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Clavicipitalean Fungal Epibionts and Endophytes – Development of Symbiotic Interactions with Plants

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Abstract

The clavicipitalean fungi are parasitic and biotrophic symbionts of plants, insects, and fungi. Their associations with and their ecological impacts on symbiotic partners were examined. Particular emphasis is placed on the trend toward plant parasitism and acquisition of the endophytic habit. The effects that clavicipitalean fungi have on the structure or physiology of host species are discussed.

Keywords: Balansa, Beauveria, ecology, endophyte, Epichloë, Hyperdermium, Myriogenospora, Neotyphodium

1. Introduction

The extent to which biotrophic microbes colonize and inhabit plants is not fully appreciated. Endophytic and epibiotic microbes of various kinds colonize plant roots, leaves, seeds, and stems. Every plant in nature is inhabited to some extent by numerous microbes (Bills, 1996). Very little is known about the life

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histories of the majority of these symbiotic microbes or the ways they impact the physiology and ecology of plant hosts. Most fungal biotrophs of plants produce haustoria that are used to penetrate host cells and interface closely with the host cell cytoplasm. This enables the biotrophic parasite to extract nutrients directly from living host cells. Rusts (Uredinales), powdery mildews (Erysiphales), and downy mildews (Peronosporales) are examples of biotrophs that extract nutrients from hosts using haustoria (Alexopoulos et al., 1996). The net impact of these fungi on hosts is negative; and infections by these species are considered diseases. Clavicipitalean fungi do not form haustoria and are usually not considered plant pathogens; they are largely cryptic and their presence in plants is often detectable only through use of microscopic examination (White, 1987).

The clavicipitalean fungi are Ascomycetes of family Clavicipitaceae that are parasitic and biotrophic associates of insects, fungi, or plants (Rogerson, 1970). Over the past several years it has become increasingly clear that clavicipitalean biotrophs employ unique methods for obtaining nutrients (Sullivan et al., 2000; White and Camp, 1995). They also have significant impacts on the physiology and ecology of plants (Clay, 1987; White et al., 1996). In this article we will explore the various ways that clavicipitalean fungi obtain nutrients from plants and their various impacts on plants.

2. Insect Parasitism

Among clavicipitalean fungi are numerous species that are parasites of insects, including species of Beauveria, Cordyceps, Metarhizium, Paecilomyces, and Torrubiella (Rehner and Samuels, 1995). Many of these species are highly virulent pathogens that infect living insects, mummify them, and degrade them. Because of their virulence and effectiveness in infecting and killing insects, these species are often employed as insect biological control agents (St Leger et al., 1993). Interestingly, one of these species, Beauveria bassiana, has taken an evolutionary step toward plant biotrophy (Wagner and Lewis, 2000). It has recently been demonstrated that conidia of B. bassiana will germinate on the surfaces of plants, infect plants via stomatal openings, and become dormant within plant tissues. When insects consume plant tissue that contains the fungus, the fungus breaks dormancy and consumes the insect. B. bassiana uses its host plant as bait in an insect ambush. There is no evidence that B. bassiana possesses the capacity to obtain a significant quantity of nutrients from its plant hosts. B. bassiana and similar fungi appear to support the "bodyguard hypothesis" that holds that plants may employ other organisms to defend themselves from herbivores (Elliot et al., 2000).

Few clavicipitaleans possess the capacity to degrade plant cell walls.
Chaunopycnis alba is an asexual clavicipitalean that is commonly isolated from leaf litter (Bills et al., 2002). Elizabeth A. Lewis (unpublished) has demonstrated that C. alba has the capacity to degrade cellulose, suggesting an ability to compete as a soil saprophyte. The majority of species in the Clavicipitaceae are limited to infection of living organisms and have not been shown to be capable of a saprophytic mode of existence.

3. Transitions to Plants via Scale Insects

Some clavicipitaleans have bridged the gap between insect parasitism and plant parasitism in a novel way. Species of Hyperdermium, Hypocreella, and Dussiella infect scale insects, degrade them, and continue development and maturation as epibionts on the surface of the plant host (Hywel-Jones and Samuels, 1998; Sullivan et al., 2000). Scale insects (Coccidae; Homoptera) immobilize themselves on plants and use a stylet to penetrate surface tissues of plants and reach the nutrient transport vascular tissues. Through consumption and removal of the scale insect, the fungus becomes the recipient of the nutrients that emanate from the plant phloem through the stylet or wound left by the scale insect. Through parasitism of scale insects these clavicipitaleans have been able to make the transition from parasitism of insects to parasitism of plants - a trans-kingdom leap that they make each life cycle. Such clavicipitaleans are necrotrophs of the insects but biotrophs of plant hosts. Very little is known on the details of the life histories of these species.

4. Plant Parasitic Epibionts

Plant biotrophs may be epibiotic, with mycelium growing on the epidermal layer; or they may be endophytic, with mycelium colonizing interior tissues of the plant (White et al., 2000). Myriogenospora atramentosa is exclusively epibiotic, infecting species of the warm-season grass Paspalum (Rykard et al., 1985). This epibiont perennates on the exterior surfaces of the tiller meristems of the plant as a sparse mycelium. When the grass leaves expand, the fungus proliferates forming a yeast-like conidial state (ephelidial conidia) followed by a black linear stroma on the inner surface of the folded leaves. The epibiotic fungus alters leaf development, causing hypertrophied epidermal and mesophyll cells that are often several times larger than those of uninfected leaves (White and Glenn, 1994). The normal waxy cuticle does not form on the epidermis, resulting in leaves with unimpeded flow of water and nutrients to the fungus on the leaf surface (Smith et al., 1985). The precise mechanism by which M. atramentosa modifies plant development is still unknown; however,
it has been demonstrated that several other clavicipitaleans produce the auxin Indole Acetic Acid (IAA) (Porter et al., 1985). Fungus-produced auxins or other plant hormone-like compounds may provide the means whereby epibiotic clavicipitaleans may alter physiology and structure of the plant for the purposes of extracting nutrients. Testing this hypothesis will require a careful examination of the effects of individual fungal metabolites on plant cell and tissue development.

5. Endophytes

Some clavicipitaleans in Balansia and Epichloë developed endophytism during evolution, becoming intercellular in plant tissues. The clavicipitalean endophytes egress from plants to reproduce by forming mycelial stromata on which reproductive spores are produced (White et al., 1991; White and Owens, 1992). Balansia epichloe perennates within leaves; as leaves differentiate the fungus proliferates to form a stroma on the epidermis that is connected to mycelium within the leaf via hyphal bridges that pass between epidermal cells. Mycelium in the leaf beneath the stroma ramifies within the vascular tissues of the grass, establishing a direct connection to the nutrient conduit of plants. Species of Epichloë form their stroma on the culm, embedding the living inflorescence primordium in the stroma and, similar to B. epichloë, the mycelium invades vascular tissues of the grass. The epidermis layer of the plant leaves and inflorescence primordium are repeatedly perforated by the fungal mycelium that ramifies throughout all organs embedded in the stroma (White et al., 1991). Further, the plant tissues enveloped within the stroma develop abnormally: the waxy cuticle layer does not develop and epidermal cell walls fail to harden (White et al., 1997). As in M. atramentosa, it is evident that plant tissues within the stroma are modified to the extent that they cannot prevent the flow of nutrients into the mycelial stroma.

The auxin IAA has also been demonstrated to be produced by Epichloë festucae (Yue et al., 2000) and may be involved in modifying plant development. However, the possible involvement of other ergot alkaloids in affecting host physiology should be considered. The report that ergot alkaloids inhibit ATPases (Moubarak et al., 1993) is consistent with the hypothesis that ergot alkaloids act as plant growth regulators to influence physiology and development of the host. Although nutrients continuously leak from cells, cells normally reabsorb those nutrients using active transport proteins that require ATP. Inhibition of ATPases would prevent active transport and leave the nutrients available to the fungal endophytes. In Epichloë the extraction of nutrients from grass culms by the stroma has been proposed to involve the "evaporative-flow mechanism" (White and Camp, 1995; White et al., 1997),
whereby water evaporates from the surface of the stroma and is replaced by water from the vascular tissues. A flow of water and nutrients from plant to fungal mycelium is thus established that results in the continual nutrition of the stroma. The importance of this proposed mechanism for nutrient absorption is yet to be fully evaluated.

6. Sexual, Parasexual, and Clonal Reproduction in Endophytes

Species of *Epichloe* are common in cool-season grasses as cryptic endophytes. Cryptic endophytes of the deuteromycete genus *Neotyphodium* are species of *Epichloe* that have lost the capacity to reproduce sexually through formation of stromata. One study estimated that as many as 10% of the cool-season grass species bear widespread infection by these endophytes (White, 1987). These species propagate clonally by mycelial growth from the maternal plant into ovules and subsequent infection of the embryos as they develop. Evidence of parasexual genetic recombination has been demonstrated in the form of multiple gene copies in genomes of many clonal species (Tsai et al., 1994). Parasexual reproduction is a mode of recombination that does not involve meiosis and gametangium development. Cryptic endophytes in *Neotyphodium* may not be genetically isolated within plants as has been previously believed, but instead may be interacting parasexually to regularly produce new genome combinations that allow them to evolve and adapt (Chung and Schardl, 1997). Parasexual interactions between endophytes may occur on surfaces of plant leaves where epiphytic stages of endophytes have been documented (Moy et al., 2000; Dugan et al., 2002). Mejier and Leuchtmann (1999) demonstrated multistrain endophytic infections of single individuals of the grass *Brachypodium sylvaticum* by *Epichloë sylvatica*. Different strains of endophytes inhabiting the same plant would also have an ample opportunity to undergo parasexual recombination.

7. Distribution of Endophytes in Plants

Endophytic mycelium is detectable in leaf sheaths, culms, and frequently in seeds of grasses (White and Cole, 1986). Azevedo and Welty (1995) demonstrated that the endophyte *Neotyphodium coenophialum* could be isolated from roots of tall fescue grass. Studies to visualize clavicipitalean endophytes in roots have yielded negative results. Recently, we used a PCR based assay to examine roots for evidence of mycelial invasion by endophytic mycelium. In this unpublished study fine fescue (*Festuca rubra*) clones, infected with endophyte *Epichloë festucae* and uninfected clones were screened for DNA
of the endophyte in leaves and roots. The result of this experiment was that endophyte DNA could be amplified from the above ground plant parts but not from roots. This result suggests that minimal if any mycelium of *Epichloë festucae* colonizes roots. However, it is possible that other species of endophytes may have some capacity to colonize roots. Recently, Vandenkoomhuyse et al. (2002) amplified DNA belonging to 49 species of fungi from a single grass species. Perhaps, Azevedo and Welty (1995) were able to isolate the endophyte *N. coenophialum* from roots because this endophyte may possess the capacity to colonize roots. Alternatively, it may be that very early stages of root growth in seedlings may contain remnants of endophytic mycelium since the endophyte was present in tissues from which the seedlings differentiated. Such endophytic remnants are expected to be sparse and difficult to visualize but on occasion may be isolated if seedlings are grown on nutrient-containing media. Such a mycelium emerges from randomly-located areas of the root, rather than uniformly along its length (White, personal observation). It seems likely that endophytic remnants do not survive in the mature roots. This question merits further evaluation.

8. Ecological Impacts

*Epichloë/Neotyphodium* endophytes have been demonstrated to positively impact fitness of plant hosts. They have been shown to increase the ability of some grasses to tolerate drought conditions (White et al., 1992; West et al., 1993). The mechanism of increased drought tolerance is not understood, but has been proposed to be due to at least two possible factors, including osmotic adjustment of tissues in infected grasses (West et al., 1990) and/or increased root development in endophyte-containing plants (Kelrick et al., 1990). It is a reasonable hypothesis that endophyte-produced auxins or alkaloids with auxin-like effects may be secreted by endophytes growing in meristematic tissues of plants and could influence the allocation of resources between shoots and roots. Considerably more research into the mechanisms of enhanced drought tolerance is needed.

9. Herbivore Resistance

Considerable evidence has been gathered that demonstrates that endophytes of the *Epichloë/Neotyphodium* group deter herbivores from consumption of grass hosts. Lewis et al. (1993) demonstrated that the European grasses *Dactylis glomerata, Holcus lanatus,* and *Festuca rubra,* containing the endophytes *Epichloë typhina, E. clarkii,* and *E. festucae,* respectively, showed
enhanced resistance to feeding by migratory locusts. Locusts were observed to briefly sample endophyte-infected plants but not to consume quantities. In contrast, the locusts showed no hesitation in consumption of endophyte-free plant material of the same species. It should be noted that not all endophytes showed deterrence properties. *Epichloë baconii* infection in bentgrass (*Agrostis stolonifera*) had no effect on feeding by migratory locusts. Apparently this endophyte does not produce deterrence compounds. Tall fescue and perennial ryegrass infected by the endophytes *Neotyphodium coenophialum* and *N. loliiformis*, respectively, have been shown to develop resistance to feeding by Argentine stem weevils, aphids, sod webworms, and several additional insects (Breen, 1994; Bush et al., 1997; Schardl, 2001). More recently it has been found that endophytes may confer resistance to seed-harvesting ants (Knoch et al., 1993).

In a study of the distribution of endophytes in the Andes Mountains, White et al. (2001) demonstrated that populations of *Bromus setifolius* in regions inhabited by leaf-cutting ants contained high levels of the endophyte *Neotyphodium tembladerae*. The correlation between presence of herbivorous ants and endophytes was hypothesized to be due to deterrence of ant herbivory by endophytes. It is believed that endophyte-produced alkaloids such as lolines, ergovaline, and peramine deter insect herbivory (Schardl, 2001). Recent advances in transformation of endophytes to eliminate secondary metabolite production makes it possible to test this hypothesis (Panaccione et al., 2001). Some endophytes appear to increase resistance of grasses to consumption by mammalian herbivores (Hance, 1876, 1877). In South Africa the grass known locally as drunk grass (*Melica decumbens*) is infected by an endophyte that will cause an intoxication in cattle (White, 1987). It has been reported that after having been intoxicated, animals avoid drunk grass. Similarly, in South America cattle that consume grasses infected by *Neotyphodium tembladerae* experience a toxicosis that results in their avoidance of infected grass (Cabral et al., 1999). The North American grass *Achnatherum robustum* (sleepygrass) often bears an endophyte that produces lysergic acid amide, which induces a profound sleep that may last for days in horses that ingest a sufficient quantity of the grass (Petroski et al., 1992). Horses and other animals avoid the grass after experiencing a toxic episode (Marsh and Clawson, 1929). There are also reports that sleepygrass is employed by native peoples in Central America as a hypnotic and sleeping agent (Emboden, 1976).

10. **Endophyte Defense of Root Systems**

Previous studies (West et al., 1988; Gwinn and Bernard, 1993) have suggested that endophyte infection may result in enhanced resistance to nematodes in
grasses. However, little is known about this phenomenon and possible mechanisms have not been fully explored. Because nematodes are deterred from feeding on plant roots that do not appear to be colonized by clavicipitalean endophytes, it is logical to suggest that translocation of deterrent compounds from above ground plant organs inhabited by the endophytes to roots must occur (West et al., 1988). It is also possible that nematode resistance may be the result of increased grass vigor or increased root growth that results in grass individuals that are hardier and more tolerant to feeding by nematodes.

11. Fungus Disease Resistance

It has been shown that some grasses infected by clavicipitalean fungi resist fungal-induced diseases better than those that are free of endophytes (Tajima, 1990; Stovall and Clay, 1991). Fine fescue turfs infected by the endophyte *E. festucae* were highly resistant to the dollar spot disease caused by *Sclerotinia homeocarpa* (unpublished data). The grass *Phleum pratensis* infected by the endophyte *E. typhina* demonstrated enhanced resistance to leaf spot caused by *Cladosporium phlei* (Tajima, 1990).

Two hypotheses have been proposed to explain enhancement of fungus disease resistance by endophytes. One hypothesis is the 'antimicrobial compound hypothesis.' This proposes that endophytes are secreting antifungal compounds into plants that protect hosts from fungal invaders (Yue et al., 2000). Evidence that many compounds produced by endophytes are inhibitory to fungal pathogens in culture appears to support this hypothesis (Yue et al., 2000). However, it has not been shown that endophytes secrete alkaloids or other inhibitory compounds into tissues of plants. If antifungal compounds are retained within endophytic mycelium, this proposed mechanism is unlikely. Another hypothesis is the 'niche exclusion hypothesis' where endophytes occupy niches on or in plants that pathogens would need to enter to incite disease (Moy et al., 2000). The recent finding that some endophytes produce a network of mycelium (termed "defensive net") on the surfaces of leaf blades would seem to support this hypothesis. Pathogens that cause leaf diseases would first need to colonize the surfaces of leaves before entering to parasitize the tissues. The superficial network of hyphae on leaf blades may prevent pathogen mycelium from colonizing the surface of the blade, thus excluding the pathogen and preventing disease progression. It is unknown how common such defensive nets are among endophyte infected grasses.

To demonstrate that the endophyte *Epichloe typhina* of the grass host *Poa ampla* was the source of the superficial network of mycelium on leaves, the endophyte was transformed using a GUS (beta-glucuronidase) reporter gene using previously published methods (Chen et al., 1994; Cullen et al., 1987; Choi
The transformed endophyte was used to infect seedlings of *P. ampla*. Plants were permitted to grow for six months after which the superficial mycelium on leaves was screened for the GUS reporter gene using the dye X-gluc (5-brom-4-chloro-3-indolyl-beta-D-glucuronide). Presence of the transformed endophyte on the surfaces of plants was demonstrated by blue staining of the hyphal cytoplasm as the X-gluc was cleaved. These results confirm those of Moy et al. (2000) who reported surface localization of fungal endophytes infecting fine fescues.

The surface hyphae may limit the ability of fungal pathogens to attack the plants. Fungal defensive molecules released by the surface hyphae would also be able to contact pathogenic organisms before they invaded the plant. We have detected expression of endophytic secreted hydrolytic enzymes proteinase, endochitinase, and β-1,6-endoglucanase in infected *P. ampla* (Moy, 2002). The homologous enzymes from the biocontrol fungus *Trichoderma harzianum* are all components of its mycoparasitic activity (Chet, 1987; de la Cruz et al., 1995; Garcia et al., 1994; Geremia et al., 1993). We are currently investigating the function of these enzymes in the endophytic system. It is possible that they play roles in the active defense of plants against invading pathogenic fungi. This would be consistent with the defensive mutualism concept proposed by Clay (1988) who hypothesizes that the endophyte-plant mutualism is defensive in nature. It is alternatively possible that endophyte-infection induces resistance to other fungi by boosting the plant's own defensive systems or improving general vigor.

The precise mechanisms by which symbiotic endophytes impact fitness of their plant hosts is an area of investigation that is currently poorly developed but which holds promise for providing an understanding of natural strategies to defend plants from stress.

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