

# FUNGAL ENDOPHYTES OF GRASSES

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## INTRODUCTION

Mutualistic interactions between species are receiving increased attention from ecologists, although research lags far behind analogous work on competition or predator-prey interactions. Most research has focused on rather showy mutualisms such as pollination or fruit dispersal and has suggested that mutualisms are more important in tropical communities than in temperate communities (67). Plant-microbial mutualisms, in contrast, have prompted little ecological research. Plant-microbial associations are more difficult to observe and manipulate than plant-animal associations. Many plants are always infected (e.g. legumes by rhizobia, forest trees by mycorrhizal fungi), so it is easy to consider the microorganisms merely as a special type of plant organ. Further, plant-microbial mutualisms historically have been outside the realm of ecology, in other areas of biology like microbiology and mycology.

Recent research has revealed a widespread mutualistic association between grasses, our most familiar and important plant family, and endophytic fungi. Asymptomatic, systemic fungi that occur intercellularly within the leaves, stems, and reproductive organs of grasses have dramatic effects on the physiology, ecology, and reproductive biology of host plants. Through the production of toxic alkaloids, endophytic fungi defend their host plants against a wide range of insect and mammalian herbivores. Poisoning of domestic livestock has spurred a great deal of research on endophytic fungi in pasture grasses. This research has shown clearly that plants benefit from

infection by endophytes under most circumstances. This review examines the comparative ecology of endophyte-infected and uninfected grasses and identifies areas for future research.

## CLAVICIPITACEOUS FUNGAL ENDOPHYTES OF GRASSES

Endophyte is a general term that refers to any organism that lives inside of a plant, including organisms as diverse as mycorrhizal fungi and mistletoes. Many types of microorganisms live within grasses as endophytes (106), and many other plant groups support endophytic microorganisms (23). I use endophyte in this review to refer to a particular group of closely related fungi that systemically infect grasses.

### *Taxonomy*

The endophytes considered here are members of the Ascomycete family Clavicipitaceae (tribe Balansieae) and their anamorphs (asexual derivatives). This family also includes the ergot fungi (*Claviceps* spp.) and *Cordyceps*, pathogens of other fungi or insects (47). The taxonomy of the group is based on the morphology of fruiting structures on host plants and the types of conidia and ascospores produced. Endophyte genera include *Atkinsonella* (two species), *Balansia* (approximately 15 species), *Epichloe* (less than 10 species), and *Myriogenospora* (two species). A fifth genus *Balansiopsis* was recognized by Diehl (47), but its validity has been questioned (110). Our knowledge of endophytes is based primarily on North American and European hosts; additional studies are needed on tropical and/or southern hemisphere endophytes.

The sexual state of many endophytes has never been observed, so they cannot be classified as Ascomycetes. However, these endophytes are similar to the asexual form of other species with known sexual states. The asexual endophytes thus have probably been derived from sexual ones, particularly from the species *Epichloe typhina* (34, 126). Asexual endophytes have been grouped in the form genus *Acremonium* section *Albo-lanosa* (92, 131). They do not fruit on their hosts and are transmitted through the seed of infected plants (36). Sexual and asexual endophytes have similar host ranges, growth forms in the plant, and conidial morphology in culture. Molecular data will be useful in determining phylogenetic relationships among endophyte genera and species, and between sexual and asexual forms.

### *Distribution Within Hosts*

Endophytes grow as elongate, convoluted, sparsely branched hyphae running through intercellular spaces parallel to the long axis of leaves and stems (36, 125). Within the aerial plant body, endophyte hyphae are most dense in the

leaf bases surrounding the intercalary meristems of grasses. During flowering, *Acremonium* endophytes grow into ovules and become incorporated into seeds (95, 112). Endophytes are able to absorb nutrients from freely available materials found in the intercellular spaces. They do not occur in roots although they can be found in rhizomes and stolons of several hosts (31, 85).

At least four endophyte species (in the tribe Balansieae) are exceptional because they occur on the surface of young leaves and inflorescences without penetrating host tissues (78, 85). At least one of these epiphytic species, *Balansia cyperi*, occurs in the same genus as endophytic species. Technically they should not be called endophytes, but I do so here to emphasize the taxonomic affinities of the fungi rather than their particular growth form. Hyphae of epiphytic species are most dense on the surface of young, tightly appressed leaves near the meristems where the cuticle is poorly developed (78). During host flowering, the fungi proliferate and differentiate into a fruiting body surrounding the young inflorescence, aborting it in the process.

### *Endophyte Reproduction and Dispersal*

Sexual endophytes produce fruiting bodies (stromata) on the leaves or inflorescences of host plants coincident with the hosts' flowering period. Fruiting bodies generally produce both conidia and ascospores. Diehl (47) suggested that one mode of contagious spread of the fungus is infection of ovules through the germination of spores on stigmata and their growth through the style. However, the mechanism and rate of contagious spread of endophytes has not been well documented in any species (but see 73). *Atkinsonella* and *Epichloe* are heterothallic, requiring the transfer of conidia between fruiting bodies of opposite mating types before ascospores can be produced (81, 127). The conidia therefore act as spermatia or gametes in sexual reproduction. Asymptomatic, seed-borne *Acremonium* endophytes do not fruit or produce ascospores.

Endophytes are dispersed in one of three general ways. Sexual species that fruit on their hosts can infect new plants contagiously. Contagious spread is not epidemic as in many plant pathogens, but it has been observed in the field (37, 47, 73, 109). Dispersal through the seeds of its host is the only means of spread for asexual *Acremonium* endophytes, but it also occurs in plants infected by *Atkinsonella* and *Epichloe* (41, 112). Both sexual and asexual endophytes can also be disseminated through vegetative structures of host plants including rhizomes, tillers, tubers, and viviparous plantlets (30, 31, 60, 109).

## HOST RANGE OF ENDOPHYTES

A recent survey indicated that endophytes infect at least 80 grass genera and several hundred species, including several important grain crops (36). En-

dophytes infect grasses in most subfamilies and tribes of the grass family (36). However, endophyte species are specialized on different groups of grasses. *Epichloe* infects primarily hosts in the subfamily Pooideae, where it is a common parasite. Asexual *Acremonium* endophytes also infect grasses only from this group, supporting the hypothesis that they are derived from *Epichloe* (Table 1, classification following 55). Species in the grass genera *Agrostis*, *Festuca*, *Lolium*, and *Poa* (poid grasses) commonly are infected by *Acremonium* endophytes, suggesting that the endophytes were present early in the history of the subfamily and have diversified concomitant with host speciation (42, 77, 128). The only tribes in the Pooideae that do not contain known hosts for *Acremonium* endophytes are very small with few species (55). Two species of *Atkinsonella* each infects only a single host genus (*Danthonia* and *Stipa*), although the two host genera are in different tribes (80, 81). Species of *Balansia* and *Myriogenospora* primarily infect panicoid grasses with the C4 photosynthetic pathway (47, 85, 109). Host ranges of individual species vary considerably, e.g. *Balansia obtecta* infects only sandbur grass *Cenchrus echinatus*, while *B. epichloe* infects many panicoid genera and species (47). Concordant with the differential distribution of C3 and C4 grasses with latitude, *Balansia*- and *Myriogenospora*-infected hosts are more common in tropical and subtropical areas, while *Acremonium*-, *Atkinsonella*-, and *Epichloe*-infected hosts are more frequent in temperate areas. Thus, the mutualistic seed-borne *Acremonium* endophytes predominate in temperate areas, in opposition to the oft stated generalization that mutualisms are more common in the tropics.

The host ranges and genetic similarity of asexual seed-borne endophytes may provide information about phylogenetic relationships of host grasses. Grasses infected by closely related endophytes should be more closely related themselves than grasses infected by more distantly related endophytes. The large number of species of *Festuca* and *Lolium* infected by similar seed-borne endophytes suggests close affinity of the two genera, in agreement with current taxonomic concepts (90). Endophytes might also provide evidence for the affinities of difficult genera within the grass family. A case in point is the genus *Brachyelytrum* which has alternatively been considered a poid or bambusoid grass (90). A recent study found that this species commonly was infected by *Acremonium* or *Epichloe typhina* endophytes (42), a fact supporting its placement as a poid grass. Fungal parasites have been used in other plant groups as indicators of host relationships (64, 114).

While most hosts of clavicipitaceous endophytes are grasses, a secondary diversification has occurred on the Cyperaceae. In some respects the sedge family represents a wetland analog to the grass family. *Balansia cyperi* infects a number of New World *Cyperus* species (30, 47, 50), including *C. rotundus*, a widespread agricultural weed (31). *Balansia cyperaceum* also infects only

**Table 1** Distribution of Balansiae and *Acremonium* endophytes in subfamilies and tribes of the Poaceae

Grass		Endophyte	
Subfamily	Tribe	Balansiae	Acremonium
Pooideae <sup>a</sup>	Poeae	+	+
	Aveneae	+	+
	Triticeae	+	+
	Meliceae	+	+
	Stipeae	+	+
	Brachyelytreae	+	+
	Diarrheneae		
	Nardeae		
Panicoideae	Monermeae		
	Paniceae	+	
Chloridoideae	Andropogoneae	+	
	Eragrostae	+	
	Chlorideae	+	
	Zoysiae		
	Aeluropodeae		
	Unioleae	+	
	Pappophoreae		
	Orcuttiae		
Bambusoideae	Aristideae	+	
	Bambuseae	+	
	Phareae		
Oryzoideae	Oryzeae	+	
Arundinoideae	Arundineae		
	Danthonieae	+	
	Centosteceae	+	

<sup>a</sup>Classification follows Gould & Shaw (55)

sedges (47). There is one reported case of *Epichloe typhina* infecting a population of the rush *Juncus effusus* in New Hampshire (70). This probably represents an unusual and transitory case of host range expansion since rushes are not known to serve as hosts for other endophytes elsewhere. Thus, clavicipitaceous endophytes infect species in only three plant families, with grasses making up the greatest number of hosts, paralleling the host range of the related *Claviceps*, which also infects primarily grasses, but also sedges and rushes (20).

Many other nonclavicipitaceous fungi occur as asymptomatic endophytes that cause localized infections of grass leaves. For example, 200 fungal species have been isolated from leaves of healthy winter wheat (106) and endophytes have been isolated from other grass species (39, 75, 78). There is little evidence yet that these endophytes of grasses are biologically signifi-

cant. Other plant groups also serve as hosts to a wide range of endophytic fungi of diverse taxonomic affinities (23). Nonclavicipitaceous endophytes are beyond the scope of this review; interested readers are referred to Carroll (23) and references therein.

## EFFECTS OF ENDOPHYTE INFECTION ON HOST PLANTS

### *Reproductive Biology of Infected Grasses*

Grasses infected by seed-borne *Acremonium* endophytes are similar to uninfected plants in their production of normal, healthy inflorescences. However, infected plants may produce more inflorescences and seeds than do uninfected plants, reflecting their greater vegetative vigor (33, 37). Seeds of tall fescue and perennial ryegrass germinate more rapidly and to higher levels when infected, and resulting seedlings grow faster than seedlings from uninfected seeds (26, 33). Endophyte-infected seeds also contain high concentrations of alkaloids and are less likely to be eaten by vertebrate and invertebrate seed feeders (25, 132; see also section on herbivory). However, differences in seed survival, dormancy, and germination have not been examined in field situations for any *Acremonium*-infected grass.

More dramatic differences between infected and uninfected plants can be observed in the reproductive biology of grasses infected by sexual Balansieae endophytes that fruit on their hosts. In many cases, infected plants do not produce seed and so are constrained to vegetative reproduction (30, 47, 72, 73). The inflorescences may be initiated normally but abort before maturity as the fungal fruiting body develops and surrounds the developing inflorescences. Examples are found in plants infected by *Epichloe*, *typhina*, *Balansia cyperi*, *B. obtecta*, and *B. strangulans* (47, 112). In other species infected by endophytes such as *B. epichloe* and *B. henningsiana*, inflorescence development is normally inhibited so that infected plants remain vegetative while surrounding uninfected plants are in full flower (39, 47). In these species, endophyte-infected individuals can be found easily in the field by looking for large, vigorous, nonflowering plants during the peak of the flowering season.

For grasses with well-developed means of vegetative or clonal reproduction, loss of seed production may be of little detriment. The lack of seed production may contribute to the vegetative vigor of infected plants by freeing resources to be used for growth rather than reproduction. Early work by Bradshaw, Diehl, and Harberd (21, 47, 60) suggests that grasses infected by *Epichloe* and *Balansia* endophytes tiller more profusely and spread horizontally more vigorously by means of rhizomes or stolons. Greater tillering of infected plants has been subsequently noted in several other grasses (29, 33,

39, 60). In grazed or mown situations, the prostrate vegetative growth of infected plants, relative to the more upright growth of flowering uninfected plants, may provide a selective advantage to infected plants by reducing damage. Sedges in the genus *Cyperus* produce subterranean tubers or bulbils, in addition to aerial vegetative shoots. In a greenhouse study with *C. rotundus*, plants infected with *Balansia cyperi* produced significantly more tubers and significantly fewer inflorescences than did uninfected plants (120). Tubers are the major source of reproduction in this species (seeds are rarely produced), and the fungus occurs around the buds on tubers (31). The location of the fungus on tuber buds enables it to infect new shoots or tubers that originate from that bud.

The most dramatic example of endophyte infection altering the balance between sexual and asexual reproduction occurs in those host species where infection induces vivipary, the vegetative proliferation of inflorescences (not the precocious germination of seeds as in mangroves). In the sedge *C. virens*, infection by *B. cyperi* normally causes the complete abortion of every inflorescence (30). Later in the growing season the aborted inflorescences of many infected plants become viviparous, producing up to 25 plantlets per inflorescence (30). The plantlets, which arise from dormant buds in the inflorescence and not from seed, root quickly in the marshy soils where the sedge typically occurs. Plants grown from plantlets are themselves infected with *B. cyperi* (30). Because of their large size compared to seedlings, plantlets are likely to have higher probability of establishment, in addition to the other possible advantages of endophyte infection. Viviparous plantlets have also been observed growing from aborted inflorescences of the grasses *Andropogon glomeratus* and *A. virginicus* infected by *Myriogenospora atramentosa* (30), suggesting that induced vivipary may be widespread. Sampson & Western (113) reported that infection by *E. typhina* caused *Poa bulbosa*, a viviparous grass, to produce plantlets that were themselves infected. However, uninfected plants were also viviparous. Endophyte infection is very common in grass genera where vivipary is well established (e.g. *Poa*, *Festuca*) (2, 36, 42, 63, 125, 128, 130, 133).

Suppression of flowering is not complete in many host species. Individual plants infected by *E. typhina* and *B. henningsiana* sometimes produce a mixture of healthy and aborted inflorescences (39, 112, 126). Aborted inflorescences are rare in *E. typhina*-infected red fescue (*F. rubra*), where the fungus is primarily transmitted by seed (112). Similar patterns of sporadic inflorescence abortion have been noted in the genera *Agrostis*, *Brachyelytrum*, *Elymus*, and *Sphenopholis*, when infected by *E. typhina* (42). These grasses apparently represent an intermediate condition between grasses such as tall fescue and perennial ryegrass where the endophyte is completely seed-borne (95, 117) and other grasses where infection causes the abortion of

all inflorescences (47). In contrast, *Panicum henningsiana* infected by *B. epichloe* produces primarily aborted inflorescences and only a minority of healthy inflorescences (39). Resultant seeds are uninfected. The production of an occasional healthy inflorescence on an otherwise heavily infected plant, noted in other hosts as well (47), probably represents the "escape" of a single tiller from the systemic growth of the fungus. This is unlike the situation with *E. typhina*-infected red fescue where all inflorescences, both healthy and aborted, contain the endophyte (112).

Several host grasses have a well-developed floral dimorphism where potentially outcrossed chasmogamous flowers are produced at the apex of flowering culms while obligately self-pollinated cleistogamous flowers are produced in the lower axils of the leaf sheaths (22, 24, 28). Species of the fungus *Atkinsonella* infect several species in the genus *Danthonia* and *Stipa leucotricha*, all of which produce dimorphic flowers (29, 49, 81). Infected *Danthonia* regularly produce viable cleistogamous flowers and seeds while the apical panicles are completely aborted (29). Thus, infected plants are completely cleistogamous and are incapable of exchanging genes with uninfected members of the populations. Moreover, the cleistogamous seeds are infected by *Atkinsonella* and give rise to infected plants (41). Cleistogamous flowers and seeds in infected *S. leucotricha*, in contrast, are usually aborted although an occasional seed may be produced (K. Clay, personal observation). Other species with dimorphic reproductive systems are known to serve as hosts (22, 47), but the effect of endophyte infection on the production of chasmogamous and cleistogamous flowers and seeds has not been critically examined.

Recent theoretical and empirical work suggests that pathogens may select for sexual reproduction in host populations (58, 82, 84). The production of genetically variable progeny may make it more difficult for pathogen populations to specialize on particular genotypes, which would be easier in asexual host populations. Inflorescence-aborting Balansieae endophytes may exhibit a counteradaptation to the host strategy of producing genetically variable offspring. The fungi suppress outcrossing and enforce complete self-fertilization or asexual reproduction, promoting a more genetically uniform host population (34). For example, viviparous plantlets produced by *B. cyperii*-infected *C. virens* are genetic copies of the maternal plant and are infected by the same fungal genotype as the maternal plant. Genetic analyses of the magnitude and spatial scale of variation in natural populations containing mixtures of infected and uninfected individuals would support or refute this hypothesis.

### *Physiological Ecology of Infected Grasses*

One of the least understood aspects of the grass/fungal endophyte symbiosis is the comparative physiological ecology of infected versus uninfected plants.



Most work has focused on tall fescue, the most important pasture grass in eastern North America. Endophyte infection causes changes in host plant physiology that stimulate growth under controlled environmental conditions (33, 76). Enhanced growth of infected plants also occurred in perennial ryegrass and purple nutsedge grown in controlled environments (33, 76, 120). In tall fescue the growth advantage of endophyte-infected plants was reversed under conditions of severe nutrient stress, suggesting that the metabolic cost of supporting the endophytic fungus has a negligible detrimental effect except under extreme conditions (26).

In response to increasing irradiance, net photosynthetic rates of endophyte-infected tall fescue were found to be less than endophyte-free clones of the same genotypes, although infected clones produced more biomass and tillers (15). We have found that endophyte-infected tall fescue maintained significantly higher net photosynthetic rates at temperatures above 25°C than did uninfected plants when both were grown under well-watered conditions in the greenhouse (S. Marks and K. Clay, unpublished). Further studies have indicated that infected plants tend to have greater stomatal resistances (4, 51). These results are consistent with experimental work showing that infected tall fescue maintains higher productivity than do uninfected plants under drought conditions (4, 105, 124). Arachevaleta et al (4) showed that infected plants exhibited leaf rolling under drought stress conditions much more rapidly than did uninfected plants, and that under extreme drought conditions all uninfected plants died while all infected plants survived. Other studies have indicated that infected plants exhibited greater osmotic adjustment than uninfected plants, which allowed the infected plants to maintain higher turgor pressures under drought conditions (51, 124). Extreme environmental stresses, such as described above, may provide intermittent strong selection favoring infected plants and causing dramatic changes in population composition. The proportion of endophyte-infected tall fescue and other grasses may have increased substantially during the drought conditions experienced over much of North America in 1988.

Mechanisms by which photosynthesis and water relations might be altered by endophyte infection include changes in the plant's hormonal balance and changes in source-sink relationships. Auxin has been detected in pure cultures of the endophyte *Balansia epichloe* (99). Morphological and/or physiological changes observed in a number of host species (i.e. inhibition of flowering) are consistent with altered hormone metabolism (30, 47). Studies of photosynthate metabolism in two different host-endophyte associations have revealed that the fungi rapidly convert plant sucrose into sugar alcohols that the plants are unable to metabolize (119, 121). By continually depleting the plant's sink of available photosynthate, endophyte infection may reduce or prevent feedback inhibition of photosynthetic rates, thereby allowing higher average growth rates.

## HERBIVORY OF ENDOPHYTE-INFECTED GRASSES

### *Mammalian Herbivory*

Grasses are relatively free of toxic secondary compounds, compared to most plant families. Crop or pasture grasses infected by species of *Claviceps*, the ergot fungus, have poisoned livestock (and humans) for hundreds of years (20, 87). Many grasses toxic to mammalian herbivores in the absence of ergot infection are now known to be endophyte infected (11, 25, 93). Endophyte-infected grasses, including species in the genera *Andropogon*, *Festuca*, *Lolium*, *Melica*, *Paspalum*, *Sporobolus*, and *Stipa* (10, 12, 25, 35, 93, 96), can be extremely toxic to a range of insect and mammalian herbivores (7, 40, 118). Animals often refuse to graze on infected grasses (19, 59, 89) and develop a number of circulatory, muscular, and neurological disorders if they do (10, 66, 93). Because most research has been conducted within an agricultural context, essentially nothing is known about how endophyte-infection affects vertebrate herbivory in natural communities. Fairly simple experiments where known infected and uninfected individuals (or ramets) of the same species are planted in random arrays in the field would provide evidence on whether differential herbivory occurs. It seems unlikely that domestic animals would react differently from wild animals in their behavioral or physiological responses to endophyte-infected grasses.

Attempts to replant heavily on endophyte-infected pastures with endophyte-free seed have been made in tall fescue pastures in the United States and perennial ryegrass pastures in New Zealand. However, eliminating the endophyte reduces the vigor of plants and their resistance to a range of biotic and abiotic stresses (4, 13, 105, 124). This is apparently the reason that endophyte-infected pasture grasses were so widely planted in the first place. Artificial selection for superior plant vigor and persistence inadvertently selected for endophyte-infected plants. Similar processes appear to occur in nature.

### *Insect Herbivory*

An important factor favoring endophyte-infected grasses in natural and agricultural communities is their enhanced resistance to insect herbivores. Field observations of fescue and ryegrass species indicate reduced herbivory of endophyte-infected plants in experimental plots and lower populations of the herbivores (54, 103, 104, 111, but see also 71, 83). Insect feeding trials and food plant preference studies conducted in the laboratory with a variety of grass species have shown that endophyte infection acts as a feeding and oviposition deterrent (61, 68) and/or reduces the survival, growth, and developmental rates of feeding insects (1, 25, 40, 74, 115). This topic has been reviewed extensively (35, 36, 118). As in the case of mammalian herbivory,

evidence for insecticidal effects of endophyte infection in the field comes primarily from domesticated grasses. There is a need for careful field studies of possible differential herbivory in wild grasses similar to the work of Marquis (88).

### *Other Pests*

Endophyte infection also may protect host plants against other pests. Reduced nematode populations were associated with endophyte infection in tall fescue, both in pot culture and in field soils (97, 124). Inhibition of plant pathogens in vitro by endophyte cultures has also been documented (14, 129). Two studies have found a negative correlation between endophyte and pathogen infection in field situations. *Panicum agrostoides* infected by *Balansia henningsiana* had significantly fewer lesions of the leaf spot fungus *Alternaria triticina* than did neighboring endophyte-free plants in a natural population (39). Another study with tall fescue found reduced levels of crown rust (*Puccinia coronata*) in endophyte-infected plots (53). Possible mechanisms by which endophytes could inhibit plant pathogens include competition for resources, induction of generalized defense responses, and the production of antimicrobial compounds (32, 46).

### *Endophyte Toxins*

Alkaloids produced by endophytes within plant tissues are in part responsible for the poisonings of mammalian grazers and plant resistance to insect herbivores. Endophyte-infected grasses contain a variety of alkaloids not found in uninfected conspecifics; these alkaloids include ergot alkaloids, loline alkaloids, lolitrems, and peramine (7, 8, 38, 68, 101, 102, 107, 108). Detailed reviews on the chemistry of endophyte alkaloids are available (7, 45). Most of these alkaloids, or their precursors, have been detected in liquid cultures of the fungi (1, 5, 6, 98). Further, feeding trials using pure alkaloids have revealed insecticidal activity against several herbivores (38, 45, 68).

Alkaloid content of endophyte-infected grasses is known to vary among host species, plant parts (leaf blades, sheaths, flowering culms), within the same parts of different age, at different times of the growing season, and with fertilization and grazing history (7, 16, 17, 62, 86). In tall fescue the greatest concentration of loline alkaloids is in the seeds, suggesting a role in preventing seed predation (25). In adult plants of tall fescue, alkaloid concentrations are higher in young than in old leaves and in leaf sheaths than in leaf blades (62, 86). These patterns coincide with endophyte hyphal density, as well as with the nutritive value of the tissues (62, 65). Fungal strains also differ in the amounts and kinds of alkaloids produced in pure culture (9, 98, 100), suggesting that between-plant variation could exist in alkaloid content resulting from genetic differences between endophyte strains.

The bitter taste of many alkaloids may allow herbivores to discriminate among plants and avoid endophyte-infected individuals. Where a species is completely endophyte infected, herbivores may shift to other species. Populations differing historically in the level of herbivory would provide evidence as to whether selection favors endophyte-infected plants or plants infected with high alkaloid-producing endophyte strains where herbivory is common or chronic. Results would also bear on the question of whether grasses benefit from herbivory (18, 43, 91).

## DEMOGRAPHY OF ENDOPHYTES AND THEIR HOSTS

### *Frequency of Infection Within Populations*

As previously indicated, some grass species are never endophyte-infected, some always are infected, and others consist of mixtures of infected and uninfected individuals. However, the majority of grass species have not been examined for endophyte infection, especially grasses from tropical regions. Complete infection of all individuals in host populations occurs only in grasses infected by asexual, seed-borne *Acremonium* endophytes (42, 125), implying a strong selective advantage for infected plants. Infection can be lost by occasional production of uninfected seed or by extended seed dormancy but cannot be gained within a lineage (122). Herbarium specimens have been examined for *Acremonium* endophyte infection. High levels of infection, often 100% of specimens, were observed in many species of *Agrostis*, *Elymus*, *Festuca*, *Lolium*, *Poa*, *Sphenopholis*, and *Stipa*, as well as numerous other genera with fewer host species (42, 125, 128, 130). While surveys of herbarium specimens may not reflect the level of infection within populations, they provide an overview of infection levels throughout the range of the host. Further, samples are unbiased since there is no way to tell, without microscopic examination, if the plants collected were infected.

Field surveys, which have focused on pasture grasses, have also revealed very high levels of *Acremonium* endophyte infection. Neill (94, 95) found that 16 of 20 perennial ryegrass (*Lolium perenne*) varieties in New Zealand were 100% infected. High levels of endophyte infection have been found in other varieties of perennial ryegrass (54, 56, 57, 77), other species of *Lolium* (77), and in *Festuca rubra* and *F. longifolia* (111, 112, 123). *Festuca arundinacea*, tall fescue, has been extensively sampled in the United States. In Tennessee, 67 of 79 counties contained endophyte-infected plants (each county represented by six plants from three locations) with an overall average of 35% infection. A total of nearly 1500 samples of tall fescue from 26 states throughout the United States had an average of approximately 50% infection, with 30% of the samples containing 90% or more infected plants (116a).

Estimates of infection level have been made for several grasses infected by

sexual *Balansia* endophytes. In Britain up to 40% of *Agrostis tenuis* and 80% of *Dactylis glomerata* plants were infected by *Epichloe typhina* in some sites (21, 72, 73). Over one half of *Cyperus virens* plants sampled in coastal Louisiana were infected by *Balansia cyperi*, with up to 80% of plants infected in some sites (30). Similarly, the majority of *Panicum agrostoides* plants in most sites in Indiana was infected by *B. henningsiana* (39), while only 15% of *Danthonia spicata* plants in a North Carolina population were infected by *Atkinsonella hypoxylon* (29). In Sierra Leone, cultivated rice becomes infected by *Balansia pallida*; 2% of panicles were destroyed by the fungus at the most heavily infected study site (52). Anecdotal observations suggest that infection levels can be high in many other species as well (K. Clay, personal observation).

Populations of grasses completely infected by sexual endophytes that suppress host seed production might be expected to have higher extinction probabilities than ones where seed are produced, even though they could persist through vegetative reproduction alone (see 21, 60). Infection levels of *Acremonium* endophytes, in contrast, are not subject to the same constraints.

### *Dynamics of Infection*

Changes in levels of endophyte infection occur in many host populations over time. Early workers noted the tendency for *Atkinsonella* and *Epichloe* to be more prevalent in older populations (21, 47, 60, 73). *Acremonium* endophyte-infection levels in *Lolium* species were higher in older pastures than in younger pastures, and in natural populations compared to domesticated varieties (44, 77, 83, 94). In field plots of *Festuca longifolia* the percentage of endophyte infection went from less than 50% to over 90% in seven years (111).

Increasing levels of endophyte infection with increasing age of the host population may be due to contagious spread of the fungus, increased survival and/or reproduction of infected plants, or both, depending upon the ability of the endophyte to sporulate and the host plant to produce seed. For seed-borne *Acremonium* endophytes contagious spread is impossible; the fungus does not produce spores and never occurs outside the interior plant body. Increasing infection over time implies a fitness benefit to infected plants, which must live longer or produce more offspring than do endophyte-free plants. Population dynamics of endophyte infection within populations could be modeled simply by equating infection with a maternally inherited gene with two alternative alleles of different selective value.

In contrast to *Acremonium* endophytes, increasing levels of infection by *Atkinsonella* or *Epichloe* can result from contagious spread of the endophyte and/or from differential host survival. In *Dactylis glomerata* fields, levels of infection rose dramatically from the first year following establishment up

through seven years. It seems likely that contagious spread is initially important since the fields were initially founded from endophyte-free seed, but increased host survival could become more important over time. In a study with marked plants in a grassland community, nearly 20% of uninfected *Sporobolus poiretti* became infected by *Balansia epichloe* in each of two years (37). Studies of other host species have found little or no contagious spread of spore-producing endophytes (3, 29, 37). Despite a low level of contagious spread, infection levels can still increase due to demographic differences between conspecific hosts and nonhosts.

### *Host Demography*

Four detailed studies of the comparative demography of endophyte-infected and uninfected plants have been conducted. Infection of *Danthonia spicata* by *Atkinsonella hypoxylon* increased from 25% to 30% of a study cohort over three years owing to greater survival of infected plants (29). Infected plants grew significantly larger but produced significantly fewer seeds than did uninfected plants. Infected plants also were superior competitors in field experiments against the cooccurring grass *Anthoxanthum odoratum* (69). In experimental populations of the sedge *C. virens* and the fungus *B. cyperi*, an initial infection level of 50% increased to 90% infection over three years owing to the greater survival of plants infected with *B. cyperi* (37). However, similar demographic experiments with *Sporobolus poiretti* revealed equivalent survival rates for *Balansia*-infected and uninfected plants (37).

Demographic studies of tall fescue, which flowers and sets seed normally when infected by endophytes, have demonstrated a nearly two-fold increase in fitness of infected plants compared to uninfected conspecifics. In field studies where ramets of known infection status were planted out into a grassland community in Louisiana, members of the infected cohort had significantly higher survival and growth rates than did uninfected plants, and a higher percentage of infected plants flowered (37). Over the long term, endophyte-infected tall fescue plants should come to dominate the population.

While increased growth of infected plants in controlled environments must result from direct effects on plant physiology, demographic and competitive differences in field situations may result from changes both in host physiology and in resistance to herbivores and other plant pests. Field competition or demographic experiments where levels of herbivory are manipulated should provide evidence on the relative importance of induced changes in host physiology and resistance to plant pests for the comparative demography of endophyte-infected plants.

### *Mutualistic and Pathogenic Endophytes*

Closely related fungi infecting various grasses can enhance host fitness by increasing host survival, growth, and reproduction, or reduce host fitness by

suppressing seed production. However, even the more pathogenic endophytes like *Atkinsonella* or *Epichloe*, which abort host inflorescences, have positive effects on plant survival, growth, and resistance to herbivory. The critical difference between the two forms of endophyte infection is the production of spore-producing fruiting bodies which abort inflorescences by the more pathogenic Balansieae endophytes versus the unimpaired flowering and the concomitant seed transmission by the more mutualistic *Acremonium* endophytes (34).

A range of endophyte effects on host reproduction occurs within and among hosts of *E. typhina*. A survey of woodland grasses infected by *E. typhina* revealed that in *Glyceria striata* all flowering culms of infected plants were aborted; in *Elymus virginicus* only a minority of flowering culms were aborted (and the endophyte was seed-borne in the other culms); and in *Agrostis hiemalis* fruiting bodies were rarely produced in most populations, and the endophyte was almost entirely seed-borne (42). A similar range of variation also occurs within single host species. Sampson (112) and White (126) observed *Festuca rubra* and *E. virginicus*, respectively, and found infected plants within the same population that either (a) produced a high proportion of aborted culms, (b) a low proportion of aborted culms, or (c) no aborted culms, with seed transmission of the endophyte in normal culms.

These relationships illustrate a transition between mutualistic and pathogenic associations and offer the potential for determining the underlying genetic and/or environmental basis for the variation in seed transmission and inflorescence abortion (34, 126). Reciprocal inoculations of seedlings or tillers from plants in categories *a*, *b*, and *c* above with fungal isolates from the same categories of plants should reveal whether the observed variation is a function of plant genotype, fungal genotype, or an interaction of both. Evidence for an environmental influence could be obtained from common garden experiments where plants in categories *a–c* are cloned and ramets planted into different microenvironments. Similar responses by all clones of a genotype would suggest a genetic basis for endophyte seed transmission versus inflorescence abortion. The ecological and genetic consequences of variation in host and fungal reproductive systems for both the host and the fungus are important areas for future research that will provide insights into how symbiotic relationships move along a continuum of mutualistic and pathogenic interactions.

## CONCLUSIONS AND FUTURE DIRECTIONS

The grass-endophyte symbiosis has important implications for many areas of ecology and evolution, both applied and basic. Endophytes are significant economic problems in the beef and dairy industries because of their detrimental effects on livestock (7, 118). Simultaneously, endophytes are attractive

potential biocontrol agents for reducing pest damage in nongrazed grassland communities such as lawns, athletic fields, golf courses, and roadsides (36). Turfgrass variables available on the commercial market often contain high levels of endophyte infection (56, 77, 111). Endophytes could be utilized to "vaccinate" crop grasses against pests if toxins did not accumulate in seeds, and they could be used as vectors for the genetic engineering of grasses (36).

Endophytes infecting grasses provide the best example of a defensive mutualism where the parasite protects its host against enemies (35). This association appears to be widespread, although detailed studies have been conducted only in a very few systems. Additional research needs to be conducted in several areas. We know little about the potential for herbivores to evolve tolerance or resistance to fungal alkaloids in grasses. Selection for tolerance in herbivore populations should be strongest in communities where a high percentage of individuals and species are endophyte-infected. In contrast, selection for inherent chemical defenses in grass populations would be strongest in communities where a low percentage of individuals and species are infected by endophytes. The degree of differential herbivory in mixed populations of infected and uninfected plants needs to be examined in several host species in different natural communities. It is not known whether different fungal genotypes infecting the same host population (see 80) vary in the amounts and kinds of alkaloids produced and whether hosts of the most toxic fungal genotypes are most successful when herbivory is common. There may be costs to the host, which cannot be recouped in the absence of herbivory, of supporting a high alkaloid-producing endophyte compared to one that produces smaller amounts of alkaloids. Comparisons of levels of endophyte infection and secondary defensive compounds (of either fungal or plant origin) between communities differing historically in the levels of herbivory would be valuable. Further, species that differ in the inherent nutritional content of foliage may be affected disproportionately by endophyte-infection such that the most nutritious and palatable species gain the greatest benefit from infection. The high levels of endophyte infection in pasture grasses support this hypothesis (36, 77, 118). Could our entire system of agriculture, based primarily on grasses, have originated through the accidental elimination of endophytes from grasses that, undefended, became especially nutritious or palatable (77)?

Levels of endophyte infection within and among species are known from few communities. Two community-level studies (42, 77) have revealed high levels of endophyte-infection in North American deciduous woodland communities and in central European perennial grassland communities. We do not know whether the levels of infection in these communities differ from infection levels in tropical grasslands, arctic tundra, or temperate prairie communities. Large-scale surveys in a diversity of community types through-



out the world are needed to answer the basic question of how common endophytes are.

Endophyte infection of grasses affects, and can be affected by, community characteristics. A number of studies reviewed here suggest that infection levels within species increase with succession and community age (37, 105, 111). Infection-enhanced competitive abilities (69) can affect the displacement of endophyte-free species by endophyte-infected species, especially if herbivory is severe (54, 111, 116). Anecdotal report suggests community changes may be rapid. Shaw (116) reported that the endophyte-infected grass *Melica decumbens* (drunk grass) increased dramatically in South African grasslands following the introduction of European livestock, presumably because they preferentially fed on uninfected species. Similar community-level increases of endophyte-infected *Sporobolus poiretii* in heavily grazed southeastern pastures also have been reported (7). The dynamics of endophyte infection within and among host species during and following insect outbreaks or the introduction of grazers would be of particular interest.

There remain many unanswered questions regarding the population and evolutionary dynamics of endophyte infection in grass populations. The rate at which asexual seed-borne endophytes increase in host populations versus sexual Balansieae endophytes that suppress sexual reproduction of their hosts needs to be quantified. Plant survival and seed production, and the rate of contagious spread of infection, are the most important parameters to be determined. Models that reveal conditions favoring one form of transmission over the other may provide insights into how mutualistic and pathogenic associations evolve and coevolve.

The inverse relationship between endophyte sexuality and host sexuality (see 34) has important implications for gene flow and the genetic structure of both host and endophyte populations. For example, *Danthonia* grasses infected by *Atkinsonella* are reproductively isolated from uninfected plants in the population. Infected plants reproduce sexually only by self-fertilized cleistogamous seeds, which are themselves endophyte infected (29, 41). The fungus, in contrast, is cross-fertilized with other genotypes (81). Do infected plants consist of genetically similar individuals interspersed within a panmictic, uninfected subpopulation? At the other extreme are situations where the endophyte is completely asexual and seed-borne, and the host flowers normally. How many fungal genotypes exist within a single host population (80)? Do seed-borne endophytes have narrower host ranges than sexual endophytes that must contagiously infect new hosts? Are there plant genes for resistance to endophyte infection? Limited data suggest that resistance to infection, as found in many crop plants towards pathogens, does not exist in endophyte host species (79).

In conclusion, much has been learned over the last ten years about fungal

endophytes of grasses, but many additional questions have been raised. Given the importance of grasses in agricultural and plant communities, the implications of endophyte symbiosis should not be underestimated. Further, endophyte-infected grasses represent ideal model systems for approaching questions about the coevolutionary origin of mutualisms from pathogenic associations, and the role of herbivory in plant population and community dynamics. We need to begin to think about many grasses not as independent organisms but as partners in a symbiosis, similar to lichens or legumes infected by N-fixing rhizobia.

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