

# SYMBIOSES OF GRASSES WITH SEEDBORNE FUNGAL ENDOPHYTES

Christopher L. Schardl,<sup>1</sup> Adrian Leuchtmann,<sup>2</sup>  
and Martin J. Spiering<sup>3</sup>

<sup>1</sup>Department of Plant Pathology, University of Kentucky, Lexington,  
Kentucky 40546-0312; email: schardl@uky.edu

<sup>2</sup>Geobotanisches Institut ETH, CH-8008 Zurich, Switzerland;  
email: leuchtmann@geobot.umnw.ethz.ch

<sup>3</sup>Department of Plant Pathology, University of Kentucky, Lexington,  
Kentucky 40546-0312; email: mspier2@uky.edu

**Key Words** clavicipitaceous fungi, *Epichloë*, mutualism, *Neotyphodium*, Poaceae

■ **Abstract** Grasses (family Poaceae) and fungi of the family Clavicipitaceae have a long history of symbiosis ranging in a continuum from mutualisms to antagonisms. This continuum is particularly evident among symbioses involving the fungal genus *Epichloë* (asexual forms = *Neotyphodium* spp.). In the more mutualistic symbiots, the *epichloë* endophytes are vertically transmitted via host seeds, and in the more antagonistic symbiots they spread contagiously and suppress host seed set. The endophytes gain shelter, nutrition, and dissemination via host propagules, and can contribute an array of host fitness enhancements including protection against insect and vertebrate herbivores and root nematodes, enhancements of drought tolerance and nutrient status, and improved growth particularly of the root. In some systems, such as the tall fescue *N. coenophialum* symbioses, the plant may depend on the endophyte under many natural conditions. Recent advances in endophyte molecular biology promise to shed light on the mechanisms of the symbioses and host benefits.

## CONTENTS

INTRODUCTION .....	316
COORDINATED LIFE HISTORIES .....	316
Symbiotic Continuum and Life Cycles .....	316
The Third Mutualist and Fungal Pollination .....	320
REGULATING ENDOPHYTE GROWTH IN PLANTA .....	321
EFFECTS ON OTHER ORGANISMS .....	322
Anti-Insect Activities .....	322
Effects on Vertebrates .....	323
Effects on Nematodes .....	325
Effects on Fungi and Plants .....	325
MOLECULAR BIOLOGY OF ENDOPHYTE SECONDARY METABOLISM .....	325

EFFECTS ON HOST PHYSIOLOGY .....	326
Effects on Growth and Abiotic Stress Tolerance .....	326
Effects on Photosynthesis .....	327
EVOLUTION OF GRASS-ENDOPHYTE SYSTEMS .....	328
Host Specificity .....	328
Epichloë Speciation and Co-Phylogeny with Hosts .....	328
Interspecific Hybrids .....	329
CONCLUDING REMARKS .....	331

## INTRODUCTION

Symbiosis is the typical state for large organisms, either plant or animal. The most widely studied symbioses are those that cause disease in the host. Other, more benign, symbioses are less obvious, but mutualistic symbioses are of major ecological and evolutionary importance. For example, most plants form mycorrhizae, interactions of roots with various fungi that facilitate nutrient uptake from soil (71). In the aerial parts of plants, endophytic and epiphytic fungi can be abundant, though inconspicuous (27, 28). The major subfamily of temperate grasses, the Poöideae, have many species possessing fungal endophytes that systemically infect much or all of the plant, some of which are also seed transmissible (141). This review focuses on the grass symbioses with the most widespread and beneficial of these endophytes, the *Epichloë* and *Neotyphodium* species (phylum Ascomycota, order Hypocreales, family Clavicipitaceae) (5, 86).

Note that the name *Neotyphodium* refers to asexual derivatives of *Epichloë* spp. (66). Here, we introduce “epichloë” as a general reference to these endophytes and use the Latin genus names only when specifying asexual or sexual species.

By definition, a mutualism must involve exchange of beneficial currencies between the partners, and must also exact mutual costs, but benefits must outweigh costs for both host and symbiont. Often, however, the relative benefits and costs depend on environmental or ecological contexts. For example, mycorrhizal symbioses show the most mutual benefit under low phosphate conditions (71). In contrast, benefits of clavicipitaceous endophytes to grass hosts have most often been observed under conditions of herbivory or parasitism (40), or in some cases under drought stress (101). Yet other benefits are also documented, including improved acquisition of phosphate and utilization of nitrogen (95, 101). The grass-endophyte symbioses demonstrate that benefits can be multifarious, and by coevolution of the partners an ever-expanding array of currencies may evolve.

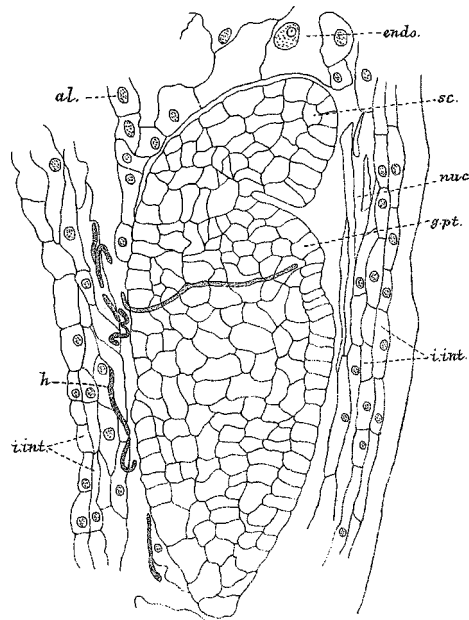
## COORDINATED LIFE HISTORIES

### Symbiotic Continuum and Life Cycles

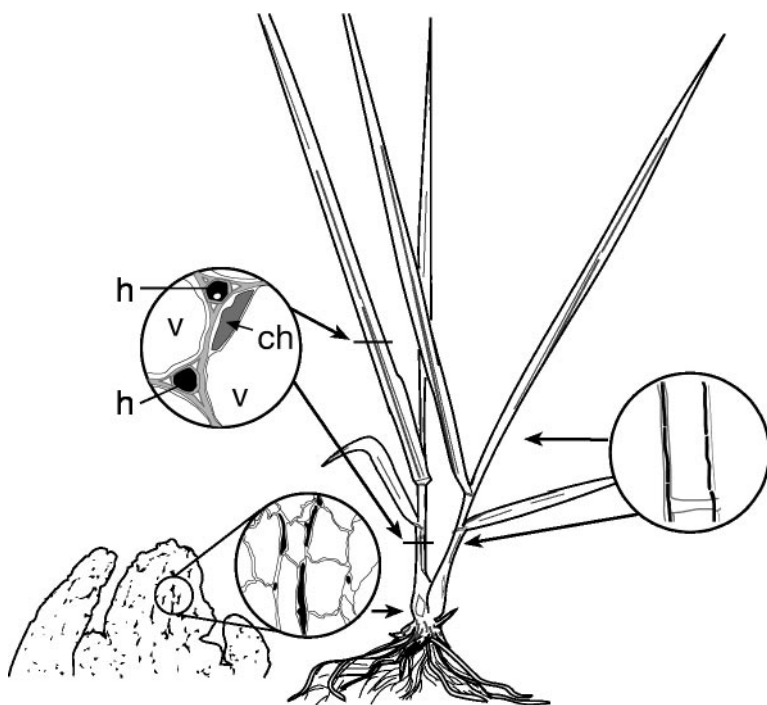
Symbioses of grass plants with epichloë fungi can be mutualistic, antagonistic, or exhibit both mutualistic and antagonistic characteristics. Where a symbiotum sits on that continuum is strongly influenced by the symbiont life cycle, which in

turn strongly influences host reproduction. In the more antagonistic symbiots, host seed production is completely suppressed (choke disease) and the symbiont only transmits horizontally via sexually derived spores (ascospores) (150). In contrast, the more mutualistic associations are characterized by vertical transmission of the fungal hyphae via the seeds, which become the common diaspore for plant and symbiont. This represents an asexual form of endophyte reproduction. Completing the continuum are plant-epichloë symbiots that have both choked and symptomless inflorescences (130, 133), and exhibit both antagonistic and mutualistic effects.

Although *Epichloë* spp. had been known of for centuries, their strictly seed-borne relatives (in their inimitably subtle fashion) long eluded the attention of botanists. The first accounts of such endophytes were published near the end of the nineteenth century. Vogel (148) identified a densely interwoven layer of fungal hyphae in the remains of the nucellus between the aleurone and the seed coat of the grain in seeds of darnel (*Lolium perenne* subsp. *temulentum* = *L. temulentum*). In 1898 Guérin (67) suggested that the hyphal layer in the grain indicates mutualistic, rather than antagonistic, symbiosis. Among the studies of the darnel seed fungus that ensued, it was Freeman (62) who, in 1904, first detailed the entire coordinated plant-endophyte life history, observing that hyphae gain entrance to the embryo long before seed maturation (Figure 1). After seed germination the endophyte coordinates its growth with the above-ground plant tissues (Figures 2 and 3),



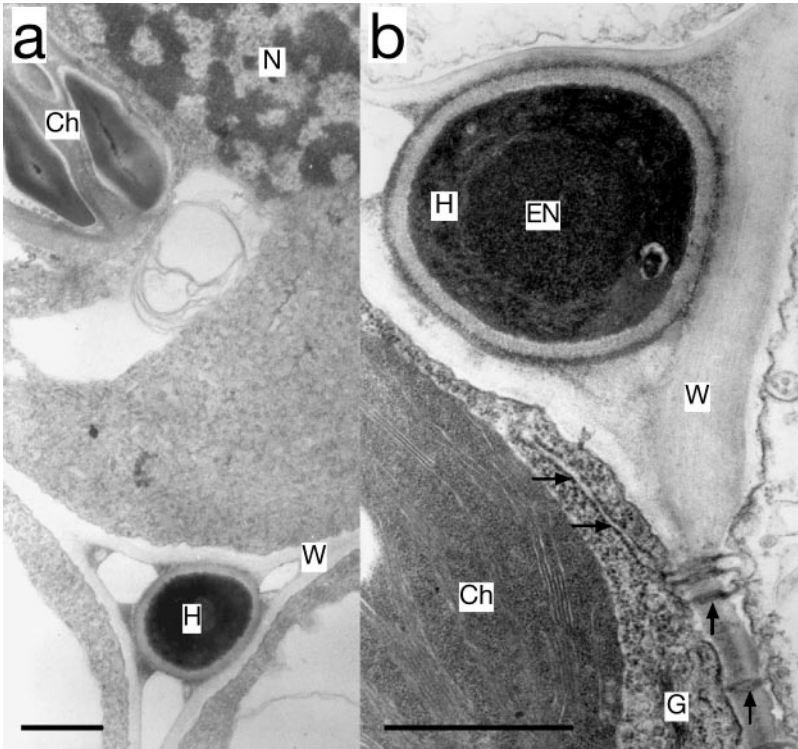
**Figure 1** Colonization by a *Neotyphodium occultans* hypha (h) of the embryo of *Lolium perenne* subsp. *temulentum* early in its development. Reprinted with permission (62).



**Figure 2** Endophyte growth in a grass plant. Bottom left: fungal growth in the true stem and leaf primordia. The fungal hyphae are shown darkly stained with osmium as they would appear in TEM (transmission electron microscopy). Upper left: A cross section of leaf or leaf sheath reveals hyphae (h) of the epichloë endophyte between host cells. Also shown are a chloroplast (ch) and vacuoles (v). Right: the endophyte as it appears in a leaf epidermal peel, stained for hyphae, which are arranged mainly along the longitudinal axis of plant cells. Unstained septa separate individual fungal cells, each of which bears a single haploid nucleus (not shown). Micrographs are at different scales. For comparison, epichloë hyphae are approximately 1–2  $\mu\text{m}$  thick.

colonizing lateral buds and, later, inflorescences. Vertical transmission of the asexual *Neotyphodium* spp. is remarkably efficient. In most natural associations the endophyte is present in essentially all seeds and, if the seed is freshly germinated, all progeny seedlings bear the symbiont (139). The hyphae never extend into roots or invade anthers. With the Freeman study (62) the systemic nature and maternal-line vertical transmission were clearly established for this representative of the clavicipitaceous endophytes.

In the 1930s Sampson (130, 131) made a key discovery concerning sexual endophytes. She compared an endophyte in *Festuca rubra* [now known as *Epichloë festucae* (92)] with *Epichloë typhina* on *Dactylis glomerata*. Whereas the latter



**Figure 3** Electron micrographs showing hyphae of (a) *Neotyphodium aotearoae* and (b) *N. australiense* in transverse leaf sheath sections of *Echinopogon ovatus*. Annotations are as follows: hypha (H), endophyte nucleus (EN), host nucleus (N), chloroplast (Ch), host cell wall (W), Golgi apparatus (G), plasmodesmata (vertical arrows), and endoplasmic reticulum (horizontal arrows). The plant cells appear normal, with no obvious damage or response to the endophytes. Scale bars represent 1  $\mu$ m. Reprinted with permission (108).

represents a textbook example of choke disease affecting all inflorescences, the *F. rubra* symbiont exhibits both the ability to cause choke and the ability to disseminate via seeds in a fashion similar to the strictly seedborne endophytes of perennial ryegrass (*L. perenne* subsp. *perenne*) and darnel. Remarkably, *E. festucae* can manifest its sexual cycle or asexual cycle on different tillers of the same plant, a characteristic later shown for most *Epichloë* spp. in poïd grasses (Figure 4). This finding connected the asexual endophytes with their sexual relatives.

The *Epichloë* sexual cycle (Figure 5) commences with accelerated fungal proliferation when the host forms inflorescence primordia (80). Grass internodes

elongate, raising the immature inflorescence, which is initially surrounded by a leaf sheath. Immediately prior to the stage when the inflorescence would emerge from the boot, the white endophyte mycelium proliferates specifically on the leaf sheath surrounding the young inflorescence, and mycelia thoroughly ramify the inflorescence. This fungal structure is the young stroma, which is loaded with conidia (mitotic spores). With stroma formation, the underlying inflorescence fails to emerge, and no seeds are produced on that tiller. If conidia (acting as spermatia) of one mating type are transferred to a stroma of opposite mating type, fruiting structures develop over the next three to six weeks. Eventually, fruiting bodies (perithecia) develop with scores of sacs (asci), each with eight ascospores. The ascospores are ejected into the air and mediate infection of neighboring plants (19) or developing seeds produced on neighboring plants (39).

### The Third Mutualist and Fungal Pollination

The *Epichloë* spp. require fly “pollinators” (Figure 5). The opposite mating types, mat1 and mat2, are cross-fertilized by flies of the genus *Botanophila* (order Diptera, family Anthomyiidae) (1, 24, 72, 82). Female flies transfer spermatia within their gut as they feed and oviposit. Immediately following oviposition, the fly fertilizes the stroma by a stereotypical fly behavior: She drags her abdomen while excreting feces on the stroma surface (24). Her larvae grow by feeding on the developing perithecial stroma and ultimately drop to pupate in the soil. This appears to be an obligate mutualism having much in common with associations between plants and their pollinating parasites (2, 21, 116, 146).

At least five described species are associated with *Epichloë* hosts in Europe (49, 72, 105). Additional fly taxa genotypically distinct from the European species were identified in North America (A. Leuchtmann, unpublished data). Two or more different *Botanophila* species can co-occur at a particular locality (49).

This unique association of *Epichloë* with *Botanophila* spp. is a delicately balanced symbiosis. The *Epichloë* fungi benefit from the flies as reliable vectors of spermatia, and the larvae depend on fertilized stromata as food (24). Larvae on unfertilized stromata seldom reach pupation (A. Leuchtmann, personal observation). As in pollinating parasites of flowering plants, the fly mutualism imposes a cost to the fungus by reducing the potential output of ascospores (149). However, flies do not overly exploit stromata, and the net effect of *Botanophila* on ascospore production is clearly positive (23, 24). One possible explanation for how the symbiotic balance is maintained is the observation that greater fly visitation with multiple eggs laid on a single stroma increases larval mortality. The causes for mortality are unknown but could be diseases or the parasitoids that commonly emerge from *Botanophila* pupae (82; A. Leuchtmann, personal observation).

The nutritional dependence of *Botanophila* larvae on fertilized stromata suggests that females should maximize cross-fertilization of stromata. This could be promoted through species-specific visitation habits by flies. Analysis of ascospore

progeny from experimental field plots indicates prevalence of specific matings between stromata of the same host *Epichloë* species, suggesting that fly visitations can be species-specific (89, 90). Genotypes of spermatia contained in feces of individual flies support this hypothesis (22). Although most flies carry a mixture of spermatia of different *Epichloë* spp., individual flies tend to carry spermatia predominantly from one species. Thus, flies may adopt “majoring” and “minoring” specificities for their host fungi. Fly selectivity may be one of the mechanisms promoting reproductive isolation among some *Epichloë* species or host races.

## REGULATING ENDOPHYTE GROWTH *IN PLANTA*

A crucial characteristic predisposing epichloë endophytes to evolution of mutualism is their strictly controlled intercellular growth throughout the host plant. The hyphae never breach host cell walls nor develop conspicuous feeding structures such as haustoria or arbuscules (62, 75, 118). Some but not all epichloë endophytes colonize vascular bundle tissues without ill effects (33). Only in association with stroma development are host cells typically damaged. White (151) suggests that the collapse of epidermal cells in the stromal leaf helps direct transpiration and, with it, nutrient flow through the stromal tiller.

Within the grass leaf the endophyte follows very distinct basal to apical hyphal concentration gradients (35, 73, 77). The hyphae appear to originate from profusely branching mycelium in the basal meristems (34), required to form new leaves, tillers, and inflorescences (34, 62, 118, 144, 151, 152). In the expanding and mature leaf tissues the hyphae are rarely branched and are arranged mainly along the longitudinal axis of adjacent plant cells. What causes the transition from highly branched hyphae in the plant base to single, thread-like hyphae in the leaf sheaths and blades is unknown, but may involve physical restrictions to branching as well as biochemical cues released from the surrounding plant cells. Within the plant, the meristems represent strong sinks providing a nutrient-rich environment for hyphal growth. In such an environment the endophyte may not need to alter or damage the host cells to obtain the necessary nutrients for profuse growth. Several enzymes putatively involved in nutrient acquisition were recently discovered (84, 85, 93, 94, 111, 123), though some of these could also be involved in hyphal growth and branching, and perhaps even in suppressing potential host defenses.

It has been suggested that colonization of newly developing leaves occurs by apical growth of hyphae into the elongating leaf (136). Christensen and colleagues (74) questioned this model, noting that hyphae appear to be intimately attached to host cell walls and the intercellular matrix, which are often interdigitated with the outer cell wall of the hyphae (see Figure 3) (118). Also, hyphae in the tips of expanding leaves appear to be older, not younger, than those at the basal parts of the same leaf. Christensen hypothesized that the hyphae undergo intercalary elongation as leaf cells also expand, thus preventing destruction of the hyphae (74). This proposal is intriguing because intercalary growth is very rare in fungi.

Endophyte growth is strictly synchronized with grass plant growth; hyphae actively grow in expanding leaves but cease to grow as the leaf completes elongation (144). Such closely coordinated growth also characterizes embryo colonization (62). The exception is the extensive proliferation and ramification during stroma formation to initiate the fungal sexual cycle. Even this stage appears to be highly regulated because it occurs only during flowering and only between the flag leaf collar and its underlying node (80, 132, 133, 152).

Because epichloë colonize all above-ground organs of the plant, they must adjust their growth rates and forms as the plant tissues differentiate. The most dramatic example of such a developmental switch is stroma initiation versus benign infection of developing inflorescences leading to seed transmission. Colonization of the plant and endophyte differentiation is most likely governed by multiple factors: biochemical changes such as phytohormone or metabolite concentrations, and/or physical changes such as movement of dividing or elongating plant cells (144). The observed host specificity of grass endophytes (37, 81) strongly suggests that host and symbiont communicate by specific signals.

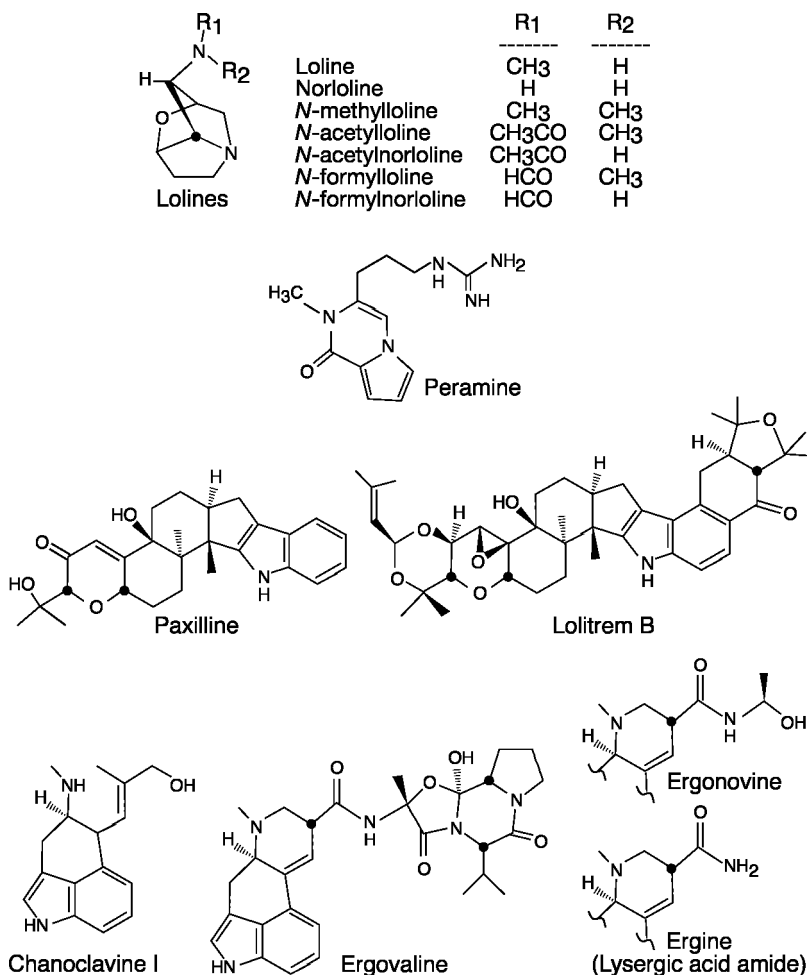
## EFFECTS ON OTHER ORGANISMS

### Anti-Insect Activities

Endophyte symbiosis exerts effects on numerous species including herbivorous and parasitoid insects, nematodes, granivorous and herbivorous birds and mammals, mycorrhizal and pathogenic fungi, and neighboring plants. A remarkable characteristic of many epichloë is their ability to produce several distinct classes of biologically active alkaloids (Figure 6). Activities of epichloë alkaloids on insects are well documented, and have been the subject of several major reviews (26, 41, 43, 44, 138). The neurotropic activities of lolines, and the activity of peramine as a feeding deterrent, can significantly enhance competitiveness of grasses with endophytes that produce these alkaloids. One of the most dramatic examples involves the Argentine stem weevil (*Listronotus bonariensis*), a devastating pest of perennial ryegrass in New Zealand. If undeterred, this exotic pest feeds on the crowns, and in much of New Zealand can eradicate entire stands. *Neotyphodium lolii* effectively protects the grass from this and several other insect herbivores (120), and the endophyte alkaloid, peramine, is primarily responsible for feeding deterrence (128). Peramine production in various endophyte-infected grass species also correlates with activity against the aphid *Schizapus graminis* (140).

Loline alkaloids exhibit a broader range and more overt toxicity to insects than does peramine (25, 126). For example, lolines but not peramine in symbiots correlates with, and is genetically linked to, activity against the aphids *Rhopalosiphum padi* and *S. graminis* (140, 154), whereas no activity of peramine against *R. padi* was evident in no-choice feeding tests (140). Deterrent activities of lolines and peramine against sucking insects may also help to reduce infections by plant viruses vectored by those insects (45, 97). Loline alkaloid levels can increase





**Figure 6** Representatives of the four classes of alkaloids produced by epichloë endophytes of grasses, namely, 1-aminopyrrolizidine alkaloids (lolines), peramine (the only known pyrrolopyrazine), indole diterpenes (here represented by paxilline and lolitrem B), and ergot alkaloids (ergovaline, ergonovine, and ergine). Also shown is chanoclavine I, a clavine alkaloid precursor to the ergot alkaloids.

dramatically in response to clipping (mock herbivory) (26, 50). Thus, lolines represent an inducible defense in the symbiotum.

## Effects on Vertebrates

Two other classes of endophyte alkaloids, indole diterpene and ergot alkaloids, possess both anti-insect and antivertebate activities, and are implicated in

livestock problems such as ryegrass staggers and fescue toxicosis (83). In the 1930s, tall fescue (*Lolium arundinaceum* = *Festuca arundinacea*) was bred and widely disseminated in the United States, touted as excellent forage with considerable longevity, stress tolerance, and capacity to prevent soil erosion. However, by the mid-1970s the problem of fescue toxicosis in cattle and other livestock had been recognized, with symptoms resembling ergot poisoning caused by *Claviceps purpurea*. Initially, researchers were unaware of the early work by Neill (112, 113) on endophytes in both tall fescue and perennial ryegrass. Bacon et al. (66) rediscovered the tall fescue endophyte now known as *Neotyphodium coenophialum*, and associated it with ergot alkaloids (12). Of particular note was ergovaline (96), an ergopeptine similar but not identical to toxins in *C. purpurea*-contaminated grain. An abundance of experimental evidence indicates that *N. coenophialum* produces alkaloids that cause the symptoms associated with livestock grazed on tall fescue (145).

In New Zealand and Australia, where perennial ryegrass is a major component of the agronomic system, toxicosis to grazing livestock was a long and persistent problem. There, the common symptomology mimicked paspalum staggers, caused by ingesting indolediterpene alkaloids produced by *Claviceps paspali* (47). On the heels of the tall fescue endophyte discovery, *N. lolii* was identified as the ryegrass endophyte causing staggers (60). The *N. lolii* strains most common in Australia and New Zealand produce indolediterpene alkaloids called lolitrems, referring to their tremor-inducing neurotropic activity (64, 127).

In addition to the problems associated with perennial ryegrass and tall fescue, endophyte-infected grasses native to several continents cause related symptoms. Most notable are drunken horse grass (*Achnatherum inebrians*) in Asia, sleepygrass (*Achnatherum robustum*) in North America, dronkgras (*Melica decumbens*) in South Africa, and *Poa huecu* (causing huecú toxicosis) in Argentina. The former two are associated with ergot alkaloids—specifically lysergic acid amide (ergine) and ergonovine—which induce stupor and aversion to future grazing (106, 117). The latter two examples are associated with tremors that suggest indolediterpene effects (46, 108, 119). Unlike ryegrass staggers, huecú toxicosis can be lethal (119).

Correlations of endophyte infection frequencies with grazing pressure on *Festuca* species were investigated in two distinctly different ecosystems. In the southwestern United States, most comparisons indicated no significant differences in endophyte incidence between paddocks from which large grazers had been excluded for 5–14 years versus adjacent stands that remained available to wildlife (129). However, infection frequencies in almost all cases were extremely high, suggesting a different basis for endophyte maintenance such as drought tolerance effects (110).

For *F. rubra* on the Scottish islands of St. Kilda and Benbecula, infection frequencies correlate with grazing from feral Soay sheep, supporting the hypothesis that the endophyte enhances survivability under grazing pressure, perhaps by deterrence (14). Also, population crashes in the feral sheep may be due in part to epichloë toxicity (14). In the same study, ergot alkaloid levels greatly increased in *F. rubra*-endophyte symbiota upon mock herbivory (clipping), again showing that an epichloë metabolite can represent an inducible plant defense.

Endophyte effects on populations of small mammals are well documented, as are deterrent effects on granivorous birds (reviewed in 43). Clay & Holah (42) observed that tall fescue with *N. coenophialum* dominated the field populations of plants to a much greater extent than endophyte-free tall fescue, and attributed this to reduced herbivory on the *N. coenophialum*-infected grass. Supporting this hypothesis, vole reproduction was significantly reduced in plots with endophyte-infected versus endophyte-free tall fescue (61). As *L. arundinaceum*, mainly *N. coenophialum*-infected, has become dominant in large areas of the continental United States, populations of small mammals appear to be negatively affected (48, 65).

## Effects on Nematodes

Among the most dramatic effects of *N. coenophialum* in tall fescue is reduced parasitism by root-knot nematodes (*Meloidogyne marylandi*) and the migratory nematode *Pratylenchus scribneri* (58, 79). This is interesting because the endophyte is completely absent (or nearly so) in roots (10, 75), so whatever is responsible for antinematode activity must either be translocated from the endophyte-infected tissues or induced in the plant by endophyte. In fact, an induced structural change in the root—namely, thickening of endodermal cell walls—might reduce the ability of *M. marylandi* to penetrate the steele, induce giant cells (which provide nutrients to *M. marylandi*), and reproduce (68, 79). The reduced parasitism helps enhance effects associated with drought tolerance, namely, osmotic adjustment in growing points of the plant, and root growth (58).

Unlike the root-knot nematode, *Pratylenchus scribneri* penetrates roots of *N. coenophialum*-infected and endophyte-free tall fescue equally well, yet *P. scribneri* reproduction is also inhibited by endophyte presence (79). Thus, antinematode activity may be due to both a physical barrier and other mechanisms not yet elucidated.

## Effects on Fungi and Plants

Reports of *in symbio* antifungal effects of endophytes are rare. Some protection of seedlings against the soilborne pathogen *Rhizoctonia zeae* has been documented (69), and *E. typhina* protects against *Cladosporium phlei* (137). Also, there are inconsistent indications that tall fescue allelopathy against clover may be enhanced by *N. coenophialum* (143). These aspects need greater attention in future.

## MOLECULAR BIOLOGY OF ENDOPHYTE SECONDARY METABOLISM

In some symbiota, lolines accumulate to very high levels, up to 2% plant dry mass (25, 26), probably well exceeding endophyte biomass. Recently, *N. uncinatum* cultures were demonstrated to produce comparable levels of lolines (17a).

Additionally, Mendelian and molecular analysis identified polymorphism at a single locus (*LOL*) in *E. festucae* that determines whether or not lolines are produced (154). A *LOL* locus gene was found to be related to genes for synthesis of cystathionine (a methionine precursor), and to dihydrorhizobitoxine synthase (142a). Further analysis of *LOL* in *E. festucae* and *N. uncinatum* indicated a cluster of at least nine genes, all appearing to be unique to loline alkaloid producing strains, and most having significant similarity to metabolism genes (142a; M.J. Spiering, H.H. Wilkinson & C.L. Schardl, unpublished data). These gene relationships, along with results of precursor feeding studies (J.D. Blankenship & C.L. Schardl, unpublished data), suggest a pathway for loline alkaloid biosynthesis that differs substantially from any known metabolic pathway. Thus, lolines represent a fungal trait characterized by a specialized biosynthetic pathway culminating in an abundant and potent defense of the host plant.

An etiological role for ergot alkaloids in fescue toxicosis seems likely but has yet to be proven. Recent advances in molecular genetics of ergot alkaloid biosynthesis open the door to more rigorous tests. The *dmaW* gene for the first step in clavine and ergot alkaloid biosynthesis has been cloned, first from *Claviceps fusiformis* (147a), then from a *N. lolii* × *E. typhina* hybrid endophyte of *L. perenne* (148a). The *lpsA* gene for the penultimate step in ergovaline production was cloned from the same endophyte (115a). Directed mutagenesis of these genes in the endophyte has confirmed their roles in ergot alkaloid biosynthesis (115a, 148a). Symbiota with such mutant and corresponding wild-type endophytes can be used to investigate the roles of these alkaloids in livestock toxicoses and other host fitness enhancements.

## EFFECTS ON HOST PHYSIOLOGY

### Effects on Growth and Abiotic Stress Tolerance

Many observations, made in controlled environments on single cultivars and natural ecotypes of tall fescue, meadow fescue (*Lolium pratense* = *Festuca pratensis*), and perennial ryegrass, suggest that their epichloë endophytes (*Neotyphodium coenophialum*, *N. uncinatum* and *N. lolii*, respectively) have positive effects on plant growth. Enhanced biomass production, tiller numbers, seed production, and root growth have been reported (16, 54, 63, 87).

Substantial (>50%) stand losses in tall fescue were reported after removing the endophyte from this grass (122). These losses are typically associated with drought periods, and endophyte-infected tall fescue exhibits improved recovery after drought compared to endophyte-free tall fescue (7). It was proposed that grass endophytes, particularly *N. coenophialum* in tall fescue, affect plant water relations, nutrient acquisition, as well as allocation and photosynthetic assimilation (11, 63). Most investigations of epichloë effects on stress tolerance focus on osmotic adjustment, water relations, and drought recovery (17, 31, 55–57, 99, 110,

121), accumulation of drought-protective osmolytes in the grass tissues (124), and photosynthetic rates under water or heat stress (4, 103, 125). Under water stress, the tall fescue endophyte is also associated with a significant increase in cell wall elasticity as measured by bulk modulus tissue elasticity, and by turgid weight to dry weight ratio (TW/DW) (153). Likewise, *N. uncinatum* increases TW/DW in water-stressed meadow fescue (98). Endophytes can induce in tall fescue and meadow fescue increased root growth and longer root hairs, and decreased root diameter (100, 102).

Taken together, there appears to be a trend toward improved physiological responses of endophyte-infected grasses to adverse environmental conditions. However, studies with several grass species confirm complex interactions between endophyte status, plant genotype, water and nutrient availability, and spatial competition (3, 13, 17, 20, 29–31, 59).

Little is known about the biochemical basis for endophyte-induced changes that cause growth effects and increase stress tolerance. Production of phytohormones by the endophytes—e.g., auxin [indoleacetic acid (IAA)]—may play a role in plant growth alterations (53, 155). However, levels of free IAA in the whole plant are unaffected by endophyte infection (53). Nonetheless, localized or transient changes might occur in the plant but could be difficult to detect.

Osmotic protection is more likely than stomatal conductance to be involved in drought protection in tall fescue (9, 56, 57, 125), but reduced stomatal conductance might be important to conserving water in *Festuca arizonica*-*Neotyphodium* sp. interactions (110). Some speculation regarding osmoprotectants centers around the fungal loline alkaloids, which are abundant in those symbiota for which the endophyte has a documented and consistent positive effect on drought tolerance (101). Lolines fit several prerequisite criteria, being nontoxic to plant cells, highly water soluble, and generally increasing in response to heat or drought. However, it is unclear if lolines reach sufficient levels to significantly affect osmotic balance. If these alkaloids are involved, they might protect macromolecules from denaturation and/or scavenge reactive oxygen species associated with drought stress, possibilities not yet tested. Other potential osmoregulators and protectants are soluble sugars and sugar alcohols, produced by the endophyte, plant, or both (124).

## Effects on Photosynthesis

Although it is useful to evaluate endophyte effects on photosynthesis, one should consider that photosynthesis is not always a reliable predictor of the productivity of grasses and crops (88, 114). In fact, limiting the rates of photosynthesis improves forage quality in terms of nitrogen content and digestible dry matter (78). It is often difficult to distinguish the effects of fungal symbionts from other factors, such as plant species, age, and environmental conditions; photosynthetic responses are not uniform among grass species, and epichloë interactions

with plant genotype and age may be significant (4, 9, 15, 18, 52, 103, 107, 110, 115, 121, 125, 142). Most studies have measured rates of net photosynthesis and/or photochemical efficiencies in grass leaves, but little or no information exists about endophyte-induced changes in amounts and/or activities of photosynthesis enzymes. Identifying enzymes secreted by *epichloë* in planta, namely, invertases, glucanases and proteinases (84, 111, 123) might provide some leads. Higher invertase and glucanase activities can increase levels of sugar monomers, causing decreased enzymatic activities in the Calvin cycle (70, 109) and, consequently, decreasing rates of photosynthesis. Surprisingly, however, amounts of ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco), a key enzyme and rate-limiting step in the Calvin cycle (6), were unaffected by endophyte status (M.J. Spiering & J. Schmid, unpublished data), despite significantly lower rates of net photosynthesis of endophyte-infected grasses (142).

Given the wide variation in physiological responses of grasses to endophyte infection, using endophyte-grass associations well characterized for growth and photosynthesis and grown under carefully standardized environmental conditions would be essential in future experiments seeking to unravel the physiological responses to putative *epichloë* signals as well as biochemical and biophysical effects of the endophytes.

## EVOLUTION OF GRASS-ENDOPHYTE SYSTEMS

### Host Specificity

Natural host *Neotyphodium* symbioses and (in the vegetative state) host *Epichloë* symbioses exhibit little or no obvious host cell response to endophyte presence (see Figure 3). Possible explanations are either that the endophyte simply fails to trigger a response, or that the host response is actively suppressed. Although there is no evidence yet to distinguish these possibilities, abundant evidence indicates that compatible interactions are highly specific. In artificial inoculation studies endophytes have been exchanged between related host species, namely *L. perenne*, *L. pratense*, and *L. arundinaceum* (32, 81). Fungal mycelium was introduced into the apical meristematic region of seedlings or mature tillers. Some of the resulting novel associations elicited incompatibility reactions such as premature death of hyphae, death of host cells in the stem apex, and stunting of surviving tillers. Often osmiophilic material was deposited in host cell walls adjacent to the hyphae. Host specificity of *E. typhina* strains is heritable and under multigenic control (38).

### Epichloë Speciation and Co-Phylogeny with Hosts

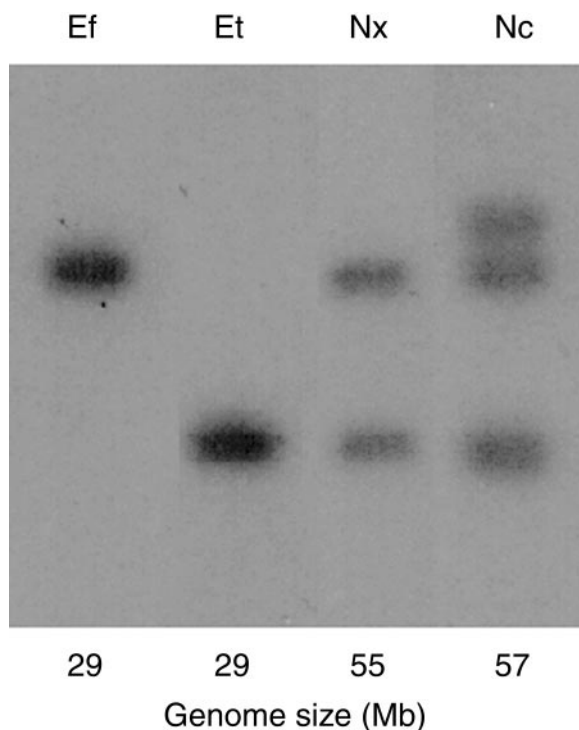
Current knowledge about the evolution of *Epichloë* and *Neotyphodium* species has been reviewed thoroughly in two recent publications (43, 135) and is summarized

here. Prior to 1993 the *Epichloë* spp. on grasses of subfamily Poöideae were all classified as *E. typhina*. Since then, nine new species have been described with the aid of molecular phylogenetic analysis and mating tests. All the new species exhibit limited host range, usually restricted to individual host genera or related genera within a host tribe. The exception is *E. typhina* as currently circumscribed, which includes interfertile strains associated with many grass species in at least three tribes. Most *Epichloë* species are capable of vertical transmission. Of these, the most intensely studied is *E. festucae*, for which vertical transmission dominates (8) and which has strong mutualistic character (133). Molecular phylogenetic analysis suggests that the seed-transmissible *Epichloë* spp. have a history of cocladogenesis (diffuse cospeciation) with the Poöideae (134). If so, then the grass-epichloë system is approximately 40 million years old.

### Interspecific Hybrids

Some asexual endophytes appear to have evolved from their *Epichloë* spp. ancestors by losing the capability to produce stromata and thereby initiate the sexual cycle. However, most asexual endophytes analyzed to date are interspecific hybrids, with combined genomes or partial genomes of two or sometimes three ancestors (135, 147). The genotypes of certain hybrids in relation to nonhybrid asexual endophytes suggest that hybridization followed loss of sexual expression (though in some cases the process may have caused the change to a strictly asexual state). The common tall fescue endophyte, *N. coenophialum*, is a dramatic example of an interspecific hybrid, having three *Epichloë* species in its ancestry (147). This is illustrated, for example, by its three  $\beta$ -tubulin alleles (Figure 7), which are in contrast to the single allele for this gene found in every known sexual species. One of the *N. coenophialum* alleles is derived from *E. festucae*, another from *E. typhina*, and another from a third species yet to be identified. Hybrid endophytes appear to retain much of their ancestral genomes, having substantially larger genome sizes than sexual *Epichloë* spp. (Figure 7) (82b).

The abundance of interspecific hybrids in surveys of endophyte species and the dominance of some hybrids in host populations strongly indicate that they are selectively favored (135). The basis for that selection is not obvious, but it seems likely that hybridization provides similar benefits to sexual reproduction. These may include the ability to purge or mask deleterious mutations, or the pyramiding of genes for host fitness enhancements. The fates of epichloë lineages that are only transmitted vertically are inextricably bound to the fates of their individual host plants and maternal-line descendants of those plants, so that host fitness should be more important in their evolution than it is for horizontally transmissible epichloë. Another possibly selective factor is that hybridization enhances the pace of evolution to keep up with host evolution (note that many grass hosts are also interspecific hybrids). Without a sexual cycle to adapt to host evolution, clonal



**Figure 7** Southern analysis indicating single  $\beta$ -tubulin gene alleles in *Epichloë festucae* (Ef) and *E. typhina* (Et), two alleles in an *Neotyphodium lolii*  $\times$  *E. typhina* hybrid (Nx) from perennial ryegrass, and three alleles in *N. coenophialum* (Nc), a complex hybrid endophyte from tall fescue. Equal amounts of nuclear DNA were loaded in each lane. Genome size estimates were from quantitative Southern analysis and electrophoretic karyotyping (82b).

endophytes might not maintain these associations for long unless they have this additional evolutionary mechanism.

Evolutionary diversification of endophytes adds to the evolution of hosts to enhance the genetic diversity of symbiots. As surveys of endophytes continue, it is apparent that grass species often harbor two or more seedborne endophytes of distinct genotype and ancestry (36, 135). Combined plant and endophyte hybridization results in symbiots loaded with genes evolved in various ancestors under various ecological conditions. The tall fescue-*N. coenophialum* symbiotum represents one of the world's most complex, heritable genetic systems. The plant has three nuclear genomes (as an allohexaploid) (76), two organellar genomes (plastid and mitochondrion), and an endophyte with contributions from three different *Epichloë* species (147).



## CONCLUDING REMARKS

The highly efficient heritability of many epichloë endophytes, and the dominance of endophyte-containing plants in populations of tall fescue (36), meadow fescue (36, 50), and other grasses, present the possibility that many symbiotes have evolved as grass-endophyte units over thousands or even millions of generations. In the process, the symbiotes have colonized diverse environments. For example, tall fescue with *N. coenophialum* naturally ranges from northern Europe into Morocco (51, 104). The broad range of endophyte benefits might be due to such long-term coevolution. The origins of these mutualisms are undoubtedly rooted in significant contributions by the symbionts of one or more benefits, such as protection from herbivory. However, after a long coevolutionary history the contributions of host and symbiont to many adaptations—such as to drought, nematodes, or low phosphate availability—should be much more entangled than if the plant were rarely or transiently associated with these endophytes. Intricate signaling systems would evolve, evidenced, for example, by the dramatic induction of endophyte alkaloids upon clipping of host leaves (50). Effects of these foliar endophytes on root architecture, apparently adaptive for drought tolerance and nematode resistance (58), also support this coevolutionary scenario.

Recent years have seen major advances in molecular biology and genetics of the endophytes and their hosts, placing these systems prominently among the models of mutualism in plants. Crucial aspects of the genetic systems and genome compositions of sexual and asexual epichloë endophytes have been elucidated, and the application of Mendelian and molecular genetic techniques have identified genes for alkaloids that play major roles in the mutualisms (115a, 142a, 148a, 154). Technologies for genetic and molecular genetic manipulations of the host grasses have steadily advanced as well (82a, 144a), and new functional genomic studies of endophytes and their hosts have been undertaken (54a, 131a). These advances position us to elucidate the specific mechanisms of grass-endophyte interactions and their mutualistic effects.

We now understand these systems sufficiently to start addressing some broader questions as well. For example, (a) Precisely what are the economic and environmental implications of grass endophytes, including but not limited to those in agronomic grasses? (b) How can the endophyte benefits best be used for agricultural and amenity grasses, and how can livestock detriments be minimized? (c) What is the ecological significance of grass endophyte diversity, and is this something that needs special attention for conserving germplasm and habitat?

## ACKNOWLEDGMENTS

We thank Uljana Hesse for helpful suggestions in the preparation of this manuscript and Rosmarie Honegger and Tom Bultman for providing photographs. Relevant studies by the authors were supported by the U.S. National Science Foundation

(NSF grants MCB-0213217, IBN-9808554, and DEB-9707427 to C.L.S.), the U.S. Department of Agriculture National Research Initiative (2001–35319-10930 to C.L.S.), and the Swiss NSF (grant 31–52728.97 to A.L.).

**The Annual Review of Plant Biology is online at <http://plant.annualreviews.org>**

## LITERATURE CITED

1. Ackland DM. 1972. A further note on anthomyiid flies associated with *Epichloë* fungus. *Entomologist* 105:231–32
2. Addicott JF. 1986. Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. *Oecologia* 70:480–94
3. Ahlholm JU, Helander M, Lehtimäki S, Wali P, Saikkonen K. 2002. Vertically transmitted fungal endophytes: different responses of host-parasite systems to environmental conditions. *Oikos* 99:173–83
4. Amalric C, Sallanon H, Monnet F, Hitmi A, Coudret A. 1999. Gas exchange and chlorophyll fluorescence in symbiotic and non-symbiotic ryegrass under water stress. *Photosynthetica* 37:107–12
5. An Z-q, Siegel MR, Hollin W, Tsai H-F, Schmidt D, Schardl CL. 1993. Relationships among non-*Acremonium* sp. fungal endophytes in five grass species. *Appl. Environ. Microbiol.* 59:1540–48
6. Andrews TJ, Lorimer GH. 1987. Rubisco: Structure, mechanisms, and prospects for improvement. In *The Biochemistry of Plants—A Comprehensive Treatise*, ed. NK Boardman, pp. 131–218. San Diego: Academic
7. Arachevaleta M, Bacon CW, Hoveland CS, Radcliffe DE. 1989. Effect of the tall fescue endophyte on plant response to environmental stress. *Agron. J.* 81:83–90
8. Arroyo García R, Martínez Zapater JM, García Criado B, Zabalgogea I. 2002. Genetic structure of natural populations of the grass endophyte *Epichloë festucae* in semiarid grasslands. *Mol. Ecol.* 11:355–64
9. Assuero SG, Matthew C, Kemp PD, Latch GCM, Barker DJ, Haslett SJ. 2000. Morphological and physiological effects of water deficit and endophyte infection on contrasting tall fescue cultivars. *N. Z. J. Agric. Res.* 43:49–61
10. Azevedo MD, Welty RE. 1995. A study of the fungal endophyte *Acremonium coenophialum* in the roots of tall fescue seedlings. *Mycologia* 87:289–97
11. Bacon CW. 1993. Abiotic stress tolerances (moisture, nutrients) and photosynthesis in endophyte-infected tall fescue. *Agric. Ecosyst. Environ.* 44:123–41
12. Bacon CW, Porter JK, Robbins JD, Luttrell ES. 1977. *Epichloë typhina* from toxic tall fescue grasses. *Appl. Environ. Microbiol.* 34:576–81
13. Barker DJ, Hume DE, Quigley PE. 1997. Negligible physiological responses to water deficit in endophyte-infected and uninfected perennial ryegrass. In *Neotyphodium/Grass Interactions*, ed. NS Hill, pp. 137–39. New York: Plenum
14. Bazely DR, Vicari M, Emmerich S, Filip L, Lin D, Inman A. 1997. Interactions between herbivores and endophyte-infected *Festuca rubra* from the Scottish islands of St. Kilda, Benbecula and Rum. *J. Appl. Ecol.* 34:847–60
15. Belesky DP, Devine OJ, Pallas JE Jr, Stringer WC. 1987. Photosynthetic activity of tall fescue as influenced by a fungal endophyte. *Photosynthetica* 21:82–87
16. Belesky DP, Stringer WC, Hill NS. 1989. Influence of endophyte and water regime

- upon tall fescue accessions. I. Growth characteristics. *Ann. Bot.* 63:495–503
17. Belesky DP, Stringer WC, Plattner RD. 1989. Influence of endophyte and water regime upon tall fescue accessions. II. Pyrrolizidine and ergopeptine alkaloids. *Ann. Bot.* 64:343–49
  - 17a. Blankenship JD, Spiering MJ, Wilkinson HH, Fannin FF, Bush LP, Schardl CL. 2001. Production of loline alkaloids by the grass endophyte, *Neotyphodium uncinatum*, in defined media. *Phytochemistry* 58:395–401
  18. Bonnet M, Camares O, Veisseire P. 2000. Effects of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll *a* fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. cv Apollo). *J. Exp. Bot.* 51:945–53
  19. Brem D, Leuchtman A. 1999. High prevalence of horizontal transmission of the fungal endophyte *Epichloë sylvatica*. *Bull. Geobot. Inst. ETH* 65:3–12
  20. Brem D, Leuchtman A. 2002. Intraspecific competition of endophyte infected vs uninfected plants of two woodland grass species. *Oikos* 96:281–90
  21. Bronstein JL. 1992. Seed predators as mutualists: ecology and evolution of the fig/pollinator interaction. In *Insect-Plant Interactions*. Vol. IV, ed. EA Bernays, pp. 1–44. Boca Raton, Fla.: CRC
  22. Bultman TL, Leuchtman A. 2003. A test of reproductive isolation among entomophilous fungal species. *Oikos*. 103:681–87
  23. Bultman TL, Welch AM, Boning RA, Bowdish TI. 2000. The cost of mutualism in a fly-fungus interaction. *Oecologia* 124:85–90
  24. Bultman TL, White JF Jr, Bowdish TI, Welch AM, Johnston J. 1995. Mutualistic transfer of *Epichloë* spermatia by *Phorbia* flies. *Mycologia* 87:182–89
  25. Bush LP, Fannin FF, Siegel MR, Dahlman DL, Burton HR. 1993. Chemistry, occurrence and biological effects of saturated pyrrolizidine alkaloids associated with endophyte-grass interactions. *Agric. Ecosyst. Environ.* 44:81–102
  26. Bush LP, Wilkinson HH, Schardl CL. 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. *Plant Physiol.* 114:1–7
  27. Carroll GC. 1995. Forest endophytes: pattern and process. *Can. J. Bot.* 73:S1316–S24
  28. Carroll GC. 1988. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* 69:2–9
  29. Cheplick GP, Cho R. 2003. Interactive effects of fungal endophyte infection and host genotype on growth and storage in *Lolium perenne*. *New Phytol.* 158:183–91
  30. Cheplick GP, Clay K, Marks S. 1989. Interactions between infection by endophytic fungi and nutrient limitation in the grass *Lolium perenne* and *Festuca arundinacea*. *New Phytol.* 111:89–97
  31. Cheplick GP, Perera A, Koulouris K. 2000. Effect of drought on the growth of *Lolium perenne* genotypes with and without fungal endophytes. *Funct. Ecol.* 14:657–67
  32. Christensen MJ. 1995. Variation in the ability of *Acremonium* endophytes of perennial rye-grass (*Lolium perenne*), tall fescue (*Festuca arundinacea*) and meadow fescue (*F. pratensis*) to form compatible associations in three grasses. *Mycol. Res.* 99:466–70
  33. Christensen MJ, Bennett RJ, Schmid J. 2001. Vascular bundle colonisation by *Neotyphodium* endophytes in natural and novel associations with grasses. *Mycol. Res.* 105:1239–45
  34. Christensen MJ, Bennett RJ, Schmid J. 2002. Growth of *Epichloë/Neotyphodium* and p-endophytes in leaves of *Lolium* and *Festuca* grasses. *Mycol. Res.* 106:93–106
  35. Christensen MJ, Easton HS, Simpson WR, Tapper BA. 1998. Occurrence of the fungal endophyte *Neotyphodium*

- coenophialum* in leaf blades of tall fescue and implications for stock health. *N. Zeal. J. Agric. Res.* 41:595–602
36. Christensen MJ, Leuchtmann A, Rowan DD, Tapper BA. 1993. Taxonomy of *Acremonium* endophytes of tall fescue (*Festuca arundinacea*), meadow fescue (*F. pratensis*), and perennial rye-grass (*Lolium perenne*). *Mycol. Res.* 97:1083–92
  37. Christensen MJ, Simpson WR, Al Samarrai T. 2000. Infection of tall fescue and perennial ryegrass plants by combinations of different *Neotyphodium* endophytes. *Mycol. Res.* 104:974–78
  38. Chung K-R, Hollin W, Siegel MR, Schardl CL. 1997. Genetics of host specificity in *Epichloë typhina*. *Phytopathology* 87:599–605
  39. Chung K-R, Schardl CL. 1997. Sexual cycle and horizontal transmission of the grass symbiont, *Epichloë typhina*. *Mycol. Res.* 101:295–301
  40. Clay K. 1990. Fungal endophytes of grasses. *Annu. Rev. Ecol. Syst.* 21:275–95
  41. Clay K. 1991. Endophytes as antagonists of plant pests. In *Microbial Ecology of Leaves*, ed. JH Andrew, SS Hirano, pp. 331–57. Berlin: Springer Verlag
  42. Clay K, Holah J. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* 285:1742–44
  43. Clay K, Schardl CL. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Nat.* 160:S99–S127
  44. Clement SL, Kaiser WJ, Eichenseer H. 1994. *Acremonium* endophytes in germplasm of major grasses and their utilization for insect resistance. In *Biotechnology of Endophytic Fungi of Grasses*, ed. CW Bacon, JF White Jr, pp. 185–99. Boca Raton, Fla.: CRC
  45. Clement SL, Wilson AD, Lester DG, Davitt CM. 1997. Fungal endophytes of wild barley and their effects on *Diuraphis noxia* population development. *Entomol. Exp. Appl.* 82:275–81
  46. Coetzer JAW, Kellerman TS, Naude TW. 1985. *Neurotoxicoses of livestock caused by plants and fungi in southern Africa. Rep. 199*, Tech. Commun., Dep. Agric., South Africa, Onderstepoort, South Africa
  47. Cole RJ, Dorner JW, Lansden JA, Cox RH, Pape C, et al. 1977. Paspalum staggers: isolation and identification of tremorgenic metabolites from sclerotia of *Claviceps paspali*. *J. Agric. Food Chem.* 25:1197–201
  48. Coley AB, Fribourg HA, Pelton MR, Gwinn KD. 1995. Effects of tall fescue infestation on relative abundance of small mammals. *J. Environ. Q.* 24:472–75
  49. Collin JE. 1967. Notes on some British species of *Pegohylemyia* (Dipt., Anthomyiidae) with descriptions of four new species. *Entomol. Monthly Mag.* 102:181–91
  50. Craven KD, Blankenship JD, Leuchtmann A, Hignight K, Schardl CL. 2001. Hybrid fungal endophytes symbiotic with the grass *Lolium pratense*. *Sydowia* 53:44–73
  51. Craven KD, West CP, Schardl CL. 1998. Endophyte phylogeny and tall fescue evolution. *Phytopathology* 88:S19
  52. Dahl Jensen AM, Deleuran LC. 2001. Effect of endophyte-infection on photosynthesis in *Lolium perenne* grown at different nitrogen levels: preliminary results. In *Grassland Conference 2000: 4th International Neotyphodium/Grass Interactions Symposium Proceedings*, ed. VH Paul, PD Dapprich, K Krohn, pp. 369–73. Soest, Germany: Universität-GH Paderborn
  53. de Battista JP, Bacon CW, Severson R, Plattner RD, Bouton JH. 1990. Indole acetic acid production by the fungal endophyte of tall fescue. *Agron. J.* 82:878–80
  54. de Battista JP, Bouton JH, Bacon CW,

- Siegel MR. 1990. Rhizome and herbage production of endophyte-removed tall fescue clones and populations. *Agron. J.* 82:651–54
- 54a. de Jong EV, Guthridge KM, Spangenberg GC, Forster JW. 2003. Development and characterization of EST-derived simple sequence repeat (SSR) markers for pasture grass endophytes. *Genome* 46:277–90
55. Eerens JPJ, Lucas RJ, Easton S, White JGH. 1998. Influence of the endophyte (*Neotyphodium lolii*) on morphology, physiology, and alkaloid synthesis of perennial ryegrass during high temperature and water stress. *N. Zeal. J. Agric. Res.* 41:219–26
56. Elbersen HW, West CP. 1996. Growth and water relations of field-grown tall fescue as influenced by drought and endophyte. *Grass Forage Sci.* 51:333–42
57. Elmi AA, West CP. 1995. Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue. *New Phytol.* 131:61–67
58. Elmi AA, West CP, Robbins RT, Kirkpatrick TL. 2000. Endophyte effects on reproduction of a root-knot nematode (*Meloidogyne marylandi*) and osmotic adjustment in tall fescue. *Grass Forage Sci.* 55:166–72
59. Faeth SH, Sullivan TJ. 2003. Mutualistic asexual endophytes in a native grass are usually parasitic. *Am. Nat.* 161:310–25
60. Fletcher LR, Harvey IC. 1981. An association of *Lolium* endophyte with ryegrass staggers. *N. Zeal. Vet. J.* 28:185–86
61. Fortier GM, Bard N, Jansen M, Clay K. 2000. Effects of tall fescue endophyte infection and population density on growth and reproduction in prairie voles. *J. Wildl. Manage.* 64:122–28
62. Freeman EM. 1904. The seed fungus of *Lolium temulentum* L., the darnel. *Philos. Trans. R. Soc. London B* 196:1–27
63. Funk CR, White RH, Breen J. 1993. Importance of *Acremonium* endophytes in turfgrass breeding and management. *Agric. Ecosyst. Environ.* 44:215–32
64. Gallagher RT, Hawkes AD, Steyn PS, Vleggaar R. 1984. Tremorgenic neurotoxins from perennial ryegrass causing ryegrass staggers disorder of livestock: structure elucidation of lolitrem B. *J. Chem. Soc. Chem. Commun.* 1984:614–16
65. Giuliano WM, Elliott CL, Sole JD. 1994. Significance of tall fescue in the diet of the eastern cottontail. *Prairie Naturalist* 26:53–60
66. Glenn AE, Bacon CW, Price R, Hanlin RT. 1996. Molecular phylogeny of *Acremonium* and its taxonomic implications. *Mycologia* 88:369–83
67. Guérin P. 1898. Sur la présence d'un champignon dans l'ivraie. *J. Botanique* 12:230–38
68. Gwinn KD, Bernard EC. 1993. *Interactions of Endophyte-Infected Grasses with the Nematodes Meloidogyne marylandi and Pratylenchus scribneri*. Proc. 2nd Intl. Symp. Acremonium/Grass Interact. Plenary Pap., Palmerston North, New Zealand
69. Gwinn KD, Gavin AM. 1992. Relationship between endophyte infestation level of tall fescue seed lots and *Rhizoctonia zeae* seedling disease. *Plant Dis.* 76:911–14
70. Hall JL, Williams LE. 2000. Assimilate transport and partitioning in fungal biotrophic interactions. *Aust. J. Plant Physiol.* 27:549–60
71. Harrison MJ. 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50:361–89
72. Hennig W. 1976. *Fliegen der Palearktischen Region, Vol. 7(1), Fam. 63a. Anthomyiidae*. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung. 680 pp.
73. Herd S, Christensen MJ, Saunders K, Scott DB, Schmid J. 1997. Quantitative assessment of in planta distribution of metabolic activity and gene expression

- of an endophytic fungus. *Microbiology* 143:267–75
74. Hesse U, Christensen MJ, Schardl CL. 2003. *Tissue Specificity of Endophyte Development in Epichloë/Neotyphodium Symbioses with Grasses*. Presented at XI Molecular Plant-Microbe Interactions Congress, St. Petersburg, Russia
  75. Hinton DM, Bacon CW. 1985. The distribution and ultrastructure of the endophyte of toxic tall fescue. *Can. J. Bot.* 63:36–42
  76. Humphreys MW, Thomas HM, Morgan WG, Meredith MR, Harper JA, et al. 1995. Discriminating the ancestral progenitors of hexaploid *Festuca arundinacea* using genomic in situ hybridization. *Heredity* 75:171–74
  77. Keogh RG, Tapper BA, Fletcher RH. 1996. Distributions of the fungal endophyte *Acremonium lolii*, and of the alkaloids lolitrem B and peramine within perennial ryegrass. *N. Zeal. J. Agric. Res.* 39:121–27
  78. Kephart KD, Buxton DR. 1993. Forage quality responses to shade. *Crop Sci.* 33:831–37
  79. Kimmons CA, Gwinn KD, Bernard EC. 1990. Nematode reproduction on endophyte-infected and endophyte-free tall fescue. *Plant Dis.* 74:757–61
  80. Kirby EJM. 1961. Host-parasite relations in the choke disease of grasses. *Trans. Br. Mycol. Soc.* 44:493–503
  81. Koga H, Christensen MJ, Bennett RJ. 1993. Cellular interactions of some grass/*Acremonium* endophyte associations. *Mycol. Res.* 97:1237–44
  82. Kohlmeyer J, Kohlmeyer E. 1974. Distribution of *Epichloë typhina* (Ascomycetes) and its parasitic fly. *Mycologia* 66:77–86
  - 82a. Kuai B, Dalton SJ, Bettany AJE, Morris P. 1999. Regeneration of fertile transgenic tall fescue plants with a stable highly expressed foreign gene. *Plant Cell, Tissue and Organ Culture* 58:149–54
  - 82b. Kuldau GA, Tsai H-F, Schardl CL. 1999. Genome sizes of *Epichloë* species and anamorphic hybrids. *Mycologia* 91:776–82
  83. Lacey J. 1991. Natural occurrence of mycotoxins in growing and conserved forage crops. In *Mycotoxins and Animal Foods*, ed. JE Smith, RS Henderson, pp. 363–414. Boca Raton, Fla.: CRC
  84. Lam CK, Belanger FC, White JF Jr, Daie J. 1994. Mechanism and rate of sugar uptake by *Acremonium typhinum*, an endophytic fungus infecting *Festuca rubra*: Evidence for presence of a cell wall invertase in endophytic fungi. *Mycologia* 86:408–15
  85. Lam CK, Belanger FC, White JF Jr, Daie J. 1995. Invertase activity in *Epichloë/Acremonium* fungal endophytes and its possible role in choke disease. *Mycol. Res.* 99:867–73
  86. Latch GCM, Christensen MJ, Samuels GJ. 1984. Five endophytes of *Lolium* and *Festuca* in New Zealand. *Mycotaxon* 20:535–50
  87. Latch GCM, Hunt WF, Musgrave DR. 1985. Endophytic fungi affect growth of perennial ryegrass. *N. Zeal. J. Agric. Res.* 28:165–68
  88. Lawlor DW. 1995. Photosynthesis, productivity and environment. *J. Exp. Bot.* 46:1449–61
  89. Leuchtmann A. 2003. Taxonomy and diversity of *Epichloë* endophytes. In *Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol and Cultural Impacts*, ed. JF White Jr et al., pp. 169–94. New York: Marcel Dekker
  90. Leuchtmann A, Bultman TL. 2001. *Epichloë* grass endophytes and their interaction with a symbiotic fly. In *Grassland Conference 2000: 4th International Neotyphodium/Grass Interactions Symposium Proceedings*, ed. VH Paul et al., pp. 103–7. Soest, Germany: Universität-GH Paderborn
  91. Deleted in proof
  92. Leuchtmann A, Schardl CL, Siegel MR.

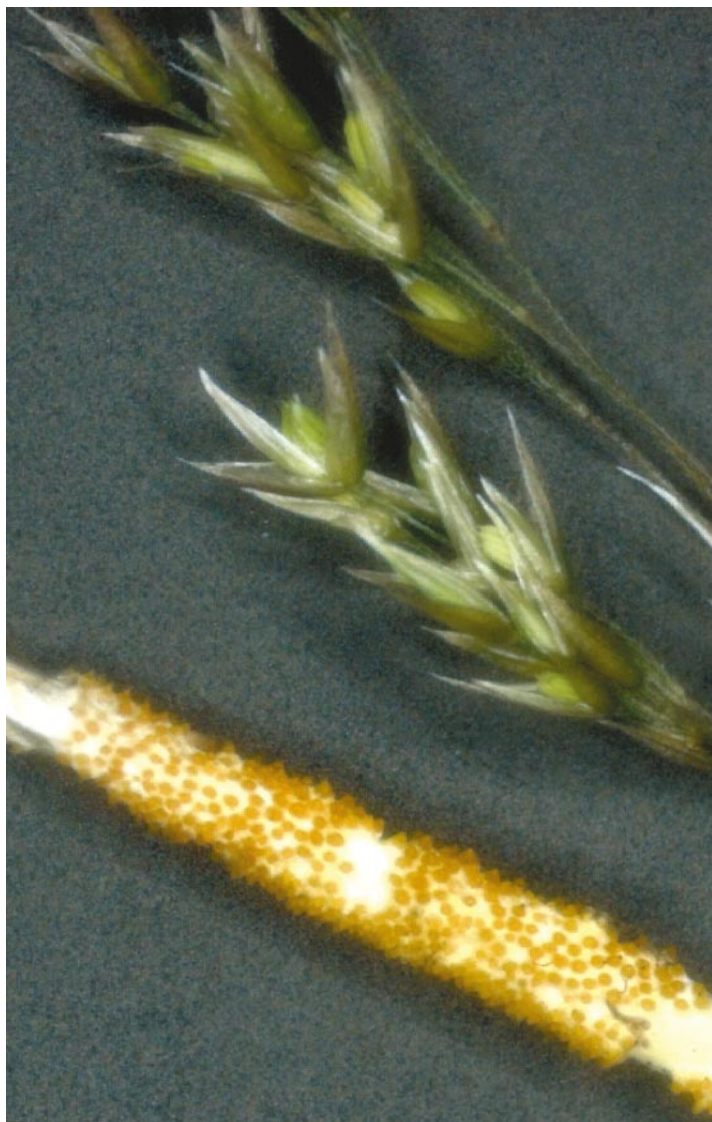
1994. Sexual compatibility and taxonomy of a new species of *Epichloë* symbiotic with fine fescue grasses. *Mycologia* 86:802–12
93. Lindstrom JT, Belanger FC. 1994. Purification and characterization of an endophytic fungal proteinase that is abundantly expressed in the infected host grass. *Plant Physiol.* 106:7–16
94. Lindstrom JT, Sun S, Belanger FC. 1993. A novel fungal protease expressed in endophytic infection of *Poa* species. *Plant Physiol.* 102:645–50
95. Lyons CP, Evans JJ, Bacon CW. 1990. Effects of the fungal endophyte *Acremonium coenophialum* on nitrogen accumulation and metabolism in tall fescue. *Plant Physiol.* 92:726–32
96. Lyons PC, Plattner RD, Bacon CW. 1986. Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. *Science* 232:487–89
97. Mahmood T, Gergerich RC, Milus EA, West CP, D'arcy CJ. 1993. Barley yellow dwarf viruses in wheat, endophyte-infected and endophyte-free tall fescue, and other hosts in Arkansas. *Plant Dis.* 77:225–28
98. Malinowski D. 1995. *Rhizomatous Ecotypes and Symbiosis with Endophytes as New Possibilities of Improvement in Competitive Ability of Meadow Fescue (Festuca pratensis)*. PhD thesis. Swiss Fed. Inst. Tech., Zurich.
99. Malinowski D, Leuchtman A, Schmidt D, Nösberger J. 1997. Growth and water status in meadow fescue (*Festuca pratensis*) is affected by *Neotyphodium* and *Phialophora* endophytes. *Agron. J.* 89:673–78
100. Malinowski D, Leuchtman A, Schmidt D, Nösberger J. 1997. Symbiosis with *Neotyphodium uncinatum* endophyte may increase the competitive ability of meadow fescue. *Agron. J.* 89:833–39
101. Malinowski DP, Belesky DP. 2000. Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. *Crop Sci.* 40:923–40
102. Malinowski DP, Brauer DK, Belesky DP. 1999. The endophyte *Neotyphodium coenophialum* affects root morphology of tall fescue grown under phosphorus deficiency. *J. Agron. Crop Sci.* 183:53–60
103. Marks S, Clay K. 1996. Physiological responses of *Festuca arundinacea* to fungal endophyte infection. *New Phytol.* 133:727–33
104. Marlatt ML, West CP, McConnell ME, Slepser DA, Buck GW, et al. 1997. Investigations on xeriphytic *Festuca* spp. from Morocco and their associated endophytes. In *Neotyphodium/grass interactions*, ed. CW Bacon, NS Hill, pp. 73–75. New York/London: Plenum
105. Michelsen V. 1985. A revision of the Anthomyiidae (Diptera) described by J. W. Zetterstedt. *Steenstrupia* 11:37–65
106. Miles CO, Di Menna ME, Jacobs SWL, Garthwaite I, Lane GA, et al. 1998. Endophytic fungi in indigenous Australasian grasses associated with toxicity to livestock. *Appl. Environ. Microbiol.* 64:601–6
107. Monnet F, Vaillant N, Hitmi A, Coudret A, Sallanon H. 2001. Endophytic *Neotyphodium lolii* induced tolerance to Zn stress in *Lolium perenne*. *Physiol. Plant.* 113:557–63
108. Moon CD, Miles CO, Jarlfors U, Schardl CL. 2002. The evolutionary origins of three new *Neotyphodium* endophyte species from grasses indigenous to the Southern Hemisphere. *Mycologia* 94:694–711
109. Moore BD, Cheng S-H, Rice J, Seemann JR. 1998. Sucrose cycling, Rubisco expression, and prediction of photosynthetic acclimation to elevated atmospheric CO<sub>2</sub>. *Plant Cell Environ.* 21:905–15
110. Morse LJ, Day TA, Faeth SH. 2002. Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of

- Arizona fescue under contrasting water availability regimes. *Environ. Exp. Bot.* 48:257–68
111. Moy M, Li HJM, Sullivan R, White JF Jr, Belanger FC. 2002. Endophytic fungal beta-1,6-glucanase expression in the infected host grass. *Plant Physiol.* 130:1298–308
  112. Neill JC. 1940. The endophyte of ryegrass (*Lolium perenne*). *N. Zeal. J. Sci. Technol.* 21:280A–91A
  113. Neill JC. 1941. The endophytes of *Lolium* and *Festuca*. *N. Zeal. J. Sci. Technol.* 23:185A–93A
  114. Nelson CJ, Asay KH, Horst GL. 1975. Relationship of leaf photosynthesis to forage yield of tall fescue. *Crop Sci.* 15:476–78
  115. Newman JA, Abner ML, Dado RG, Gibson DJ, Brookings A, Parsons AJ. 2003. Effects of elevated CO<sub>2</sub>, nitrogen and fungal endophyte-infection on tall fescue: growth, photosynthesis, chemical composition and digestibility. *Global Change Biol.* 9:425–37
  - 115a. Panaccione DG, Johnson RD, Wang JH, Young CA, Damrongkool P, et al. 2001. Elimination of ergovaline from a grass-*Neotyphodium* endophyte symbiosis by genetic modification of the endophyte. *Proc. Natl. Acad. Sci. USA* 98:12820–25
  116. Pellmyr O. 1989. The cost of mutualism: interaction between *Trollius europaeus* and its pollinating parasites. *Oecologia* 78:53–59
  117. Petroski R, Powell RG, Clay K. 1992. Alkaloids of *Stipa robusta* (sleepygrass) infected with an *Acremonium* endophyte. *Nat. Toxins* 1:84–88
  118. Philipson MN, Christey MC. 1986. The relationship of host and endophyte during flowering, seed formation, and germination of *Lolium perenne*. *NZ. J. Bot.* 24:125–34
  119. Pimilio AB, Rofi RD, Gambino MP, Mazzini CA, Debenedetti de Langenheim RT. 1989. The lethal principle of *Poa huecu* (coirón blanco): a plant indigenous to Argentina. *Toxicon* 27:1251
  120. Prestidge RA, Gallagher RT. 1988. Endophyte fungus confers resistance to ryegrass: Argentine stem weevil larval studies. *Ecol. Entomol.* 13:429–35
  121. Ravel C, Courty C, Coudret A, Charmet G. 1997. Beneficial effects of *Neotyphodium lolii* on the growth and the water status in perennial ryegrass cultivated under nitrogen deficiency or drought stress. *Agronomie* 17:173–81
  122. Read JC, Camp BJ. 1986. The effect of the fungal endophyte *Acremonium coenophialum* in tall fescue on animal performance, toxicity, and stand maintenance. *Agron. J.* 78:848–50
  123. Reddy PV, Lam CK, Belanger FC. 1996. Mutualistic fungal endophytes express a proteinase that is homologous to proteases suspected to be important in fungal pathogenicity. *Plant Physiol.* 111:1209–18
  124. Richardson MD, Chapman GW Jr, Hoveland CS, Bacon CW. 1992. Sugar alcohols in endophyte-infected tall fescue under drought. *Crop Sci.* 32:1060–61
  125. Richardson MD, Hoveland CS, Bacon CW. 1993. Photosynthesis and stomatal conductance of symbiotic and nonsymbiotic tall fescue. *Crop Sci.* 33:145–49
  126. Riedell WE, Kieckhefer RE, Petroski RJ, Powell RG. 1991. Naturally occurring and synthetic loline alkaloid derivatives: insect feeding behavior modification and toxicity. *J. Entomol. Sci.* 26:122–29
  127. Rowan DD. 1993. Lolitrems, paxilline and peramine: mycotoxins of the ryegrass/endophyte interaction. *Agric. Ecosyst. Environ.* 44:103–22
  128. Rowan DD, Latch GCM. 1994. Utilization of endophyte-infected perennial ryegrasses for increased insect resistance. In *Biotechnology of Endophytic Fungi of Grasses*, ed. CW Bacon, JF White Jr, pp. 169–83. Boca Raton, Fla.: CRC
  129. Saikkonen K, Helander M, Faeth SH, Schulthess F, Wilson D. 1999.

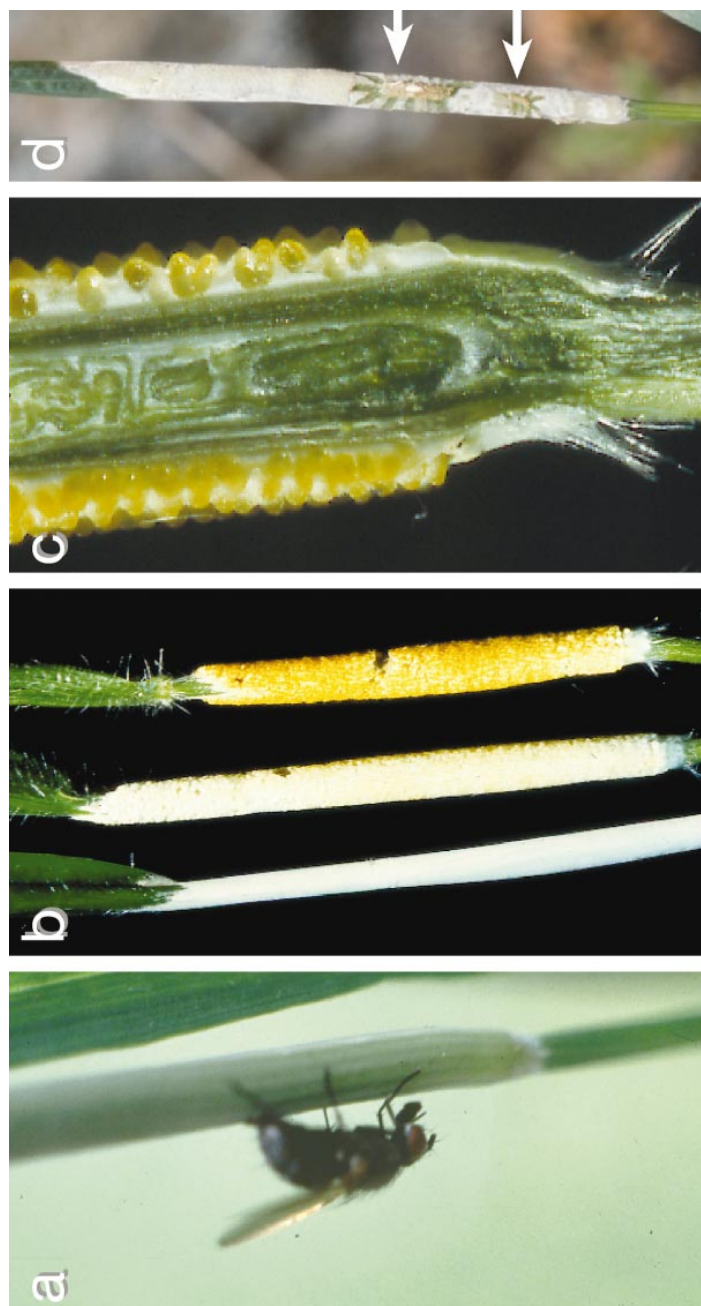


- Endophyte-grass-herbivore interactions: the case of *Neotyphodium* endophytes in Arizona fescue populations. *Oecologia* 121:411–20
130. Sampson K. 1933. The systemic infection of grasses by *Epichloe typhina* (Pers.) Tul. *Trans. Br. Mycol. Soc.* 18:30–47
  131. Sampson K. 1935. The presence and absence of an endophytic fungus in *Lolium temulentum* and *L. perenne*. *Trans. Br. Mycol. Soc.* 19:337–43
  - 131a. Sawbridge T, Ong EK, Binnion C, Emmerling M, McInnes R, et al. 2003. Generation and analysis of expressed sequence tags in perennial ryegrass (*Lolium perenne* L.). *Plant Sci.* 165:1089–1100
  132. Schardl CL. 1996. *Epichloë* species: fungal symbionts of grasses. *Annu. Rev. Phytopathol.* 34:109–30
  133. Schardl CL. 2001. *Epichloë festucae* and related mutualistic symbionts of grasses. *Fung. Genet. Biol.* 33:69–82
  134. Schardl CL, Leuchtmann A, Chung K-R, Penny D, Siegel MR. 1997. Coevolution by common descent of fungal symbionts (*Epichloë* spp.) and grass hosts. *Mol. Biol. Evol.* 14:133–43
  135. Schardl CL, Moon CD. 2003. Processes of species evolution in *Epichloë/Neotyphodium* endophytes of grasses. In *Clavicipitalean Fungi: Evolutionary Biology, Chemistry, Biocontrol and Cultural Impacts*, ed. JF White Jr, CW Bacon, NL Hywel-Jones, JW Spatafora, pp. 273–310. New York/Basel: Marcel-Dekker
  136. Schmid J, Spiering MJ, Christensen MJ. 2000. Metabolic activity, distribution and propagation of grass endophytes in planta: investigations using the GUS reporter gene system. In *Microbial Endophytes*, ed. JF White Jr, CW Bacon, pp. 295–322. New York: Marcel Dekker
  137. Shimanuki T. 1987. Studies on the mechanisms of the infection of timothy with purple spot disease caused by *Cladosporium phlei* (Gregory) de Vries. *Res. Bull. Hokkaido Natl. Agric. Exp. Stat.* 148:1–56
  138. Siegel MR, Bush LP. 1996. Defensive chemicals in grass-fungal endophyte associations. *Rec. Adv. Phytochem.* 30:81–120
  139. Siegel MR, Johnson MC, Varney DR, Nesmith WC, Buckner RC, et al. 1984. A fungal endophyte in tall fescue: incidence and dissemination. *Phytopathology* 74:932–37
  140. Siegel MR, Latch GCM, Bush LP, Fanin FF, Rowan DD, et al. 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. *J. Chem. Ecol.* 16:3301–15
  141. Siegel MR, Schardl CL, Phillips TD. 1995. Incidence and compatibility of non-clavicipitaceous fungal endophytes in *Festuca* and *Lolium* grass species. *Mycologia* 87:196–202
  142. Spiering MJ. 2000. *Distribution of Neotyphodium lolii-Endophyte Metabolic Activity in Perennial Ryegrass (Lolium perenne L.) and its Implications for Alkaloid Distribution and Photosynthesis*. PhD thesis. Massey Univ., 208 pp.
  - 142a. Spiering MJ, Wilkinson HH, Blankenship JD, Schardl CL. 2002. Expressed sequence tags and genes associated with loline alkaloid expression by the fungal endophyte *Neotyphodium uncinatum*. *Fung. Genet. Biol.* 36:242–54
  143. Springer TL. 1996. Allelopathic effects on germination and seedling growth of clovers by endophyte-free and -infected tall fescue. *Crop Sci.* 36:1639–42
  144. Tan YY, Spiering MJ, Scott V, Lane GA, Christensen MJ, Schmid J. 2001. In planta regulation of extension of an endophytic fungus and maintenance of high metabolic rates in its mycelium in the absence of apical extension. *Appl. Environ. Microbiol.* 67:5377–83
  - 144a. Thomas HM, Morgan WG, Humphreys MW. 2003. Designing grasses with a

- future—combining the attributes of *Lolium* and *Festuca*. *Euphytica* 133:19–26
145. Thompson FN, Stuedemann JA. 1993. Pathophysiology of fescue toxicosis. *Agric. Ecosyst. Environ.* 44:263–81
  146. Thompson JN, Pellmyr O. 1992. Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. *Ecology* 73:1780–91
  147. Tsai H-F, Liu J-S, Staben C, Christensen MJ, Latch GCM, et al. 1994. Evolutionary diversification of fungal endophytes of tall fescue grass by hybridization with *Epichloë* species. *Proc. Natl. Acad. Sci. USA* 91:2542–46
  - 147a. Tsai H-F, Wang H, Gebler JC, Poulter CD, Schardl CL. 1995. The *Claviceps purpurea* gene encoding dimethylallyl-tryptophan synthase, the committed step for ergot alkaloid biosynthesis. *Biochem. Biophys. Res. Commun.* 216:119–25
  148. Vogel A. 1898. Mehl und die anderen Mehlprodukte der Cerealien und Leguminosen. *Z. Nahrungsmittelunters. Hyg. Warenk.* 12:25–29
  - 148a. Wang J, Machado C, Panaccione DG, Tsai H-F, Schardl CL. 2003. The determinant step in ergot alkaloid biosynthesis by a grass endophyte. *Fung. Genet. Biol.* 41:189–98
  149. Welch AM, Bultman TL. 1993. Natural release of *Epichloëtyphina* ascospores and its temporal relationship to fly parasitism. *Mycologia* 85:756–63
  150. White JF. 1988. Endophyte-host associations in forage grasses. XI. A proposal concerning origin and evolution. *Mycologia* 80:442–46
  151. White JF Jr, Bacon CW, Hinton DM. 1997. Modifications of host cells and tissues by the biotrophic endophyte *Epichloë amarillans* (Clavicipitaceae; Ascomycotina). *Can. J. Bot.* 75:1061–69
  152. White JF Jr, Morgan-Jones G, Morrow AC. 1993. Taxonomy, life cycle, reproduction and detection of *Acremonium* endophytes. *Agric. Ecosyst. Environ.* 44:13–37
  153. White RH, Engelke MC, Morton SJ, Johnson-Cicalese JM, Ruemmele BA. 1992. *Acremonium* endophyte effects on tall fescue drought tolerance. *Crop Sci.* 32:1392–96
  154. Wilkinson HH, Siegel MR, Blankenship JD, Mallory AC, Bush LP, Schardl CL. 2000. Contribution of fungal loline alkaloids to protection from aphids in a grass-endophyte mutualism. *Mol. Plant Microbe Interact.* 13:1027–33
  155. Yue Q, Miller CJ, White JF Jr, Richardson MD. 2000. Isolation and characterization of fungal inhibitors from *Epichloë festucae*. *J. Agric. Food Chem.* 48:4687–92



**Figure 4** Simultaneous expression of diseased and benignly infected inflorescences on the same *Agrostis perennans* plant systemically infected with *Epichloë amarillans*. The normally developing inflorescences (*above*) produce seeds bearing the symbiont, whereas other inflorescences bear the sexual state of the fungus (*below*).



**Figure 5** Development of the *Epichloë* spp. sexual state, and the role of the *Botanophila* fly in cross-fertilizing the fungi. Panel a: stereo-typical behavior of the fly after ovipositing (see text). Panel b: three stromata in (from left to right) the early, middle, and late stages of maturation after fertilization by the fly. Note the distinct thickening of the stroma as perithecia (fruiting bodies) mature. Panel c: longitudinal section through a stroma. Embedded in the surface are yellow-orange perithecia. Fungal mycelium (white material) is apparent throughout the grass tissue under the stroma. Panel d: larval brood chambers (arrows) from which larvae emerge to feed on developing perithecia. Note the feeding tracks near the brood chambers.

## CONTENTS

---

AN UNFORESEEN VOYAGE TO THE WORLD OF PHYTOCHROMES, <i>Masaki Furuya</i>	1
ALTERNATIVE NAD(P)H DEHYDROGENASES OF PLANT MITOCHONDRIA, <i>Allan G. Rasmusson, Kathleen L. Soole, and Thomas E. Elthon</i>	23
DNA METHYLATION AND EPIGENETICS, <i>Judith Bender</i>	41
PHOSPHOENOLPYRUVATE CARBOXYLASE: A NEW ERA OF STRUCTURAL BIOLOGY, <i>Katsura Izui, Hiroyoshi Matsumura, Tsuyoshi Furumoto, and Yasushi Kai</i>	69
METABOLIC CHANNELING IN PLANTS, <i>Brenda S.J. Winkel</i>	85
RHAMNOGALACTURONAN II: STRUCTURE AND FUNCTION OF A BORATE CROSS-LINKED CELL WALL PECTIC POLYSACCHARIDE, <i>Malcolm A. O'Neill, Tadashi Ishii, Peter Albersheim, and Alan G. Darvill</i>	109
NATURALLY OCCURRING GENETIC VARIATION IN <i>ARABIDOPSIS</i> <i>THALIANA</i> , <i>Maarten Koornneef, Carlos Alonso-Blanco, and Dick Vreugdenhil</i>	141
SINGLE-CELL C <sub>4</sub> PHOTOSYNTHESIS VERSUS THE DUAL-CELL (KRANZ) PARADIGM, <i>Gerald E. Edwards, Vincent R. Franceschi, and Elena V. Voznesenskaya</i>	173
MOLECULAR MECHANISM OF GIBBERELLIN SIGNALING IN PLANTS, <i>Tai-ping Sun and Frank Gubler</i>	197
PHYTOESTROGENS, <i>Richard A. Dixon</i>	225
DECODING Ca <sup>2+</sup> SIGNALS THROUGH PLANT PROTEIN KINASES, <i>Jeffrey F. Harper, Ghislain Breton, and Alice Harmon</i>	263
PLASTID TRANSFORMATION IN HIGHER PLANTS, <i>Pal Maliga</i>	289
SYMBIOSES OF GRASSES WITH SEEDBORNE FUNGAL ENDOPHYTES, <i>Christopher L. Schardl, Adrian Leuchtmann, Martin J. Spiering</i>	315
TRANSPORT MECHANISMS FOR ORGANIC FORMS OF CARBON AND NITROGEN BETWEEN SOURCE AND SINK, <i>Sylvie Lalonde, Daniel Wipf, and Wolf B. Frommer</i>	341

REACTIVE OXYGEN SPECIES: METABOLISM, OXIDATIVE STRESS, AND SIGNAL TRANSDUCTION, <i>Klaus Apel and Heribert Hirt</i>	373
THE GENERATION OF $\text{Ca}^{2+}$ SIGNALS IN PLANTS, <i>Alistair M. Hetherington and Colin Brownlee</i>	401
BIOSYNTHESIS AND ACCUMULATION OF STEROLS, <i>Pierre Benveniste</i>	429
HOW DO CROP PLANTS TOLERATE ACID SOILS? MECHANISMS OF ALUMINUM TOLERANCE AND PHOSPHOROUS EFFICIENCY, <i>Leon V. Kochian, Owen A. Hoekenga, and Miguel A. Piñeros</i>	459
VIGS VECTORS FOR GENE SILENCING: MANY TARGETS, MANY TOOLS, <i>Dominique Robertson</i>	495
GENETIC REGULATION OF TIME TO FLOWER IN <i>ARABIDOPSIS THALIANA</i> , <i>Yoshihumi Komeda</i>	521
VISUALIZING CHROMOSOME STRUCTURE/ORGANIZATION, <i>Eric Lam, Naohiro Kato, and Koichi Watanabe</i>	537
THE UBIQUITIN 26S PROTEASOME PROTEOLYTIC PATHWAY, <i>Jan Smalle and Richard D. Vierstra</i>	555
RISING ATMOSPHERIC CARBON DIOXIDE: PLANTS FACE THE FUTURE, <i>Stephen P. Long, Elizabeth A. Ainsworth, Alistair Rogers, and Donald R. Ort</i>	591
INDEXES	
Subject Index	629
Cumulative Index of Contributing Authors, Volumes 45–55	661
Cumulative Index of Chapter Titles, Volumes 45–55	666
ERRATA	
An online log of corrections to <i>Annual Review of Plant Biology</i> chapters may be found at <a href="http://plant.annualreviews.org/">http://plant.annualreviews.org/</a>	