefits such as improved drought tolerance.

Class 1 endophytes of agronomic grasses that produce alkaloid mycotoxins are considered mutualists because they deter herbivore feeding, enhance resistance to pathogens, and aid in withstanding poor environmental conditions, although the effects are dependent on the fungal strain and stage of the plant [16,17]. Grass endophytes in general do not elicit a resistance response from their hosts. The hyphae do not generally penetrate host tissue or produce haustoria, however, the sexual grass endophyte Epichloë does penetrate and cause a disease of the grass inflorescence [18].

Faeth and Fagan [19] suggested that while many fungal endophytes are symbionts within grasses, they could also be neutral or pathogenic toward their hosts. If insect pressure is high, Bromus setifolia benefits from Neotyphodium infection, while if low, the infected plants are smaller than uninfected [20]. Grass endophytes have a
complicated interaction with reactive oxygen species production. Epichloe produces reactive oxygen species during infection to limit its own growth, but both the plant hosts and the fungi may produce reactive oxygen species in a more pathogenic interaction [21]. Eaton and coworkers [22] suggested that the grass endophyte E. festucae maintains a mutualistic association with perennial ryegrass through components of the MAP kinase pathway and production of reactive oxygen. When the fungal NADPH oxidase complex is disrupted, or the gene for an iron siderophore is lost, the fungus switches to a pathogenic mode. Jani and coworkers [23] showed that with native grasses (sleepy grass, Achnatherum robustum), in contrast to what was found with agronomic grasses, Neotyphodium-produced alkaloids were associated with increased numbers and types of insect herbivores and suggested that the alkaloids may negatively affect natural enemies of herbivores that are not adapted to the chemicals.

Faeth [24] proposed that endophytes of native grasses (versus cultivated grasses) do not act as defensive plant mutualists and instead function as reproductive parasites. Neotyphodium endophytes cause native perennial ryegrass to produce more reproductive structures (flowers and seeds), flower earlier, and tolerate higher insect herbivory [25]. Shifts to early growth and reproduction of the host would benefit the fungal transmission and lead to decreased life span of the plant under limiting environmental conditions. Under those conditions, the fungus would act more as a reproductive parasite than as a defensive mutualist. Faeth [24] observed that natural populations of native grasses exist as mixtures of endophyte–infected and uninfected plants. When the native grass Achnatherum robustum was infected with a high alkaloid-producing Neotyphodium, the fungal presence hurt the growth and seed production, even with supplemental water [26]. The high alkaloid-producing endophyte-infected plants had higher overwintering survival than plants infected with endophyte that did not produce alkaloids, thereby increasing the reproductive potential for the endophyte.

Class 2 endophytes colonize roots, stems, and leaves, are transmitted through seeds or rhizomes, and help reduce stress [15]. They promote plant growth through increases in root and shoot biomass, provide disease resistance, and help with drought stress and salinity [27]. Stress tolerance was highly correlated with susceptibility to reactive oxygen species and reactive oxygen protection was dependent on the habitat of the plant host [28]. Redman and coworkers [27] suggests that a variety of Colletotrichum sp. in natural systems might express a range of symbiotic lifestyles from mutualistic, commensal or pathogenic depending on the host plant genotype and environmental conditions.

Locoweed endophytes are most similar to Class 1 endophytes of native grasses in that they also have a narrow host range, colonize shoots and crowns, are found to extensively colonize the plant hosts, and are seed transmitted [29-31]. The locoweed endophytes are asexual and cause no obvious symptoms of disease in any natural infection, and produce a toxin, swainsonine, with activity against grazing animals in plants [29]. Locoweed endophytes have been classified as Undifilum sp. [32], with different species associated with different species of locoweeds [33]. Undifilum oxytropis is found infecting Oxytropis sericea and O. lambertii [32], U. cinereum infects Astragalus mollissimus, and U. fulvum infects A. lentiginosus [33]. Most of these fungi produce conidia in culture [32,33], however, the conidia have not been found in infected plants [34]. The fungus is very slow growing, less than 0.2 mm/day [32].

The nature of the symbiosis between Undifilum spp. and their locoweed hosts has been difficult to characterize. Most research has not shown the endophyte to benefit locoweeds. Undifilum produces the alkaloid swainsonine, which inhibits the enzymes α-mannosidase and mannosidase II resulting in lysosomal storage disease and altered glycoprotein synthesis [35]. While consumption of swainsonine-containing plants may deter feeding in some vertebrates, such as rats, it encourages feeding in others, which instead can lead to increased consumption [36,37]. Swainsonine does not deter grazing, in fact animals take two to three weeks to show clinical signs and continue grazing locoweeds after becoming intoxicated [38]. Furthermore swainsonine concentrations are reported not to change in response to clipping used to simulate herbivory [39].

Swainsonine does not deter invertebrate feeding [40], and weevils are just as likely to choose a high swainsonine locoweed as a low swainsonine variety [41]. High swainsonine levels in seed do not stop weevil feeding, and some insects specialize on locoweeds [40]. In addition, the presence of Undifilum endophytes does not appear to provide substantial resistance against common pathogens such as Fusarium. The presence of Undifilum in a locoweed plant does not aid in plant growth under heat stress or nitrogen deficiency [30]. However, swainsonine production was not affected by the addition of nitrogen or changes in the water status of mature Undifilum – containing locoweeds, suggesting that it doesn’t harm the plant either [42,43]. Oxytropis sericea with high levels of swainsonine and those with low levels of swainsonine grown together produced very similar biomass and levels of endophyte [44].

Undifilum will readily emerge from desiccated tissues and sporulate on them in media in laboratory conditions, although conidiation has not been noted in field conditions. There are two exceptions to this: Embellisia astragali, which is pathogenic on Astragalus adsurgens causing yellow stunt and root rot in China [45] and U. bornmeulleri which is pathogenic on vetch [32]. E. astragali, which is likely a different species of Undifilum, readily sporulates on plant tissues and causes disease in both aerial and root portions of the plant [45]. U. bornmeulleri does not produce swainsonine and is reported to function as a pathogen and sporulate within infections [32].

We propose that Undifilum endophytes are primarily commensals with their locoweed hosts that can become saprophytic toward weakened plants. This is similar to that reported for Rhabdocline parkeri on Douglas fir [46]. The presence of the endophyte confers few obvious benefits for its host. Undifilum may play a limited role in water stress of young plants [30], but not older plants [33], and appears to increase reactive oxygen, instead of reducing it. Locoweed plants do not appear to recognize Undifilum as a pathogen and do not show a resistance response toward the fungus, even at a cellular level [34]. The endophyte might possibly help the locoweed seed survive and germinate, however, natural populations of swainsonine-free (and thus endophyte free) have been found in several locations [39]. The endophyte does not aid in disease resistance, temperature or pH stress, drought stress, or invertebrate herbivory. However, retention of the endophyte does not appear to be costly for the locoweed plant.


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in that endophyte-free plants exhibit no physiological differences to endophyte-infected plants.

\textit{Undifilum} may alter the propensity for the locoweed to flower or set seed. Achata and coworkers \cite{47} found that high swainsonine concentration in plant samples was associated with high levels of endophyte. \textit{Undifilum} and its swainsonine production might affect the ability of locoweeds to withstand cold temperature. Swainsonine levels in leaves were correlated with average daily temperatures for some locoweed species at specific locations, seed pods had higher levels of swainsonine than leaves, and locoweeds of the same species contained higher levels of swainsonine in colder locations than in warmer locations \cite{47}. Cook and coworkers \cite{31,48} also found that although swainsonine concentrations vary seasonally, the flowers and pods of \textit{Astragalus mollissimus} had significantly higher levels of swainsonine than crowns, stems, or leaves, while the endophyte content was inversely related, i.e. higher levels in crowns than leaves, flowers, and pods.

Evans \cite{49} postulated that presence of mutualistic endophytes could help weedy species against natural enemies, and move with the plants even though the natural enemies do not migrate. The ‘enemy release hypothesis’ suggests that these plants would have a strong competitive advantage because the plants could put more resources toward growth and reproduction. Although \textit{Astragalus} is native to North America, perhaps locoweeds acquired the endophytes, but the natural enemies are no longer present.

The locoweed-endophyte symbiosis is ancient, since the same species of fungus can be found in \textit{Oxytropis} sp. in both North America and China. The fungus has obviously adapted to growth in a plant with dependence on seed transmission for propagation. The slow growth of the fungus, lack of sporulation on the plant, lack of production of host cell haustoria, and intercellular mycelial growth habit without puncturing plant walls, suggest a long adaptation perhaps from a more pathogenic or mutualistic relationship. This natural mycobiotic system differs from that of the Class 1 endophytes of agronomic grasses and from Class 2 endophytes in that they are primarily mutualists with their plant hosts. With the evidence thus far, the locoweed plant and the toxin-producing endophyte \textit{Undifilum} offer a new ecological platform for the study of commensalism and its origins, as well as its contrast with other plant fungal relationships.

\textbf{References}


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