

The ecology and evolution of endophytes

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ABSTRACT

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The association between tall fescue and its endophyte is the best-known case of a symbiosis that is widespread in grasses, especially in cool-season, pooid grasses. Field and herbarium surveys have shown that many grasses are endophyte infected, often at 100% frequency. Ubiquitous endophyte infection with a host species is *prima facie* evidence that the association is mutualistic since there are mechanisms by which individuals could lose the infection (e.g. extended seed dormancy). Studies have shown that the frequency of endophyte infection tends to increase over time, suggesting an ecological advantage for infected (E+) plants. A 3 year demographic experiment with tall fescue in a grassland community in Louisiana revealed that E+ plants had significantly higher survival, growth and flowering rates than E– plants. The mean fitness of E+ tall fescue was twice the mean fitness of E– plants. Greenhouse and common garden experiments have shown that E+ plants generally perform better than E– plants in mixtures. In order to separate the effects of endophyte-induced physiological changes in hosts versus protection against herbivory, a series of factorial competition experiments have been conducted where infection status, insect herbivory, density, and the identity of competing species were controlled. Preliminary results indicate that both infection and herbivory can affect competitive interactions among plants.

The high frequency of seed-borne *Acremonium* endophytes within species of genera like *Festuca*, *Lolium*, *Poa*, and *Stipa* suggests that the association originated before speciation within these genera. The association of clavicipitaceous fungi with grasses spans a great range of interactions from localized ovarian parasites (e.g. *Claviceps*), systemic, choke-inducing fungi (e.g. *Atkinsonella*, *Balansia*, *Epichloë*), epiphytic fungi (*Atkinsonella*, some species of *Balansia*, *Myriogenospora*), and completely seed-borne, asymptomatic endophytes such as in *Lolium*. The question of what type of associations are ancestral and which are derived remains unanswered. The potential of these fungi for contagious spread and the presence or absence of a sexual reproductive system may influence genetic variation within taxa, and therefore their evolution and coevolution with grasses. Seed-borne *Acremonium* endophytes do not spread contagiously or reproduce sexually. They are reproductively isolated from other endophytes and can become genetically differentiated by the accumulation of random mutations. Isozyme electrophoresis has revealed abundant genetic variation in many *Acremonium* endophytes from wild grasses but, in contrast, artificial selection of cultivated grasses apparently has eliminated much genetic variation of endophytes. Future research on endophytes will focus on quantifying genetic variation for important traits such as alkaloid production, stroma formation, and host compatibility.

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INTRODUCTION

Clavicipitaceous fungal endophytes infect many grasses (Clay, 1989). Most endophyte species, which are classified in the general *Atkinsonella*, *Balansia*, *Epichloë*, and *Myriogenospora*, produce fruiting bodies and ascospores on their hosts. In contrast, a subset of endophyte taxa classified in the form genus *Acremonium* do not sporulate on their hosts and are maternally transmitted through seeds. Relatively few endophytes are known that never produce the sexual stage, although those endophytes have received the greatest attention given their agricultural significance. Endophytes that are primarily seed borne but occasionally produce the fruiting bodies of *Epichloë typhina* (Pers.) Tul. are more common in natural communities. Thus, the seed-borne and ascospore-producing endophytes form a single phylogenetic grouping and the *Acremonium* endophytes are best viewed as derivatives of *Epichloë*. In order to gain a better understanding of their ecology and evolution, both ascospore-forming and seed-borne endophytes are considered in this review.

Samples of herbarium specimens and living plants from natural populations have shown that the infection frequency of seed-borne endophytes is typically high and reaches 100% in some species (White, 1987; Clay and Leuchtman, 1989). Simple theory predicts that these endophytes should not be able to persist in host populations if they decrease the fitness of their hosts relative to uninfected plants. A high frequency of endophyte infection in a grass species or population is itself evidence of a mutualistic association. Attempts to reduce endophyte infection in managed grasslands may be made more difficult if natural selection acts to increase infection frequencies.

The purpose of this paper is two-fold. First, the effect of endophyte infection on host plant fitness will be considered. Several different mechanisms may exist whereby endophytes affect plant fitness and the impact of infection may vary with the host's abiotic and biotic environment. Second, genetic variation in endophytes will be considered. Genetic variation provides the raw material for evolution in endophytes and is a prerequisite for biotechnological improvements in grass-endophyte associations.

DYNAMICS OF ENDOPHYTE INFECTION IN HOST POPULATIONS

Asymptomatic, seed-borne *Acremonium* endophytes are hereditary symbionts analogous to chloroplasts. Both are maternally inherited through seeds and do not possess any known mechanism of contagious transmission. An endophyte that increases its host's fitness will increase in frequency in host populations over time by virtue of the longer life and greater seed production of its host, relative to uninfected plants or plants infected by endophytes that decrease host fitness.

Population genetic models approximating the conditions found in hosts in-

ected by seed-borne endophytes have been constructed (see Crow and Kimura, 1970, for an example). The important conditions are that the population contains two types of genotypes (e.g. infected (E+) and uninfected (E-) plants); the two genotypes can have different fitnesses, and progeny have the same infection status as their mother.

Figure 1 presents the results of a simple computer simulation model where the frequency of E+ plants is plotted against the number of generations. The fitness of E+ plants was varied from 5.0 to 0.5 while the fitness of E- plants was held constant at 1.0. The results indicate that the frequency of endophyte infection in host populations will decline if E+ plants have lower average fitnesses than E- plants (Fig. 1). The rate of decline increases with an increasing fitness disadvantage but even with a small fitness disadvantage (0.90), E+ plants would be eliminated from a host population in about 40 generations. If endophyte infection enhances host fitness then the frequency of E+ plants should increase over time. A two-fold fitness advantage would

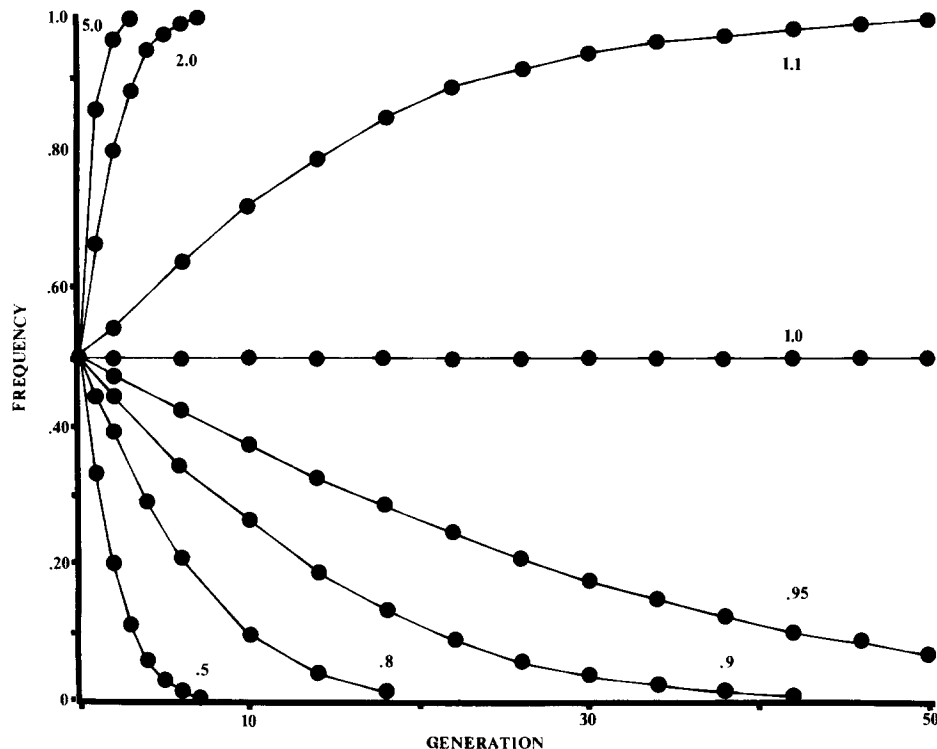


Fig. 1. Results of computer simulation where the frequency of endophyte infection in host populations over time is plotted for various relative fitnesses of infected plants where the fitness of uninfected plants was held constant at 1.0. Further details are provided in the text.

cause infection rates to approach 100% frequency within seven generations (Fig. 1).

There should be no equilibrium value of endophyte infection frequency other than 0 or 100%, except for the unlikely case where E+ and E- plants have precisely the same fitness. This is unlikely because it requires that the decrease in host fitness caused by the energetic drain from supporting an endophyte be precisely counterbalanced by an increase in host fitness caused by some other factor. Published studies have shown that the frequency of E+ plants increases over time, consistent with a mutualistic interaction (Lewis and Clements, 1986; Saha et al., 1987; Francis and Baird, 1989; Clay, 1990a). Seedling establishment in dense pastures may be very low, resulting in long generation times and slower replacement of E- by E+ genotypes.

Models are heuristic tools that are valuable not only for their predictions but also for evaluating their underlying assumptions. Exceptions to the results presented in Fig. 1 could occur if the underlying assumptions of the model were not true. If some proportion of the progeny of E+ plants escaped infection (Rykard et al., 1985; Welty et al., 1987), the approach to 100% or 0% infection would be slowed or accelerated. Similarly, if E- plants became infected contagiously then the frequency of infection could increase even if infection reduced host fitness. Thus, the frequency of infection in grasses parasitized by *Balansia* or *Epichloë*, which produce spores, is less dependent on their host's fitness (Large, 1954). Another important assumption was that the relative fitnesses of E+ and E- plants were constant, but fitness could vary with different environmental conditions. For example, infection could be detrimental to hosts in the absence of insects but advantageous when pest pressure is strong. The relative fitness of E+ and E- plants could also vary with the physical environment. Cheplick et al. (1989) showed that biomass production by tall fescue (*Festuca arundinacea* Schreb.) was enhanced by endophyte infection at high soil nutrient levels, but reduced at lower levels.

Intermediate frequencies of infection reported from tall fescue and perennial ryegrass populations (Funk et al., 1983; Long and Hilty, 1985; Shelby and Dalrymple, 1987; Latch et al., 1987) may reflect transitory states headed towards complete infection. Conditions where E- plants are favored have rarely been demonstrated (see Cheplick et al., 1989), but further research may reveal other examples. Uninfected individuals of a number of wild species of *Festuca* and *Lolium* and other genera have yet to be found, suggesting a mutualistic relationship (White and Cole, 1985a, 1986; White, 1987; Latch et al., 1987; Clay and Leuchtman, 1989). In contrast, where infection prevents seed production, host populations that are 100% infected are more likely to go extinct.

EMPIRICAL EVIDENCE OF MUTUALISM

The best evidence for mutualism comes from field studies showing that the levels of seed-borne *Acremonium* infection in host populations increase over

time. Because there is no contagious spread, infection can increase only through enhanced survival, growth, and/or reproduction of infected plants. In Britain, Lewis and Clements (1986) found that infection frequency of perennial ryegrass (*Lolium perenne* L.) was higher in pastures greater than 15 years old compared with younger pastures. The interpretation of these results is confounded by ignorance of initial infection frequencies when the pastures were established. In New Zealand, the frequency of infection of perennial ryegrass increased within pastures over time (Francis and Baird, 1989). Another study of individual plots followed over 7 years demonstrated that the frequency of infection in *Festuca longifolia* Thuill. doubled, from an initial value of 48% (Saha et al., 1987).

In a demographic study of individual plants, tillers of E+ or E- tall fescue were sprigged into a pasture community in central Louisiana, where tall fescue was a dominant component of the flora (Clay, 1990a). After 3 years, E+ plants had a 50% higher survival rate than E- plants and they produced about 50% more tillers, 40% more inflorescences, and 70% more biomass. Mean fitness of E+ plants was estimated to be twice that of E- plants under the conditions of the experiment. This is probably a conservative estimate, because the seed dispersal, germination, and establishment phases of the life cycle were not considered. Other studies have shown that the success of these stages can differ significantly between E+ and E- plants. Seed-feeding insects and birds did less damage to E+ seeds (Cheplick and Clay, 1988) (Madej and Clay, 1991); E+ seeds germinated more rapidly and to higher percentages than E- seeds; E+ seedlings grew faster than E- seedlings (Clay, 1987). More recent work demonstrated that seed production in plots of E+ tall fescue greatly exceeded that of E- plots (Rice et al., 1990).

Other evidence is consistent with a mutualistic association between host grasses and fungal endophytes including higher biomass production in field plots (Mortimer and di Menna, 1983; Read and Camp, 1986), greater growth in controlled environments (Latch et al., 1985; Clay, 1987; West et al., 1988; Arachevaleta et al., 1989; Hill et al., 1990), and increased stress tolerance (Arachevaleta et al., 1989; Elmi et al., 1989). Although infection of grass and sedge hosts by *Atkinsonella*, *Balansia*, *Epichloë*, and *Myriogenospora* has similar effects (Bradshaw, 1959; Clay, 1984, 1986; Clay et al., 1989), seed production is greatly reduced or eliminated, so that the fitness of E+ plants may be substantially lowered relative to E- plants. However, if seed production and seedling establishment is unimportant, choke-type infections may also be considered mutualistic (Bradshaw, 1959; Clay, 1986).

MECHANISMS FOR MUTUALISM

Two general classes of mechanisms appear to be important for enhancing the fitness of infected hosts. Intrinsic mechanisms are specific to the infected

plant and include changes in host biochemistry, physiology, and/or morphology (Porter et al., 1985; Belesky et al., 1987; Arachevaleta et al., 1989; Hill et al., 1990). Extrinsic mechanisms depend on the interaction of host plants with other species in their environment such as herbivores, pathogens, and competitors (Clay et al., 1985; West et al., 1988; Marks et al., 1991). The two classes of mechanisms are not necessarily independent and may interact in a complex fashion. For example, nematode damage to root systems could alter root/shoot ratios and therefore drought tolerance. Intrinsic and extrinsic mechanisms affect host fitness primarily through their impact on host competitive ability.

Several experimental studies have shown that under controlled environmental conditions E+ plants of perennial ryegrass and tall fescue can produce significantly more biomass per unit time than E- plants (Latch et al., 1985; Clay, 1987; Marks and Clay, 1990). E+ plants may have greater rates of resource uptake or greater efficiency of resource utilization. Photosynthetic rates of several tall fescue genotypes differed among E+ and E- clones, but neither group had consistently higher rates (Belesky et al., 1987). Infection also caused differences in water use; in general, E+ plants tended to maintain a more positive water potential, thus avoiding wilting (Arachevaleta et al., 1989; Elmi et al., 1989; Hill et al., 1990). With respect to mineral nutrients, biomass production of E+ and E- seedlings and adults of perennial ryegrass and tall fescue varied with soil nutrient concentrations (Chaplick et al., 1989) (Fig. 2). There was a trend for E+ plants to perform best relative to E- plants at the highest nutrient level. The advantage of infection diminished at lower nutrient levels and, in the case of tall fescue, E+ seedlings produced significantly less biomass than E- seedlings. E+ perennial ryegrass exhibited greater growth in response to elevated CO₂ levels than E- plants (Marks and Clay, 1990). The roles of infection-induced changes in host phytohormone levels (Porter et al., 1985) and carbon source-sink relationships (Thrower and Lewis, 1973; Smith et al., 1985) in the plant responses to infection described above need to be more fully explored.

Choke-inducing endophytes can also enhance the growth of host plants relative to non-hosts. Increased survival, tillering and/or biomass production has been reported for grasses infected by *E. typhina* (Bradshaw, 1959; Harberd, 1961), *Atkinsonella hypoxylon* (Pk.) Diehl (Clay, 1984; Kelley and Clay, 1987; Antonovics et al., 1987), *Balansia epichloë* (Weese) Diehl (Marks and Clay, 1990) and *Balansia henningsiana* (Moell.) Diehl (Clay et al., 1989). The same patterns have been observed in the sedges *Cyperus rotundus* L. and *Cyperus virens* Michx. infected by *Balansia cyperi* Edg., (Clay, 1986, 1990a; Stovall and Clay, 1988), indicating that mutualistic interactions with other plant families can also occur.

The most important extrinsic mechanism of mutualism in grass/endophyte associations is the protection of host plants against animal and micro-

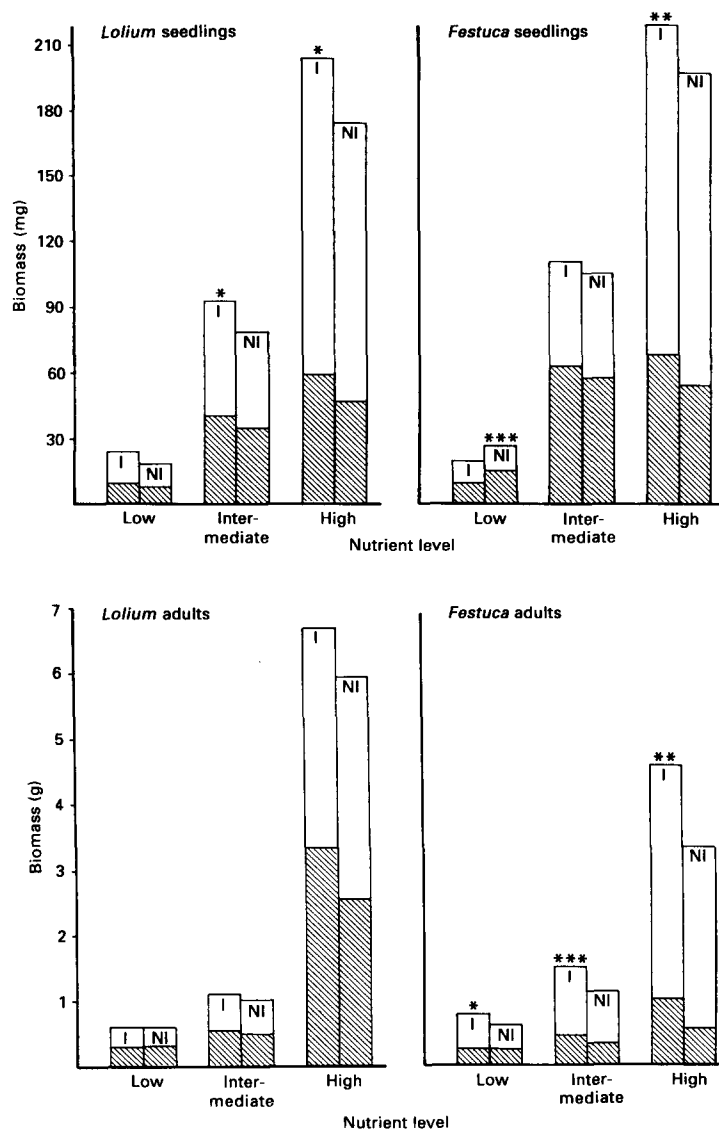


Fig. 2. Mean biomass production of infected (I) and uninfected (NI) perennial ryegrass and tall fescue grown at three nutrient levels. Shaded portion of bar represents root biomass; open portion of bar represents shoot biomass. Asterisks indicate a significant difference between biomass of infected and uninfected plants. Data from Cheplick et al. (1989).

bial pests (Clay, 1988b). Alkaloid toxins produced by the fungi appear to be the basis for protection in most situations (Bacon et al., 1986; Siegel et al., 1987a). In addition to causing livestock toxicoses, endophyte infections deter a wide range of chewing, boring, and sucking insects (Prestidge et al., 1982;

Funk et al., 1983; Clay et al., 1985), nematodes (Pedersen et al., 1988; West et al., 1988), and pathogenic fungi (White and Cole, 1985b). While much of the data is from laboratory situations, field experiments and observations have also indicated that host plants can gain considerable protection from their enemies (Prestidge et al., 1982; Funk et al., 1983). Choke-inducing endophytes (*Balansia*, *Epichloë*, etc.) can also be toxic to mammalian grazers (Nobindro, 1934; Diehl, 1950; Bacon et al., 1986), insects (Clay et al., 1985; Schmidt, 1986; Cheplick and Clay, 1988), and fungal pathogens (Yoshihara et al., 1985; Clay et al., 1989; Stovall and Clay, 1991).

INTERACTIONS AMONG PLANTS

The significance of endophyte-induced changes in host physiology and resistance to pests comes from their effects on the host's ability to capture space, water, nutrients, and light when in mixtures with other plants. One study examined *Danthonia spicata* (L.) Beauv. infected by *Atkinsonella hypoxylon*, an epiphytic member of the Balansieae. E+ and E- genotypes were collected from a field population, divided into clones, and planted back into the same field site in combination with clones of the common co-occurring grass *Anthoxanthum odoratum* L. (Kelley and Clay, 1987). Competition between the two species was ensured by planting two clones per 7.5 cm plastic pot. The bottoms of the pots were removed and the pots were imbedded in the ground so that experimental plants would root into native soil and be exposed to variations in their natural environment. The results of this 2 year experiment were that (1) *Danthonia* produced fewer tillers and inflorescences on average than *Anthoxanthum*, (2) E+ clones of *Danthonia* produced significantly more tillers and inflorescences than E- clones when in competition with *Anthoxanthum*, and (3) E+ and E- clones did not perform significantly differently from each other when grown with other E+ or E- plants, although E+ clones had higher average values (Fig. 3). The ratio of E+/E- performance in competition with *Anthoxanthum* was 1.56 for tillers and 2.08 for inflorescences (Kelley and Clay, 1987).

In a greenhouse study with tall fescue and perennial ryegrass, E+ or E- seedlings were planted in the middle of small pots along with either five, three, one or no competing seedlings. Competing seedlings were either E+ or E- individuals of either species (Marks et al., 1991). The fact that competition was occurring could be inferred because mean plant size decreased as the number of plants per pot increased (Fig. 4). In general, endophyte infection enhanced the biomass production of tall fescue plants. In nine of ten experimental combinations encompassing four densities of competitors (including no competitors), E+ plants outperformed E- plants (Fig. 4). In contrast, E- plants of perennial ryegrass produced more biomass than E+ perennial ryegrass in seven of the ten experimental combinations (Fig. 4). The effects

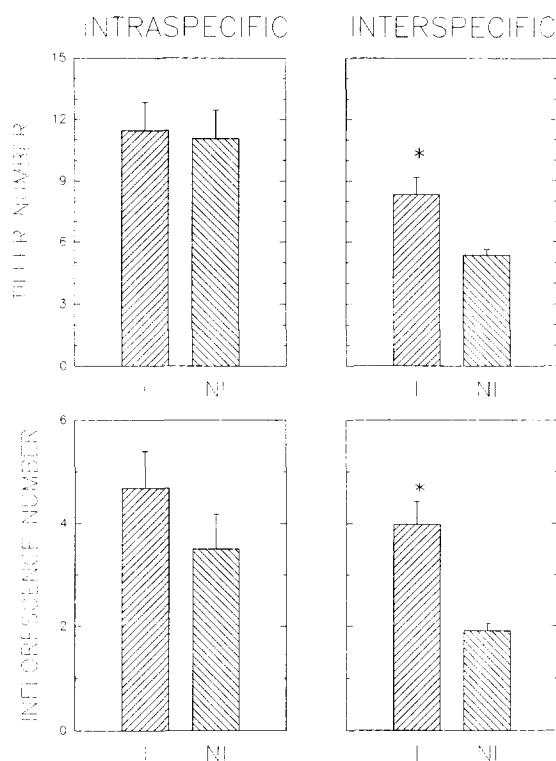


Fig. 3. Tiller and inflorescence production of the grass *Danthonia spicata* either infected (I) or uninfected (NI) by *Atkinsonella hypoxylon* when grown in competition with other *Danthonia* (intraspecific) or *Anthoxanthum odoratum* (interspecific) plants. Asterisks indicate a significant difference between infected and uninfected plants. Further details are provided in the text. Data from Kelley and Clay (1987).

of endophyte infection in this experiment were not consistent; the relative performance of E+ and E– plants often varied with the identity of the competing plants. For example, when tall fescue target seedlings were grown with five competing seedlings, E+ seedlings produced more biomass than E– seedlings when they were grown with E+ tall fescue or perennial ryegrass, but less biomass when they were grown with E– competitors (Fig. 4).

Another greenhouse competition experiment with tall fescue was conducted in which E+ or E– seedlings were transplanted to 10-cm pots at densities of four or 16 plants per pot (M. Rucker and K. Clay, unpublished data, 1989). Competition could again be inferred because of the negative correlation between mean plant size and plant density. Pots contained either only E+ plants, only E– plants, or a mixture of half E+ plants and half E– plants. Half of the pots in each planting combination were exposed to drought by allowing soil moisture to fall below 40% before rewatering (at which point

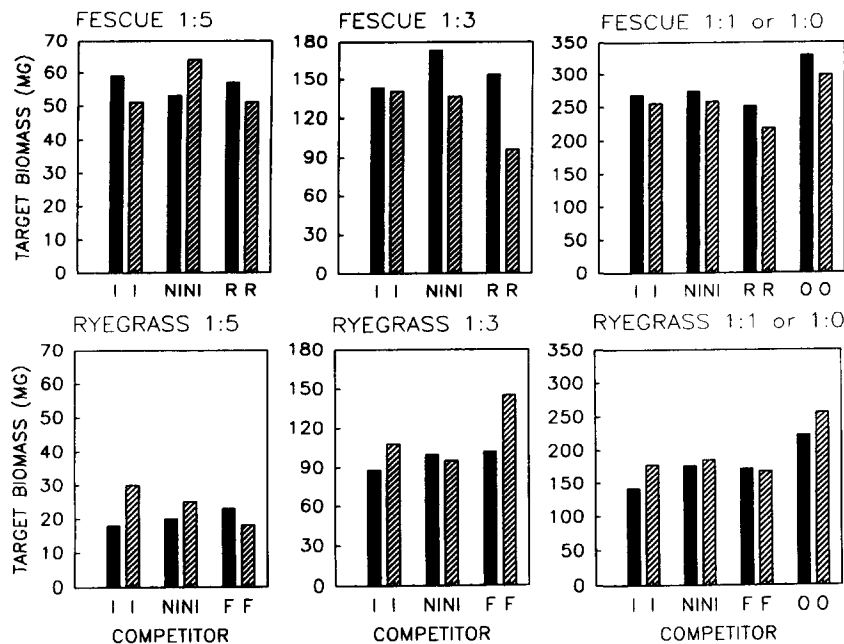


Fig. 4. Results of competition experiments where one target seedling was planted surrounded by either five, three, one or no competitor seedlings. Targets were either infected (I) or uninfected (NI) tall fescue (F, top graphs) or perennial ryegrass (R, bottom graphs). Competitors were infected or uninfected plants of the same species (I, NI) or uninfected plants of the opposite species (R for tall fescue, F for perennial ryegrass). Solid bars represent mean above-ground biomass of infected target, hatched bars represent aboveground biomass of uninfected target. Further details are provided in the text. Data from Marks et al. (1991).

Analysis of variance of mean biomass production (see graphs). *P*-values are presented for the main effects of identify of target, competitor, and their interaction.

| Source | Fescue 1:5 | Fescue 1:3 | Fescue 1:1 or 1:0 |
|------------|--------------|--------------|---------------------|
| Target | 0.1161 | 0.0001 | 0.0398 |
| Competitor | 0.4885 | 0.0082 | 0.0001 |
| T×C | 0.0008 | 0.0015 | 0.8748 |
| Source | Ryegrass 1:5 | Ryegrass 1:3 | Ryegrass 1:1 or 1:0 |
| Target | 0.0002 | 0.0001 | 0.0071 |
| Competitor | 0.0787 | 0.0001 | 0.0001 |
| T×C | 0.0001 | 0.0001 | 0.1991 |

leaves began to roll) as determined with soil moisture blocks placed in each pot. The remaining pots were maintained at 100% soil moisture. After 5 weeks of growth all aboveground plant parts were harvested, dried, and weighed. Results were analyzed with three-way analysis of variance where infection,

TABLE 1

Mean biomass production (g per pot) of E+ and E- tall fescue grown under conditions of intraspecific competition. Plants were grown at two densities and two soil moisture levels. A pure stand consisted of all E+ (or E-) plants per pot; a mixed stand consisted of half E+ and half E- plants per pot. Data from Rucker and Clay (M. Rucker and K. Clay, unpublished data, 1989)

| | Pure stand | | Mixed stand | |
|---------------|---------------|------------|-------------|------------|
| | Infected | Uninfected | Infected | Uninfected |
| High density | | | | |
| Low moisture | 3.18 | 2.92 | 3.26 | 3.22 |
| High moisture | 3.94 | 2.86 | 3.82 | 3.53 |
| Low density | | | | |
| Low moisture | 2.79 | 2.71 | 2.71 | 2.58 |
| High moisture | 3.81 | 3.06 | 3.37 | 3.03 |
| | | | | |
| | Source | | F | P < |
| | Density (D) | | 9.44 | 0.0026 |
| | Moisture (M) | | 37.49 | 0.0001 |
| | Infection (I) | | 5.15 | 0.0021 |
| | D×M | | 0.96 | 0.3291 |
| | D×I | | 2.31 | 0.0792 |
| | M×I | | 1.80 | 0.1511 |
| | D×M×I | | 0.02 | 0.9972 |

density, and watering treatment were the main effects. Biomass of all E+ or E- plants in a pot was combined and biomass of pots containing only E+ or E- plants was divided by two to facilitate comparisons with pots containing mixtures of E+ and E- plants.

The analysis indicated there were highly significant effects of all three main effects. In general, high density pots produced more biomass than low density pots and high moisture pots produced more biomass than low moisture pots (Table 1). Infection resulted in greater biomass production in eight of eight combinations of density, moisture, and competitors. The largest advantage of E+ over E- plants occurred in high moisture pots containing 16 plants of one type (Table 1). The differences were somewhat unexpected since it has been reported that E+ tall fescue is more drought tolerant than E- plants (West et al., 1988; Arachevaleta et al., 1989; Elmi et al., 1989).

IMPORTANCE OF INTRINSIC VERSUS EXTRINSIC FACTORS

Both intrinsic and extrinsic mechanisms have been implicated in the enhanced performance of E+ plants in the field. Do E+ plants perform better than E- plants because of biochemical, physiological, or morphological dif-

ferences between E+ and E- plants, or do they perform better because they are less damaged by pests? One way to separate these intrinsic and extrinsic factors is to manipulate pest pressure. Competition studies were therefore conducted where insect herbivory was increased in half of the replicates by releasing neonate fall armyworm (*Spodoptera frugiperda* J.E. Smith) larvae and decreased in the other half by routinely spraying an insecticide (K. Clay, unpublished data, 1990). Changes in herbivory were superimposed over a background level of herbivory that occurred in a greenhouse open to the outside environment.

Experimental combinations of plants were established as before where either four plants of one type (pure stand) were planted per pot or a mixture of two plants of one type and two of another (mixed stand) were planted per pot. Further, mixed stands consisted of either only individuals of the same species differing in their infection status (intraspecific) or individuals of two different species (interspecific). Three different species were utilized in the intraspecific competition studies: perennial ryegrass, tall fescue, and red fescue (*Festuca rubra* L.); in the interspecific competition studies the three species were paired with Kentucky bluegrass (*Poa pratensis* L.), orchard grass (*Dactylis glomerata* L.), and perennial ryegrass, respectively. In total there were three intraspecific experiments where mixtures consisted of two E+ plants and two E- plants of the same species, and three interspecific experiments where mixtures consisted of two plants of one species and two of another species. Insect herbivory was increased in half of the pots in each experiment and reduced in the other half. Data are presented as ratios of E+/E- biomass; values less than 1.0 indicate that E- plants produced more biomass than E+ plants in the same situation; values over 1.0 indicate that E+ plants produced more biomass than E- plants.

In the intraspecific combinations, E+ plants of both perennial ryegrass and tall fescue produced more biomass than E- plants in both pure stands and mixtures, under both high and low herbivory (Table 2, Intra columns). However, E- red fescue plants had greater or equal biomass production compared with E+ plants when herbivory was low, but E+ plants outperformed E- plants when herbivory was high (Table 2). In total, the E+/E- ratio was increased by increasing herbivory in five of six intraspecific combinations.

Considering interspecific combinations of perennial ryegrass, there was an enhancement of E+ plant performance relative to E- plant performance with increased herbivory (Table 2, Inter columns). This was especially dramatic in mixed stands with Kentucky bluegrass. In the low herbivory treatment, E+ and E- plants produced equivalent amounts of biomass in competition with Kentucky bluegrass but at the high herbivory treatment E+ ryegrass produced 64% more biomass than E- ryegrass (Table 2). Similar results were obtained with tall fescue. There were relatively small differences in biomass between E+ and E- plants in mixtures with orchard grass in low herbivory

TABLE 2

Relative biomass production of E+ to E- perennial ryegrass, red fescue, and tall fescue in intraspecific (pure stand was all E+ (or E-) plants per pot; mixed stand was half E+ and half E- plants per pot) and interspecific (pure stand as before, mixed stand was half E+ or E- plants of target species per pot and half E- plants of the competing species) competition. Biomass production is expressed as the ratio: biomass of E+ plants/biomass of E- plants. Data from Clay et al. (K. Clay, S. Marks and G.P. Cheplick, unpublished data, 1990)

| | Perennial ryegrass | | Red fescue | | Tall fescue | |
|--------------|--------------------|-------|------------|-------|-------------|-------|
| | Intra | Inter | Intra | Inter | Intra | Inter |
| Pure stand | | | | | | |
| No herbivory | 1.13 | 1.07 | 0.65 | 1.07 | 1.04 | 1.69 |
| Herbivory | 1.34 | 1.18 | 1.58 | 0.88 | 1.12 | 1.33 |
| Mixed stand | | | | | | |
| No herbivory | 1.09 | 1.00 | 1.01 | 1.28 | 1.17 | 1.11 |
| Herbivory | 1.06 | 1.64 | 1.29 | 1.16 | 1.46 | 2.03 |

conditions but in the high herbivory treatment E+ tall fescue markedly outperformed E- tall fescue (Table 2). Insect damage was not assessed directly in these experiments but it seems likely that E- plants sustained more damage than E+ plants in mixtures with Kentucky bluegrass or orchard grass. Results from red fescue were in the opposite direction where the relative performance of E+/E- plants decreased from the low to high herbivory treatments.

The results of these experiments suggest that both endophyte infection and herbivory can affect the outcome of competition among grasses, and that these two factors can interact in a complex fashion. Endophytes interacting with herbivores might therefore influence the structure of grassland vegetation. Unpalatable grasses often come to dominate overgrazed pastures, and in some cases grazers' aversion may arise in response to endophyte infection (Shaw, 1873; Bacon et al., 1986; White, 1987). Reduced herbivory of E+ plants could provide them with a competitive advantage in mixtures with E- plants.

ROLE OF ENDOPHYTES IN GRASSLAND COMMUNITIES

An assessment of the role of endophytes in grassland communities requires knowledge of the frequency of infection within and among grass species, and the effect of infection on host physiology, growth, and interactions with other species. Research has shown that *Acremonium* endophytes are limited to the grass subfamily Pooideae and that the frequency of E+ species is relatively high in eastern North American deciduous woodlands (Clay and Leuchtmann, 1989) and in central European grassland communities (Latch et al.,

1987). Nevertheless, we know virtually nothing about endophytes in a community context, and this represents an important area for future research. For example, do E+ grasses occur in major grassland regions like the Great Plains of central North America, the steppes of central Eurasia, or the Serengeti of Africa? Broad-scale surveys such as those reported by White and Cole (1985a, 1986), Latch et al. (1987), and Clay and Leuchtman (1989) are needed from many communities.

How does endophyte infection affect associated herbivore populations? One study has examined the insect communities on replicated E+ and E- tall fescue pastures (Kirfman et al., 1986), and several others have assessed particular insect populations in E+ and E- plots of several grasses (Prestidge et al., 1982; Funk et al., 1983; Lewis and Clements, 1986; Saha et al., 1987). Anecdotal reports suggest that E+ grasses increase in abundance with heavy grazing pressure (Shaw, 1873; Bradshaw, 1959; Bacon et al., 1986). What are the dynamics of E+ and E- species in mixed communities and how do they change with increasing pest pressure? How do occasional years of extreme abiotic conditions alter infection levels and community composition? Many community level processes could be greatly influenced by endophytes, but grassland ecologists have generally ignored the potential role of endophytes. An abundance of published data may be flawed because the endophyte status of experimental plants was not considered.

POSSIBLE EVOLUTIONARY ORIGIN OF ENDOPHYTES

Based on the diversity and distribution of hosts known at present, the association between grasses and fungal endophytes must be quite ancient. Plant-parasitic members of the Clavicipitaceae (*Claviceps* and members of the Balansieae) are restricted entirely to graminoids (Cyperaceae, Gramineae, and one species of Juncaceae) and primarily to grasses (Clay, 1989). Since the number of grass species acting as hosts far exceeds the number of sedge hosts, it is likely that the tribe Balansieae evolved in conjunction with the grasses and underwent a secondary radiation on *Cyperus*.

Modern grasses may have evolved from forest understory plants similar to the primitive bamboos found in the rainforests of South America (Stebbins, 1981). During the Eocene, the Earth's climate became cooler and drier, resulting in a change from forest vegetation to savannah-like vegetation in continental areas. The first fossil grasses and grazing mammals have been recorded from this time period (Stebbins, 1981; McFarlane, 1987). It is likely that the primitive forest grasses were already infected by systemic *Balansia*-type fungi that may have evolved from saprophytic fungi colonizing decaying woody culms. Supporting this idea are *Balansia* species which infect primitive bamboos from South America (Diehl, 1950).

The relationship of *Claviceps* to endophytes is unclear. One hypothesis is

that *Claviceps* is ancestral to members of the Balansieae, but an equally plausible alternative is that *Claviceps* itself was derived from a systemic, *Balansia*-like fungus. For example, *Balansia obtecta* Diehl produces stromata that detach and overwinter on the ground before producing ascospores, similar to the *Claviceps* life cycle. Other *Balansia* species produce stromata that individually mummify single florets, again similar to *Claviceps* (Diehl, 1950; Ou, 1972). Walker (1970) has described what he considers an intermediate between *Balansia* and *Claviceps* infecting an Australian species of *Phalaris*. Molecular evidence will be useful in resolving the relationships among *Claviceps* and the different endophyte genera.

Seed-borne *Acremonium* endophytes are restricted entirely to members of the grass subfamily Pooideae, with most hosts found in the tribe Poeae (Clay, 1990b, following the classification of Gould and Shaw, 1983). *Epichloë* also primarily infects members of the Pooideae, but other members of the Balansieae infect hosts in all six subfamilies. This pattern suggests that the sexual forms are ancestral to the seed-borne *Acremonium* endophytes and that the *Acremonium* endophytes share an *E. typhina*-like ancestor. Temperate cool-season grasses apparently originated in Eurasia and the ancestors of the North American Pooideae migrated from Eurasia (Stebbins, 1981). Some of the migrants must already have been infected by seed-borne *Acremonium* endophytes. The high frequency of endophyte infection among species in genera like *Festuca*, *Poa*, and *Stipa* in both Eurasia and North America is consistent with this explanation. The alternative explanation, that the seed-borne habit has arisen independently many times all over the world, is less likely.

An important unanswered question is why are seed-borne *Acremonium* endophytes apparently found only (or mostly) in the Pooideae when *Epichloë* and *Balansia* species are known from other subfamilies (Diehl, 1950; Mhaskar and Rao, 1976). A number of possible hypotheses exist, but none can be proven definitively. There may be adaptive reasons why *Acremonium* endophytes are restricted to one part of the grass family, or this distribution may represent an evolutionary 'accident'. Research investigating the factors responsible for the balance between seed transmission and stroma production in one host species or population may provide insights into the ecological conditions that favor seed transmission (or stroma production) in other species.

THE SIGNIFICANCE OF STROMA PRODUCTION

The mode of reproduction of *E. typhina* is quite labile. For example, *Glyceria striata* (Lam.) Hitchcock typically bears stromata on every flowering culm when infected and seed transmission has not been observed (Clay and Leuchtmann, 1989). In contrast, *Brachyelytrum erectum* (Schreb.) Beauv. and *Poa sylvestris* Gray bear infrequent stromata on one of every hundred or

thousand flowering culms while producing E+ seeds on the large majority of culms. Many *Acremonium*-infected grasses may produce the *Epichloë* state so rarely that it has never been observed. For a number of hosts, the fungus both regularly produces stromata and is seed borne on the same plant (White, 1988; Clay and Leuchtman, 1989). Sampson (1933) described a population of red fescue infected by *E. typhina* where some E+ plants produced only aborted, stroma-bearing culms, some plants produced a mixture of flowering and stroma-bearing culms, while other E+ plants produced only stromata-free, flowering culms bearing E+ seeds.

Understanding the environmental and/or genetic control of stroma production and the selective forces favoring one form of endophyte propagation over another is central to understanding the origin of *Acremonium* endophytes and their dynamics within host populations. Several studies suggest that the differential growth of fungus and plant can influence the production of stromata versus E+ seeds. For example, E+ chewings fescue bore a higher frequency of stromata when growing in N-poor soils compared with N-rich soils (Sun et al., 1990). Additions of gibberellic acid to E+ *Dactylis glomerata* plants caused more rapid elongation of inflorescences that escaped choke formation compared with untreated plants (Emecz and Jones, 1970). White and Chambless (1991) showed that isolates of *E. typhina* from stroma-bearing plants of *Agrostis heimalis* (Walt.) Britton, Stearns and Poggenberg grew significantly faster in culture than isolates from asymptomatic, E+ plants. Transplanted plants that were stroma-bearing in the field often cease producing stromata in the greenhouse, and asymptomatic E+ plants transplanted in the field often begin to produce stromata (K. Clay, personal observation, 1990). Cool-season grasses capable of very rapid growth during the early growing season may be simply outracing the endophyte to produce an expanded inflorescence before a stroma can be formed. Further experiments are needed in which clones of the same infected grass genotypes are grown under different environmental conditions (of light, temperature, fertilization rate, etc.) to elucidate determinants of stromata production.

In addition to environmental factors, plant genotype and/or fungal genotype may also influence stromata production. Certain endophytes could be genetically incapable of producing stromata, or certain host plants could possess biochemical characteristics that inhibit stromata development. Besides known differences among host species, there are few data bearing on possible genetic variation in stromata production among host plants. Bier (J. Bier, personal communication, 1990) has suggested that there is some underlying genetic control of stromata production. When replicated clones of 13 E+ *Poa sylvestris* genotypes were planted at random back into their native woodland habitat, only a few clones produced stroma-bearing culms and they were all clones of just a single plant genotype. However, it was unclear whether it was the fungal genotype or the plant genotype, or their interaction, that controlled

stroma production. To resolve the basis for variation in stroma production, both fungal isolates and E- tillers could be obtained from several E+ plants exhibiting varying degrees of stroma production. Reciprocal inoculations of tillers from asymptomatic E+ plants with isolates from stroma-producing plants, and E- tillers from stroma-bearing plants with isolates from asymptomatic E+ plants would decouple plant and fungal genotypes, allowing their contribution to stroma production to be assessed.

Regardless of mechanism, there must be some ultimate, or evolutionary, explanation why an endophyte like *E. typhina* should become entirely maternally transmitted through the seed. White (J. White, personal communication, 1990) has suggested that environmental conditions found at higher latitudes and altitudes (where cool-season grasses dominate) reduce the chance of contagious transmission, favoring the more certain strategy of seed transmission. Seed transmission could also represent a means of reproductive isolation. In plant communities containing several grasses infected by *E. typhina*, cross-fertilization between host-specific races or isolates would be prevented by seed transmission. A third explanation relates to the caespitose (clump-forming) growth habit of many pooid grasses. Stromata production and the lack of seed production is likely to be more detrimental for clump grasses than for rhizomatous grasses, because rhizomatous grasses have alternative means of propagation. Choke-type infections are significantly less common in caespitose grasses than in rhizomatous grasses (Clay, 1988a). Seed transmission might also be a mechanism for protecting seeds from predators deterred by the high concentrations of fungal alkaloids found in E+ seeds (Cheplick and Clay, 1988). A final hypothesis concerns the relative fitness of seed-borne versus stroma-producing endophytes as a function of infection frequency in the host population. Seed transmission may be a more successful 'strategy' if infection frequencies are high (few E- plants), because if most plants are already infected, the chance of infecting a new host plant by contagious spores is very small. This hypothesis predicts that seed-borne *Acremonium* endophytes should occur at higher frequencies within host populations than choke-inducing *Epichloë* or *Balansia* infections. Available data on infection frequencies support this prediction (White, 1987; Latch et al., 1987; Clay and Leuchtmann, 1989).

GENETIC VARIATION IN ENDOPHYTES

Endophytes vary in their morphology and host range. Recognition of this variation has resulted in the classification of many species and several genera, including the asexual form genus *Acremonium* (Morgan-Jones and Gams, 1982; Latch et al., 1984; White and Morgan-Jones, 1987). Recent studies have revealed considerable variation within accepted taxa of endophytes in alkaloid production, cultural characteristics including conidial morphology,

stroma production, host specificity, and genetic variation of isozymes and DNA sequences. Variability in all of the aforementioned characteristics provides raw material for biotechnological manipulation of the endophyte symbiosis and is the basis for adaptive evolution of herbivore defenses, reproductive strategies, and host relations.

The technique of isozyme electrophoresis has been used to examine genetic variation in a wide range of organisms including fungi (Michales et al., 1986). It is a relatively conservative measure of genetic variability since isozymes are under continuous selection for function, unlike non-coding DNA sequences. Isozyme variation in 291 isolates of *Atkinsonella hypoxylon* or *Atkinsonella texensis* from four host species was examined in a recent study (Leuchtmann and Clay, 1989b). Eleven of 13 loci examined were variable and 20 multilocus genotypes were detected (Table 3). All 20 isolates of *Atkinsonella texensis* from *Stipa leucotricha* were identical whereas there were nine genotypes found among 122 isolates of *Atkinsonella hypoxylon* from *Danthonia compressa* and 11 genotypes among 124 isolates from *Danthonia spicata* (Table 3). Two multilocus genotypes were found in common from *Danthonia compressa* and *Danthonia spicata*. Within single populations of *Danthonia compressa*, a minimum of three and a maximum of seven multilocus genotypes were found (Leuchtmann and Clay, 1989b). Isolates from single ascostromata or asci were also variable. One of two asci from which all ascospores were isolated contained two genotypes in a 4:4 ratio, confirming the Mendelian inheritance of isozyme loci (Leuchtmann and Clay, 1989b).

In another study, 219 fungal isolates of *Acremonium* or *Epichloë* from 17 host grass species were examined. Considerable isozyme variation was found among isolates from most hosts, except for tall fescue and *G. striata* (Leuchtmann and Clay, 1990). Out of 52 isolates of *Acremonium coenophialum* from tall fescue, only two multilocus genotypes were detected (Table 3). Forty-seven isolates had one multilocus genotype characteristic of isolates from cultivar 'KY-31' tall fescue while five isolates from cultivar 'Triumph' had a different genotype that was more similar to isolates of *Acremonium lolii* from perennial ryegrass cultivar 'Repell'. In contrast, endophyte isolates from woodland fescue (*Festuca obtusa*) were highly variable with five genotypes among 14 isolates (Table 3). Seven isolates of *G. striata* collected both from New York State and Canada were all identical. Otherwise, endophytes from other wild grasses (e.g. *Agrostis* spp., *Poa sylvestris*, *Sphenopholis* spp.) exhibited considerable isozyme variation, including genetic variability within a single host population. This may indicate that there are not large fitness differences among coexisting endophyte genotypes. The limited variability observed in cultivated tall fescue probably has resulted from genetic bottlenecks in endophyte populations subjected to artificial plant breeding and selection.

Studies are needed to examine endophyte population structure as a function of host plant, stromata frequency, and the number of sympatric, infected

TABLE 3

Isozyme variation in fungal endophytes and epiphytes in the genera *Acremonium*, *Atkinsonella*, and *Epichloë*. All isolates from one host were called *Epichloë* if stromata were observed on any plants. Data compiled from Leuchtmann and Clay (1989b, 1990)

| Fungus | Host species | No. genotypes | No. isolates | Ratio |
|-------------------------------|------------------------------|---------------|--------------|-------|
| <i>Acremonium</i> spp. | | | | |
| | <i>Festuca arundinacea</i> | 2 | 52 | 0.038 |
| | <i>Festuca obtusa</i> | 5 | 14 | 0.429 |
| | <i>Lolium perenne</i> | 1 | 6 | 0.167 |
| | <i>Poa autumnalis</i> | 1 | 1 | — |
| | <i>Poa wolfii</i> | 1 | 1 | — |
| | <i>Sphenopholis nitida</i> | 2 | 2 | 1.000 |
| | <i>Sphenopholis pallens</i> | 2 | 2 | 1.000 |
| | <i>Stipa robusta</i> | 1 | 1 | — |
| <i>Atkinsonella hypoxylon</i> | | | | |
| | <i>Danthonia compressa</i> | 9 | 122 | 0.074 |
| | <i>Danthonia sericea</i> | 1 | 1 | — |
| | <i>Danthonia spicata</i> | 11 | 124 | 0.089 |
| <i>Atkinsonella texensis</i> | | | | |
| | <i>Stipa leucotricha</i> | 1 | 20 | 0.050 |
| <i>Epichloë typhina</i> | | | | |
| | <i>Agrostis hiemalis</i> | 9 | 13 | 0.692 |
| | <i>Agrostis perennans</i> | 6 | 6 | 1.000 |
| | <i>Brachyelytrum erectum</i> | 3 | 18 | 0.167 |
| | <i>Elymus villosus</i> | 1 | 1 | — |
| | <i>Elymus virginicus</i> | 4 | 40 | 0.100 |
| | <i>Glyceria striata</i> | 1 | 7 | 0.143 |
| | <i>Holcus lanatus</i> | 1 | 1 | — |
| | <i>Hystrix patula</i> | 6 | 40 | 0.150 |
| | <i>Poa sylvestris</i> | 3 | 13 | 0.231 |

species. Given that stromata production varies among *Epichloë* isolates, it would be worthwhile to determine whether genetic variability within populations was positively correlated with the frequency of stromata production because multilocus genotypes are recombined via sexual reproduction. However, the data presented in Table 3 do not reveal greater variation in sexual endophytes. Sexual reproduction may also occur between endophyte isolates infecting different species. The same multilocus genotype was isolated from *Elymus virginicus* and *Hystrix patula* that were growing sympatrically in the same habitat, implying interspecific infections by single genotypes of *Epichloë typhina* (Leuchtmann and Clay, 1990). Seed-borne *Acremonium* endophytes are, by definition, reproductively isolated from other endophytes and may exhibit greater host specificity as a result. Further genetic studies are also needed to confirm the observation that cultivated grasses are infected by a

small number of endophyte genotypes. Genetically variable endophytes might best be collected from wild grasses, provided they can be transferred to new hosts.

HOST COMPATIBILITY

Specificity of endophytes for their hosts has been investigated using the technique of seedling inoculations. A fine needle is used to puncture and introduce mycelium from a culture into a seedling near the meristem (Latch and Christensen, 1985). Successful infections are evidenced by the proliferation of hyphae into tillers produced subsequent to inoculation. Several studies have shown that endophytes can be moved among hosts but usually only among closely related host species or genera. Latch and Christensen (1985) and Siegel et al. (1987b, 1990) reported intrageneric and intergeneric transfers of endophytes isolated from species of *Festuca*, *Lolium* and other pooid grasses. Leuchtmann and Clay (1988, 1989a) also transferred *Atkinsonella hypoxylon* among *Danthonia* species and *Balansia cyperi* among *Cyperus* species.

These studies assessed the compatibility of endophytes and host grasses in synthetic combinations. Specific resistance mechanisms like the hypersensitive response have not been observed with endophyte infections. The ability of an endophyte to successfully infect an alien host may therefore reflect basic metabolic compatibility between symbionts. Narrow host ranges suggest a high level of specific adaptation of endophytes for particular hosts.

The criteria for ascertaining compatibility should require that viable endophyte is seed transmitted at high frequency following the inoculation of a sterile seedling. Two or three generations of seed transmission would be more conclusive. An endophyte can be introduced into a seedling from a different species and proliferate in vegetative tissues for some period of time, but cannot survive indefinitely and/or become incorporated into seeds. In one study (A. Leuchtmann and K. Clay, unpublished data, 1988), seedlings of tall fescue were inoculated with *Acremonium* or *Epichloë* endophytes from 13 different host species. Two months following inoculation, endophytes from several host species were found infecting tall fescue. After 8 months, however, only plants originally inoculated with *Acremonium coenophialum* were still infected. Siegel (M. Siegel, personal communication, 1990) reported a similar result with *Festuca* and *Lolium* inoculated with alien endophytes. Seed-borne *Acremonium* endophytes apparently exhibit a high specificity for their original hosts in seedling inoculation experiments.

Experimental data indicate that there are limits to transferring stroma-forming endophytes among potential host species. Seedlings of three host species of *Atkinsonella* were reciprocally inoculated with isolates from each of the three species (Leuchtmann and Clay, 1989a) (Table 4). Inoculation with

TABLE 4

Compatibility of isolates of *Atkinsonella hypoxylon* from *Danthonia* species and *Atkinsonella texensis* from *Stipa leucotricha* with seedlings of *Danthonia compressa* (Dc), *Danthonia spicata* (Ds), and *Stipa leucotricha* (Sl). Percentages are of inoculated seedlings that became infected (sample sizes in parentheses). Data from Leuchtman and Clay (1989a)

| Isolate source | Host grasses | | |
|----------------|--------------|--------------|--------------|
| | Dc | Dsp | Sl |
| Dc | 99% (68) | 96% (66) | 0% (20) |
| Dsp | 61% (69) | 81% (147) | 0% (100) |
| Sl | 0% (72) | 0% (75) | 22% (225) |

Atkinsonella hypoxylon revealed that isolates from *Danthonia compressa* and *Danthonia spicata* were essentially interchangeable (Leuchtman and Clay, 1989a (table 5)). However, cross-inoculations of isolates from *Danthonia* and isolates of *Atkinsonella texensis* from *Stipa leucotricha* Trin. and Rupr. to the opposite hosts were impossible.

Given that stroma-forming endophytes may occasionally infect new hosts contagiously, we should expect that they would tend to have wider host ranges than seed-borne *Acremonium* endophytes, which should exhibit a high level of specificity and adaptation to their particular hosts. Excepting plant hybridization, it is difficult to conceive how an *Acremonium* endophyte from one host species would ever be exposed to another host species. There would be no selection to maintain functions related to the infection of a diversity of host species. This could present difficulties in manipulating the grass/endophyte symbiosis since the greatest applied interest focuses precisely on those endophytes that may be incapable of significant host shifts. The best source of *Acremonium* endophytes to introduce into a desired species are those that are isolated from that of congeneric host species.

CONCLUSIONS

The research reviewed here provides one perspective on the complex and diverse associations between grasses and fungal endophytes. Ecological research has been useful in delineating the conditions under which endophyte infection enhances plant survival and growth and those under which infections are detrimental. It has also provided considerable information on the mechanistic basis for changes in plant survival and growth. However, very few grass/endophyte associations have been examined in any detail and fewer still have been examined in natural grassland communities. The construction

and release of grasses infected with foreign or altered endophytes should proceed from a sound ecological base.

Identification of natural genetic diversity in endophytes and the creation of specific variants through genetic engineering represent key areas for future research. A thorough understanding of the genetic basis for host specificity will be necessary to exploit fully the biotechnological potential of endophytes. Broad generalizations should not be made from a narrow data base. Research on the endophytes of tall fescue and perennial ryegrass reflect their economic importance as pasture and turf-grasses. However, they represent just two of many hundreds or thousands of grasses infected by similar endophytes. If the isozyme data cited earlier are representative, then much of the published research on *Acremonium coenophialum* infecting tall fescue has been conducted primarily with a single endophyte genotype. Wild grasses should play a greater role in endophyte research, not only as repositories of genetic variability, but also as comparative systems for understanding the physiological, ecological and evolutionary interactions between plant and fungus.

Endophyte research is poised at a juncture. In one direction, endophytes could become specialized problems for the livestock industry with little research interest outside of pasture and turf-grass management. In the other direction, endophytes could be widely used model systems for studying plant/microbe symbioses and the evolution of mutualism. The long-term health of endophyte research lies in convincing the general scientific and public community that endophytes represent not only specific applied problems and benefits, but also a model system for answering basic questions in plant, animal, and fungal biology.

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