Clavicipitaceous endophytes of grasses: their potential as biocontrol agents

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Many grasses harbour systemic clavicipitaceous endophytes in their leaves, stems, and seeds. Laboratory experiments and field observations have shown that infected plants are often toxic to livestock and more resistant to insect herbivores than uninfected conspecifics. Recently developed inoculation techniques allow artificial infection of grasses and the development of new varieties with high levels of endophyte infection. The fungi have applied potential as biocontrol agents against insect pests of grasses although their utilization may be limited to non-pasture situations. Exploitation of this naturally occurring symbiosis may obviate the need for chemical pesticides in managed grasslands.

Key words: Endophytes, Biological control, Clavicipitaceae, Gramineae.

There is a growing concern with the adverse environmental impact of chemical herbicides and insecticides. Pollution of soils, water and air are frequent, unwanted side effects of chemical pesticides whose synthesis often is based on petrochemicals that are expensive and non-renewable. Single treatments are rarely sufficient so repeated applications of the chemical are necessary with concomitant economic cost and environmental impact. Moreover, chemical herbicides and pesticides provide strong selective pressures favouring resistant mutants which then can increase and dominate the pest population. As a result, the potential use of biocontrol agents has received increased attention (Waage & Greathead, 1988).

Exploitation of naturally occurring enemies to control pest populations represents an attractive alternative to chemical-based pest control. In theory, biocontrol agents exhibit greater specificity to their target organisms while having little effect on other species and their physical environment. They can be self-replicating, unlike many chemical agents, so that continual applications of the agent are not necessary. The successful control of prickly pear cactus by the *Opuntia* moth and of skeleton weed (*Chondrilla juncea*) by the rust *Puccinia chondrillina* in Australia are two well-known examples that attest to the potential of biocontrol.

Fungi have been used to successfully control populations of weeds and insects but compared to viruses, bacteria, and insects, fungi have been under-utilized as biocontrol agents. In part this results from unique characteristics of fungi, particularly environmental requirements for spore germination and establishment on hosts, that restrict distributions to a subset of potential habitats (Cullen & Hasa, 1988; Payne, 1988). Fungal pathogens often regulate the population sizes of their hosts in nature although this regulation may not be apparent without disturbance to the system (Burdon & Shattock, 1980). Their utilization for biocontrol requires a greater understanding of fungal interactions with plants and insects in natural systems, including the number and kinds of fungi infecting the pest organism of interest, their effects on the host, and the genetic and environmental bases of the interaction. Additional research promises to reveal more fungi with potential as biocontrol agents.

The purpose of this paper is to consider the relationship between grasses and clavicipitaceous fungal endophytes and the potential of the fungi for increasing the resistance of grasses to insects. Two factors make endophytic fungi prime candidates for biocontrol agents. The recent development of inoculation techniques now allow the combination of different grass and fungal strains that did not previously occur (Latch & Christensen, 1985; Leuchtmann & Clay, 1988 b). In addition, the unique feature of maternal transmission of many of these fungi through the ovule and seeds of their hosts means that the inoculation be done only once and then the symbiotic association becomes self-replicating. Prior research on this relationship has revealed a great deal of variation in basic features of both organisms' life histories in different species combinations. This variation represents the results of natural selection acting on both partners for thousands of generations under many circumstances and provides the raw material to use for biocontrol.

CLAVICIPITACEOUS ENDOPHYTES OF GRASSES

The presence of endophytic fungi occurring within the tissues of several grasses has been known for some time but it has been only recently that their detrimental effects on insect and mammalian herbivores have been realized. Before considering this aspect of the relationship I will briefly review the biology of the fungi and their host relations.

Species of fungi in the family Clavicipitaceae, tribe Balansiae systemically infect host plants in three plant families (Poaceae, Cyperaceae, and Juncaceae) with the overwhelming majority of hosts being grasses. There are five genera and about 30 species in the tribe (Diehl, 1950; Luttrell & Bacon, 1977). The genera *Atkinsonella* and *Myriogenospora* are monospecific while the genera *Balansia*, *Balansiopsis* and *Epichloë* contain more than one species; *Balansia* is the most diverse genus with approximately 15 species (Diehl, 1950). These genera are distinguished primarily on the basis of conidial fructifications (Clay, 1986a). While the fungi are generally termed endophytes, several species occur as epiphytes on meristems and young leaves and inflorescences of their hosts (Leuchtmann & Clay, 1988a; Luttrell & Bacon, 1977). In contrast, most species occur vegetatively as convoluted intercellular hyphae running parallel to the long axis of host cells in leaf and stem tissue (Fig. 1).

In addition to those species for which the teleomorph has been observed, there are many anamorphs for which teleomorphs have not been correlated. These anamorphs, generally classified in the genus *Acremonium* sect. *Albo-lanosa*, appear to be closely related to the anamorph of *E. typhina* (White & Morgan-Jones, 1987). They are restricted to cool-season grasses, often to genera that also are hosts for *E. typhina*. They do not sporulate on their hosts or produce any visible signs of infection. In culture, many produce conidia similar to those produced by *E. typhina* (White & Cole, 1985). Others have not been cultured but resemble the *Acremonium* or Balansiae endophytes in terms of their host range and morphology within their hosts (White, 1987). The endophyte of darnel, *Lolium tementulum*, is an example of this latter category (Sampson, 1935; White & Cole, 1985).

Reproduction of the fungi is closely associated with their effects on the reproduction of their hosts. Hosts infected by Balansiae teleomorphs generally are sterile, either due to inhibition of flowering or abortion of developing inflorescences. Stromata bearing ascospores and conidia (with the exception of *Balansiopsis* which is not known to produce conidia) are produced on leaves or the aborted inflorescences of host plants (Fig. 2; Diehl, 1950). Diehl (1950), Western & Cavett (1959), Bacon *et al.* (1986) and others have suggested that spores infect new hosts through the stigmata of flowering plants but attempts at artificial inoculations have met with limited success (but see Latch & Christensen, 1985; Leuchtmann & Clay, 1988b). In contrast, hosts of the anamorphs flower normally and the fungi are transmitted maternally by vegetative growth of hyphae into ovules and seeds (Fig. 3). These fungi are not known to sporulate on their hosts although many produce conidia in culture.

A progression is observed from fungi like *E. typhina* which fruit on their hosts, producing conidia and ascospores, to species like *Acremonium lolii* which never fruit, are transmitted maternally through the ovule and seed, and sporulate in culture, to fungi like the one found in *Stipa robusta*, which resembles other *Acremonium* endophytes of grasses but has

Figs 1–3. Endophytic fungi of grasses. **Fig. 1.** Endophytic hyphae of *Acremonium coenophialum* in leaf sheath of tall fescue grass. **Fig. 2.** Stroma of *Epichloë typhina* infecting *Dactylis glomerata*. **Fig. 3.** Endophytic hyphae of *A. lolii* in aleurone layer of perennial ryegrass seed.
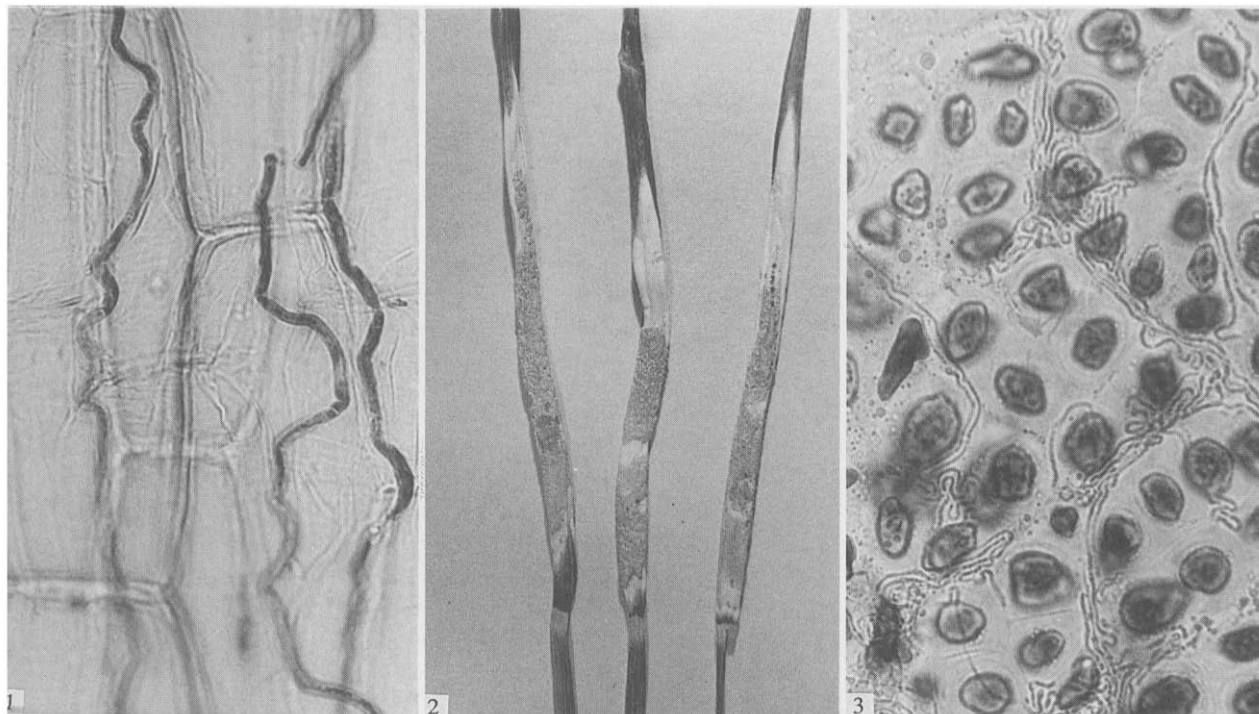
Table 1. Reported host genera for clavicipitaceous endophytes (by subfamily in the grasses). Numbers in parentheses refer to numbers of host species within the genus.   (A = *Atkinsonella hypoxylon*, B = *Balansia* spp., Bs = *Balansiopsis* spp., E = *Epichloë*, M = *Myriogenospora atramentosa*, e = non-sporulating endophyte)

Host	Fungus	Host	Fungus
CYPERACEAE		*Cenchrus* (2)	B
Cyperus (9)	B	*Cymbopogon* (2)	M
Scleria (1)	B	*Cynodon* (1)	E, B
		Cyrtococcum (1)	B
JUNCACEAE		*Eremochloa* (1)	M
Juncus (1)	E	*Hymenachne* (1)	B
		Ichnanthus (4)	B, Bs
POACEAE: POOIDEAE		*Lasiacis* (1)	Bs
Agropyron (7)	E	*Oplismenus* (2)	Bs
Agrostis (9)	E, e	*Panicum* (37)	B, Bs, M
Alopecurus (4)	E	*Paspalum* (6)	B, M
Anthoxanthum (1)	E	*Saccharum* (1)	M
Arrhenatherum (1)	E	*Sacciolepis* (1)	B
Asprella (1)	E	*Setaria* (6)	B
Brachyelytrum (1)	E	*Sorghum* (1)	B
Brachypodium (2)	E		
Bromus (3)	E, e	POACEAE: CHLORIDOIDEAE	
Bromopsis (2)	E	*Aristida* (15	B
Calamagrostis (6)	E	*Bouteloua* (2)	B, E
Cinna (2)	E, e	*Calamovilfa* (2)	E
Dactylis (1)	E	*Chasmanthium* (1)	B
Deschampsia (1)	E	*Chloris* (3)	B, Bs
Elymus (5)	E, e	*Ctenium* (1)	B
Elytrigia (3)	E	*Eragrostis* (4)	B
Festuca (24)	E, e	*Eragrostria* (1)	E
Glyceria (3)	E	*Gymnopogon* (1)	B
Hierochloe (1)	E	*Muhlenbergia* spp.	E
Holcus (2)	E	*Sporobolus* (2)	B, E
Hystrix (1)	E	*Thraysa* (1)	B
Koeleria (2)	E	*Tridens* (1)	B
Lolium (8)	e	*Uniola* (1)	Bs
Leymus (1)	E		
Melica (6)	E, e	POACEAE: BAMBUSOIDEAE	
Milium (1)	E	*Bambusa* (1)	Bs
Phalaroides (1)	E	*Chusquea* (1)	B, Bs
Phleum (1)	E	*Guadua* (1)	Bs
Poa (13)	E, e	*Olyra* (2)	B
Puccinellia (1)	E	*Orthoclada* (1)	Bs
Secale (1)	E	*Pariana* spp.	B
Sitanion (1)	e		
Sphenopholis (3)	E	POACEAE: ORYZOIDEAE	
Stipa (5)	A, e	*Leersia* (2)	B, E
Triticum (1)	E	*Luziola* (1)	B
		Oryza (2)	B
POACEAE: PANICOIDEAE			
Andropogon (6)	B, E, M	POACEAE: ARUNDINOIDEAE	
Axonopus (2)	B, M	*Danthonia* (2)	A, E

not sporulated in culture. This variation appears to represent an evolutionary trend where the fungi have reduced complexity in their life cycle and greater dependence upon their hosts for dissemination (Clay, 1988 *b*). Even within a single population of one host species there exist plants with symptoms of choke (*E. typhina*) while other plants appear healthy but are endophyte-infected and produce seeds containing the endophyte (Sampson, 1933). Because the strictly seed-borne endophytes do not produce symptoms on their hosts or suppress flowering, and because they are 'inherited' maternally from generation to generation, these

anamorphic fungi with affinities to *E. typhina* offer the greatest potential for exploitation as biocontrol agents.

Utilization of clavicipitaceous endophytes of grasses as biocontrol agents may be limited by their host range. As mentioned previously, species of the tribe Balansiae and their anamorphic derivatives infect only graminoids, especially grasses. However, within this large plant family it is clear that each fungal species is restricted to a subset of hosts and often to a single species or genus. One distinguishing feature concerns the photosynthetic pathway of their hosts. While *A. hypoxylon* and *E. typhina* primarily infect cool-season grasses

with the C-3 photosynthetic pathway, most of the other fungi in the tribe infect warm-season, C-4 grasses (Clay, 1986a). The non-sporulating endophytes are restricted to the same groups of grasses as *E. typhina*, further supporting the idea that these endophytes are derived from *E. typhina* (Clay, 1988b; Siegel, Latch & Johnson, 1987a).

An extensive literature review has revealed that over 80 genera and several hundred species of graminoids are known as hosts for clavicipitaceous endophytes (Table 1). Grass taxonomy follows Gould & Shaw (1983). This list is not exhaustive but was compiled from several sources with listings of hosts (primarily from Diehl (1950), Kohlmeyer & Kohlmeyer (1974), Pshedetskaya (1984), Sampson & Western (1954), Sprague (1950), USDA (1960), and White (1987)) as well as from personal observations. The host genera are listed here as reported in the original reference for clarity. In addition, the list is undoubtedly conservative. Most research has been conducted on a few economically important hosts in Europe and North America; extensive surveys of wild grasses have not been conducted, especially in the tropics. Moreover, hosts of seed-borne anamorphs probably are underestimated because of the lack of symptoms in the field. Despite the shortcomings in any attempt to determine completely the number of hosts on a worldwide basis, several patterns of host specificity are clear. Genera in the large subfamily Pooideae, which includes most grasses common in temperate northern climates, are infected only by *E. typhina* or symptomless, seed-borne endophytes with the exception of *Atkinsonella hypoxylon* infecting *Stipa leucotricha* (Diehl, 1950; Table 1). The subfamily Panicoideae consists of warm-season grasses with tropical affinities that frequently are infected by species of *Balansia, Balansiopsis,* or *Myriogenospora atramentosa*. The subfamily Chloridoideae also is most often infected by species of *Balansia* although *E. typhina* and *E. cineria* have been reported to infect several species.

Most major food, forage, and turf grasses occur as hosts, suggesting that relatively few grasses may be unsuitable candidates for breeding or inoculation for endophyte-enhanced resistance to insect pests. Restriction of the anamorphic endophytes to members of the Pooideae preclude their application to tropical grasses. The apparent absence of these fungi from warm-season grass hosts may be due to the lack of research conducted in tropical areas, (i.e. non-sporulating endophytes derived from *Balansia* may exist but have not been observed) but if not, it represents a fundamental evolutionary difference between members of the Balansiae infecting different subfamilies of grasses.

EFFECTS ON INSECTS AND OTHER PESTS

Considerable research has been conducted in the last 5 years on the role of endophyte infection on the interaction between host grasses and herbivores. This has been spurred by economic losses caused by poor livestock performance on pastures consisting of endophyte-infected perennial ryegrass (*Lolium perenne*) or tall fescue (*Festuca arundinacea*). These grasses are two of the most important forage grasses for livestock in temperate areas worldwide; for example, tall fescue is planted on over 10 million hectares in the southeastern

U.S.A. alone (Shelby & Dalrymple, 1987). Cattle grazing infected tall fescue exhibit toxic effects including reduced weight gain and milk production, increased respiration and body temperature, and gangrene of extremities (Bacon & Siegel, 1988; Hoveland *et al.*, 1983). Symptoms are most severe in hot summer months. Ryegrass staggers, a neurological disorder where afflicted animals exhibit severe muscular spasms, occurs in sheep and other domestic animals grazing on endophyte-infected *L. perenne* pastures (Mortimer & di Menna, 1983; Siegel *et al.*, 1987a). Other grasses with local reputations of being poisonous to livestock have proven to be endophyte-infected upon microscopic examination (Bailey, 1903; Hance, 1876; White, 1987).

Negative effects of clavicipitaceous endophytes on insects were first reported by Prestidge, Pottinger & Barker (1982), observing perennial ryegrass. We now know that many endophyte-infected grasses exhibit enhanced resistance to many insects (Clay, 1987a). Plant resistance to insects may arise from deterrent properties causing insect pests to avoid host plants or from antibiosis effects where insects feed on hosts but suffer from growth or developmental abnormalities, which ultimately reduce population growth.

Most research has focused on tall fescue and perennial ryegrass and has taken one of three major approaches: correlation of endophyte infection level in the field with diversity and abundance of insects present, laboratory feeding experiments where insects have no choice as to the type of plant they feed upon, and laboratory choice experiments where infected and uninfected plants are offered simultaneously and insect responses are observed. In field situations, Prestidge *et al.* (1982) first demonstrated a negative correlation between damage by Argentine stem weevils (*Listronotus bonariensis*) and the frequency of endophyte-infected perennial ryegrass in New Zealand pastures. In the United States Funk *et al.* (1983) found that perennial ryegrass plots with high levels of endophyte infection suffered less damage from sod webworms (*Crambus* spp.) and had fewer adults and eggs present than plots with lower levels of infection. Similarly, Ahmad *et al.* (1986) found increased resistance of infected perennial ryegrass to the bluegrass billbug (*Sphenophorus parvulus*) in field trials with different cultivars. In Missouri tall fescue pastures with varying levels of endophyte-infection, several insect species were less abundant in high endophyte pastures but two species were actually more common in infected pastures (Kirfman, Brandenburg & Garner, 1986). As yet there has been no research conducted on herbivory of endophyte-infected *vs.* uninfected wild grasses in natural plant communities.

No-choice laboratory feeding experiments consistently have demonstrated that many grasses, infected by at least 10 different clavicipitaceous endophytes, are more toxic and result in reduced insect survival, growth and developmental rate compared to uninfected conspecies (Table 2). For example, in three trials with infected and uninfected perennial ryegrass, survival to pupation of fall armyworm larvae (*Spodoptera frugiperda*), a generalist feeder on grasses and other graminoids, fed infected leaves was significantly reduced in two of three trials; in one trial using field-grown plants survival of larvae on infected plants was only half that of larvae on uninfected

Table 2. Insects reported to be negatively affected by endophyte-infected grasses

Species	Reference
Crickets (*Acheta domesticus*)	Ahmad *et al.*, 1985
Black Cutworms (*Agrotis segetum*)	Schmidt, 1986
Chinch Bugs (*Blissus leucopterus hirtus*)	Saha *et al.*, 1987
Sod Webworms (*Crambus* spp.)	Funk *et al.*, 1983
Black Beetles (*Heteronychus arator*)	Ahmad *et al.*, 1985
Argentine Stem Weevils (*Listronotus bonariensis*)	Barker *et al.*, 1984
Milkweed Bugs (*Onocopeltus fasciatus*)	Johnson *et al.*, 1985
Aphids (*Rhopalosiphum padi*)	Latch *et al.*, 1985
Aphids (*Schizaphis graminum*)	Latch *et al.*, 1985
Bluegrass Billbugs (*Sphenophorus parvulus*)	Ahmad *et al.*, 1986
Fall Armyworms (*Spodoptera frugiperda*)	Clay *et al.*, 1985 a
Southern Armyworms (*Spodoptera eridania*)	Ahmad *et al.*, 1987
Flour Beetles (*Tribolium castaneum*)	Cheplick & Clay, 1988
Corn Flea Beetles (*Chaetocnema pulicaria*)	Kirfman *et al.*, 1986
Sharpshooters (*Draeculacephala antica*)	Kirfman *et al.*, 1986

Table 3. Host grasses or sedges reported to exhibit toxicity or increased resistance to herbivores. Endophyte refers to fungi that do not sporulate on their hosts, resembling the endophytes in tall fescue and perennial ryegrass

Host Species	Fungus	Reference
Andropogon spp.	*Balansia* spp.	Nobindro, 1934
Cenchrus echinatus	*Balansia obtecta*	Clay *et al.*, 1985 a
Cyperus virens	*Balansia cyperi*	Clay *et al.*, 1986 a
Cyperus pseudovegetus	*Balansia cyperi*	Clay *et al.*, 1986 a
Cyperus rotundus	*Balansia cyperi*	Stovall, unpubl.
Dactylis glomerata	*Epichloë typhina*	Schmidt, 1986
Danthonia spicata	*Atkinsonella hypoxylon*	Cheplick & Clay, 1988
Festuca arundinacea	*Acremonium coenophialum*	Hardy *et al.*, 1986
Festuca longifolia	endophyte	Saha *et al.*, 1987
Festuca obtusa	endophyte	Cheplick & Clay, 1988
Festuca rubra	*Epichloë typhina*	Cheplick & Clay, 1988
Festuca versuta	endophyte	Cheplick & Clay, 1988
Glyceria striata	*Epichloë typhina*	Cheplick & Clay, 1988
Lolium perenne	*Acremonium lolii*	Prestidge *et al.*, 1982
Lolium tementulum	endophyte	Steyn, 1933
Melica decumbens	endophyte	Shaw, 1873
Panicum agrostoides	*Balansia henningsiana*	Cheplick & Clay, 1988
Paspalum dilatatum	*Myriogenospora atramentosa*	Clay *et al.*, 1985 a
Paspalum notatum	*Myriogenospora atramentosa*	Cheplick & Clay, 1988
Stipa leucotricha	*Atkinsonella hypoxylon*	Clay *et al.*, 1985 a
Stipa robusta	endophyte	Marsh & Clawson, 1929
Stipa mongol	endophyte	Hance, 1876
Tridens flavus	*Balansia epichloë*	Cheplick & Clay, 1988

plants (Clay, Hardy & Hammond, 1985 a; Hardy, Clay & Hammond, 1985). Larval weights were significantly lower and time to pupation was significantly increased for larvae fed infected leaves in all trials. A variety of insects is negatively affected by endophyte-infected grasses (Table 3). Ahmad *et al.* (1987) found similar patterns of antibiosis affecting the southern armyworm, *S. eridiana*, only the effects were even more dramatic than we observed for fall armyworm. In several studies insects suffered complete mortality on endophyte-infected hosts. For example, crickets (*Acheta domesticus*) fed infected ryegrass all died within 84 h while those fed uninfected plants exhibited only 20–30% mortality over the same period (Ahmad *et al.*, 1986). Johnson *et al.* (1985) found that two species of aphids were unable to survive when confined to endophyte-infected tall fescue.

Results from other grasses, insects, and endophytic fungi are consistent with those from perennial ryegrass and tall fescue reported above (Tables 2, 3). Fall armyworm larvae perform poorly on species of *Festuca* infected by various endophytes including *E. typhina*, *Acremonium coenophialum*, and two non-sporulating endophytes (Cheplick & Clay, 1988; Clay *et al.*, 1985 a). The grass *Dactylis glomerata* infected by *E. typhina* was avoided by black cutworm larvae (*Agrotis segetum*) and resulted in reduced survival and growth in no-choice

experiments (Schmidt, 1986). Clay *et al.* (1985*a*) and Cheplick & Clay (1988) looked at several wild grasses infected by species of *Balansia* and *Myriogenospora atramentosa* and in all cases there were significant negative effects on fall armyworm larvae when compared to larvae provided with uninfected conspecifics. The antibiosis affects are not limited to grasses; two species of *Cyperus* infected by *B. cyperi* had significant antibiosis effects on fall armyworm larvae (Clay, Hardy & Hammond, 1985*b*).

In laboratory or greenhouse choice experiments many insects actively discriminated between endophyte-infected and uninfected perennial ryegrass and tall fescue (Table 2). When given a choice, neonate and fourth-instar larvae of fall armyworm significantly preferred leaves from uninfected perennial ryegrass over leaves from infected plants (Hardy *et al.*, 1985). Argentine stem weevils significantly preferred uninfected perennial ryegrass in laboratory choice tests (Barker *et al.*, 1984). Latch, Christensen & Gaynor (1985*a*) demonstrated that aphids preferentially fed on endophyte-free tall fescue in a ratio of 20:1 over infected tall fescue in the greenhouse. Johnson *et al.* (1985) and Siegal *et al.* (1985) found similar insect responses on infected and uninfected tall fescue laboratory experiments. The patterns of insect distribution in field situations may also represent some degree of discrimination between infected and uninfected plants although antibiosis effects also may be present.

For those endophytes transmitted maternally through the seed of their hosts, there is evidence that seeds may gain protection from herbivores as well. Rats fed infected tall fescue seed exhibited a number of significant physiological abnormalities compared to rats fed uninfected seed (Neal & Schmidt, 1985). Considering insects, Cheplick & Clay (1988) found that survival and population growth rates of flour beetles (*Tribolium castaneum*) on ground seeds of infected perennial ryegrass and tall fescue was significantly lower than on uninfected seed; on infected tall fescue seed beetle populations rapidly went extinct.

Although less than 20 species of endophyte-infected grasses have been critically examined in controlled experiments, there is no evidence to suggest that the results from those studies are not applicable to a wider range of grasses infected by other endophytes, which are fed upon by other herbivores. Certainly, a larger number of infected grasses have been shown to be toxic to livestock (see Clay, 1988*a*). Thus, the utilization of clavicipitaceous endophytes as biocontrol agents for insect pests of grasses has widespread potential and is not restricted to a small number of species. Even if it were, the few grasses about which most is known are some of the most important commercial grasses on a worldwide basis.

There are some exceptions to the general anti-herbivore properties of endophyte-infected grasses; should these exceptions prove more widespread they may limit the exploitation of endophytes as biocontrol agents. For example, problems with livestock on endophyte-infected pastures are often seasonal or climate-dependent. Most severe effects of tall fescue and perennial ryegrass are limited to hot summer months while in the rest of the year animal performance is satisfactory. Neill (1941) and Cunningham (1958) attempted to demonstrate toxic effects of endophyte-infected ryegrass

but without success. In the U. K. toxicity of perennial ryegrass pastures is not severe, if present; moreover, Lewis & Clements (1986) found no evidence for enhanced resistance to fruit fly larvae (*Oscinella* spp.). Latch *et al.* (1985*a*) found that the aphid *Rhopalosiphum padi* was deterred by endophyte-infected tall fescue but not by infected ryegrass. At least one insect is known to be a specialist feeder only on stromata of *E. typhina* (Kohlmeyer & Kohlmeyer, 1974). Other environmental factors may also influence the degree of resistance of endophyte-infected plants. Resistance to fall armyworm larvae varied with the age of foliage of infected tall fescue (Hardy, Clay & Hammond, 1986). Similarly, feeding trials utilizing both field and greenhouse grown plants generally resulted in a greater differential between infected and uninfected plants from the field than plants from the greenhouse (Cheplick & Clay, 1988; Clay *et al.*, 1985*a*). The basis for these differences is not clear. Lyons, Plattner & Bacon (1986) found that the level of alkaloids present in endophyte-infected tall fescue varied with nitrogen application. Further research is required to define precisely the interacting factors that influence the resistance of endophyte-infected plants to herbivores.

CHEMICAL BASIS OF INSECT RESISTANCE

Clavicipitaceous endophytes of grasses produce a number of biologically active compounds both *in vivo* and *in vitro* that appear to be responsible for their effects on herbivorous animals. Other mechanisms by which fungal symbionts of plants can influence herbivory of hosts are known (see Clay, 1987*a*) but most evidence suggests that resistance results primarily from fungal secondary compounds. Species in the tribe Balansiae are known to produce ergot alkaloids, as is seed-borne endophyte *Acremonium coenophialum* from tall fescue (Bacon *et al.*, 1986; Lyons *et al.*, 1986). Ergot alkaloids are characterized by a N-substituted tetracyclic ring structure (Fig. 4). Their physiological activities against animals are well-known from previous research on ergot-poisoning by *Claviceps* species (Bove, 1970; Mantle, 1969). Symptoms of poisoning from infected tall fescue and other grasses resemble the symptoms of ergot poisoning, supporting the idea that they have the same chemical basis (Bacon *et al.*, 1986). Feeding trials have shown that these alkaloids can be extremely toxic to insects (Clay & Cheplick, 1988; Johnson *et al.*, 1985). The endophyte of ryegrass, while not known to produce ergot alkaloids, does produce peramine and lolitrems, which are

Fig. 4. Molecular structure of ergonovine, a typical ergot alkaloid produced by clavicipitaceous endophytes.
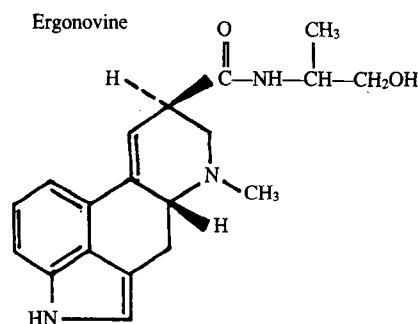
alkaloids with known antiherbivore properties (Rowan & Gaynor, 1986; Rowan, Hunt & Gaynor, 1986). Loline alkaloids also are found in endophyte-infected tall fescue and annual ryegrass (*Lolium tementulum*) (Jones *et al.*, 1983; Siegel *et al.*, 1987a).

The identities and concentrations of ergot alkaloids and other alkaloids in endophyte-infected plants is variable both within and between infected species (Bacon, Porter & Robbins, 1979, 1981; Porter *et al.*, 1981). Laboratory studies with pure fungal cultures suggest that there are genetic differences among strains within a species and among species with respect to their abilities to produce different alkaloids (Bacon *et al.*, 1981). Growth conditions within the plant and in pure culture also influence the type and quantities of alkaloids produced (Hardy *et al.*, 1986; Lyons *et al.*, 1986; Siegel *et al.* 1987a). Laboratory studies show that different ergot alkaloids are not equally toxic (Clay & Cheplick, 1988) and that different insects may respond more strongly to some alkaloids and not to others (Johnson *et al.*, 1985).

OTHER EFFECTS OF ENDOPHYTE INFECTION

In addition to the well-documented examples of insect resistance and livestock toxicity, endophyte-infected grasses may possess other properties that also are of applied value. West *et al.* (1987) demonstrated that nematode counts were significantly higher in plots of endophyte-free tall fescue than in plots of infected tall fescue. Resistance to nematodes may be present in a wider variety of endophyte-infected grasses. Infected grasses might also be more resistant to fungal pathogens. Culture studies by White & Cole (1986) have demonstrated that there is antagonism between endophytic fungi and common grass pathogens. Plants of endophyte-infected perennial ryegrass were observed to have fewer rust pustules (*Puccinia coronata*) per unit leaf area than uninfected plants growing in a common garden situation (Clay, unpubl.). There are many examples of plants infected by one fungus being more resistant to other pathogens (Clay, 1987a) so it should not be surprising to find a similar situation in endophyte-infected grasses.

Other changes in host morphology and physiology may also have significant applied potential. Several researchers have suggested that endophyte-infected tall fescue plants are more drought-tolerant than uninfected plants (Belesky *et al.*, 1987; Siegel *et al.*, 1987a; West *et al.*, 1987). Infected plants are able to persist and recover more rapidly than uninfected plants following severe water stress, providing a considerable advantage in environmentally stressful habitats. Two studies of photosynthetic rate in tall fescue report conflicting results; Belesky *et al.* (1987) report lower rates by infected plants but Bush (cited in Siegel *et al.*, 1987a) report the opposite.

Grasses infected by clavicipitaceous endophytes with an external sexual stage often exhibit morphological changes that can be advantageous in turf situations. Typically flowering is either inhibited completely by hormonal alterations (Porter *et al.*, 1985), or developing inflorescences are mechanically aborted by the development of fungal stromata on the culms

(Clay & Jones, 1984). As a result, infected plants can be smaller in stature with a more compact growth form (Diehl, 1950). Bradshaw (1959) described two *Agrostis* species infected by *E. typhina* that had a more prostrate, spreading morphology compared to uninfected plants. Clay (1984) found that plants of *Danthonia spicata* infected by *Atkinsonella hypoxylon* tillered more densely and produced fewer inflorescences, which were aborted, than uninfected plants. Many other grasses spread more vigorously when infected (Clay, pers. obs.). Such changes could reduce turf maintenance and increase competitiveness with weeds.

Endophyte-induced changes in morphology and physiology exhibited by infected grasses may, in total, contribute significantly to their vigor and persistence quite independently of their resistance to insects. Increased growth of infected plants in controlled environments lacking insects clearly results from a different mechanism (Clay, 1987b; Latch, Hunt & Musgrave, 1985b). The larger size of infected plants in nature (Bradshaw, 1959; Clay, 1984; Diehl, 1950; Harberd, 1961) and their higher survival and growth in field experiments (Clay, 1984; Read & Camp, 1986) are probably due to a combination of insect resistance and these other factors. Experiments controlling the level of herbivory are necessary to separate the consequences of endophyte-induced insect resistance from their effects on other plant properties.

DEVELOPMENT OF CLAVICIPITACEOUS ENDOPHYTES AS BIOCONTROL AGENTS

An efficient biocontrol agent should result in significant reduction in pest damage either by directly killing or damaging the pest, reducing its population growth, or by deterring the pest before it can do any damage. It also should have a narrow range of activity so that no damage to other organisms or the physical environmental results. Finally, the economic costs of applying and maintaining effective levels of a biocontrol agent should be competitive with more traditional chemical-based pest control.

Clavicipitaceous endophytes of grasses exhibit many characteristics of a good biocontrol agent. A wide variety of insects on many grasses is deterred, or suffers greater mortality and reduced population growth, from feeding on endophyte-infected grasses (Tables 2, 3). The fungus has no direct effect on any other organisms except its own host and organisms that feed upon or infect the host. The high degree of specificity exhibited by most of the fungi for particular grass hosts make it unlikely that they would spread to other hosts. The Balansiae anamorphs, which do not produce stromata and are seed-borne from generation to generation, have no mechanism for contagious spread and it is these endophytes that offer the greatest potential for biocontrol. Once host plants are established the endophytes are 'inherited' precluding the necessity of repeated applications. The endophytes that produce stromata on, and sterilize, their hosts also have potential as biocontrol agents in situations where long-lived, vegetatively spreading grasses dominate.

There are several ways that additional endophyte-infected grass strains with increased resistance to insect pests can be

produced for commercial markets. The simplest way is to select material exhibiting desirable qualities that is already endophyte-infected from natural grasslands or long-established turfs. Infected plants can be clonally propagated in test fields and seed collected for distribution. A number of grass cultivars already exist with high levels of endophyte-infection. Some were selected originally because their hosts showed good forage or turf properties, with no *a priori* knowledge of their endophyte status (Funk *et al.*, 1983; Siegel *et al.*, 1987a). For example, infected tall fescue in eastern North America was propagated and dispersed without knowledge of the endophytic fungus; today infected plants derived from one farm in Kentucky cover over 10 million hectares (Shelby & Dalrymple, 1987). Other endophyte-infected turf grasses also resulted from similar inadvertant selections (Funk *et al.*, 1983). Latch, Potter & Tyler (1987) sampled old turfs from throughout central Europe and found a very high percentage of endophyte-infected plants; similarly, in the U.S.A. infected tall fescue accounts for over 90% of the plants in many states (Shelby & Dalrymple, 1987). Now that endophyte infection is recognized as an important source of insect resistance, new cultivars are being developed; at least eleven cultivars of *Lolium perenne* and three species of *Festuca* have been purposely selected to contain high levels of endophyte-infection (Bacon & Siegel, 1988).

A second method for developing endophyte-infected grass varieties exploits the fact that the fungus is maternally inherited through the seed. Most morphological and physiological characters presumably are coded for by nuclear genes and so can be transferred between maternal backgrounds by a series of repeated backcrosses where endophyte-infected plants are crossed with fungus-free plants with good turf qualities. The maternal offspring of the infected plants are then backcrossed with the endophyte-free plants for several generations. One can rapidly move turf-quality determining genes into endophyte-infected plants; over 90% of nuclear genes will be transferred into endophyte-infected plants in just five backcrosses. In self-incompatible grasses like perennial ryegrass and tall fescue, where the endophyte does not sterilize its host, crossing programmes are relatively easy. The time required for repeated backcrosses and the necessity that both parental stocks be crossed are the drawbacks of this approach.

The third method is the most direct and potentially the one with the greatest economic significance. It involves the introduction of endophytic fungi into uninfected grasses by artifical inoculations. While this would appear to be the easiest and most straightforward mechanism for generating endophyte-infected grasses, prior attempts to do so have largely failed (Diehl, 1950; Rykard, Bacon & Luttrell, 1985; Sampson, 1933, but see Mühle & Frauenstein, 1970; Western & Cavett, 1959). Only recently Latch & Christensen (1985) reported the successful inoculation of tall fescue seedlings with several endophytes. Subsequently, Siegel *et al.* (1987b) have reciprocally inoculated perennial ryegrass and tall fescue with *Acremonium* endophytes and *E. typhina*. Leuchtmann & Clay (1988b) also have infected seedlings of several grasses and sedges with strains of *Aktinsonella hypoxylon* and *Balansia cyperi*. Seedlings were grown in aseptic culture and inoculated

by placing mycelium and/or conidia from pure fungal cultures into a small incision just above the meristematic region of the seedling. The rate of successful infections ranged from only a few percent to 100% (Leuchtmann & Clay, 1988b).

In experiments conducted thus far it has been possible to transfer clavicipitaceous endophytes from one host species into another although certain host/fungus combinations could not be synthesized. For example, Latch & Christensen (1985) could not infect perennial ryegrass with the tall fescue endophyte; similarly, strains of *A. hypoxylon* from *Stipa leucotricha* could not infect seedlings of its other host, *Danthonia spicata*, and *vice versa* (Leuchtmann & Clay, unpub.). Moreover, there appears to be a small window in time when seedlings can be infected artificially; mature plants have not been successfully inoculated (Latch & Christensen, 1985). Where successful cross-inoculations were made, increases in insect resistance and plant vigor resulted. For example, Johnson, Bush & Siegel (1986) found that inoculated plants of tall fescue were resistant to aphids and Leuchtmann & Clay (1988b) observed that growth of seedlings of *Danthonia spicata* increased significantly following infection by *A. hypoxylon* compared with control seedlings. As yet, however, only a few host grasses and endophytic fungi have been investigated. Further studies are required before we fully understand the factors limiting compatibility between different host/fungus combinations.

Another method of artificial inoculation involves tissue culture of host plants. Johnson *et al.* (1986) inoculated callus cultures of uninfected tall fescue with mycelium from its natural endophyte *A. coenophialum* and were able to successfully regenerate infected plants. However, they could not regenerate tall fescue plants infected with other endophytes, nor were they able to infect plants of Kentucky bluegrass (*Poa pratensis*) or timothy (*Phleum pratense*) using this technique. Tissue culture techniques have several advantages over backcrossing or seedling inoculations for producing endophyte-infected grasses in that infected plants can be produced more quickly and in greater numbers than with other techniques.

Inoculations with endophytes offer the possibility of introducing desirable traits, including resistance to pests, into many grasses. In addition, endophytes could also be used as vectors for introducing new genes into grasses. Transformation of grasses has proven difficult but genetic engineering of the endophytes themselves may offer fewer problems. The ability to inoculate seedlings combined with the subsequent maternal transmission of the fungus through its host's seed insure that modified fungi will be faithfully maintained and replicated in host populations. Endophyte strains from alien hosts may be introduced or, in contrast, strains modified in the laboratory may be introduced. For example, fungal strains could be mutagenized and screened for non-alkaloid-producing mutants or mutants producing only a subset of the original alkaloids. Theoretically, a strain that was toxic to insects but innocuous to livestock might be produced. Also possible is the direct modification of fungal strains by introducing alien genetic material via transformation vectors. The expression of desirable traits in endophyte culture would need to be confirmed in field trials with the host plants of interest.

POTENTIAL PROBLEMS

As with any biocontrol agent there are potential problems with the utilization of endophytes for pest control. Most notable is the toxic nature of infected grasses. The same endophyte-infected species that are more resistant to insect herbivores can be toxic to mammalian herbivores (Clay, 1988a). In pasture situations endophyte-infected grasses should be used with caution, if at all. However, in pastures with insect problems, as in perennial ryegrass pastures in New Zealand infested with Argentine stem weevils, the benefit of insect resistance may outweigh the economic losses accruing from livestock toxicity (see Prestidge et al., 1982). This problem could be solved if the chemical basis of insect resistance was different from the basis of livestock toxicity by the development of modified endophyte strains. In turf, conservation, or reclamation grasses, livestock toxicity is not a problem. Therefore, the development of endophyte-infected grasses for non-pasture situations offers the greatest potential for biocontrol of insect pests.

A second potential problem is that the aggressiveness of grasses could be increased by endophyte infection so that they become pests themselves. In the southeastern United States kudzu (*Pueraria lobata*) was introduced to control soil erosion but now has become a serious problem in its own right. Many weed grasses are already endophyte-infected and this may contribute to their aggressiveness. Purple nutsedge (*Cyperus rotundus*, Cyperaceae) is considered to be the world's worst weed (Holm et al., 1977). It produces an extensive network of subterranean tubers, bulbs and rhizomes that are resistant to herbicide applications (Clay, 1986b). It was introduced to North America where the fungus *Balansia cyperi* apparently has colonized purple nutsedge from native, sympatric species of *Cyperus* (Clay, 1986b). Greenhouse experiments have demonstrated that infected plants grow more rapidly, producing more biomass and vegetative propagules than uninfected plants (Stovall & Clay, 1988). Thus, in this case, endophyte infection contributes significantly to the species' weediness.

The contagious spread of clavicipitaceous endophytes from one species, e.g. a turf grass, to another species, e.g. a pasture or food grass, could result in significant problems with livestock or human toxicities. This would not be a problem for non-sporulating, seed-borne endophytes like those in tall fescue and perennial ryegrass. Spread to alternate hosts represents a major problem with any biocontrol agent (Waage & Greathead, 1988). As previously described, there are examples of endophytes spreading from one host to another. The fact that many important food grasses, i.e. wheat, rice, sugarcane, millet, occasionally serve as hosts for species of Balansiae demonstrates the importance of this problem (Table 1). However, several factors minimize the potential for spread to new hosts. Inoculation experiments have demonstrated that there is some genetic basis to host range so extensions of host ranges will probably be limited to closely related species (Leuchtmann & Clay, 1988b). In addition, the absence of spore production in many endophytes should preclude contagious spread. For endophytes producing stromata and dispersing spores, host plants are typically sterilized. In annual crops planted from seed produced in managed seed orchards, contamination with endophyte-infected seeds would be eliminated each year. Finally, there is no history of endophytes representing serious agricultural problems in most areas although wild, endophyte-infected grasses often occur in close proximity to crops. The infection of rice by species of *Balansia* in Africa and Asia is a notable exception. Up to 10% of all spikelets of rice grown in Sierra Leone were destroyed by infection (Fomba, 1984); local wild grasses also served as hosts and a continual source of inoculum.

Another potential problem results from the deterrent properties of endophyte-infected grasses towards many insects (Clay, 1988a). Introduction of new endophyte-infected grass cultivars could result in the shift of insect pests from one grass species to another species not protected by endophytes; i.e. pest problems are not solved but merely shifted elsewhere. In the eastern United States turf often consists of a mixture of *Festuca*, *Lolium* and *Poa* species. While endophyte-infected varieties of the first two genera are readily available, *Poa pratensis*, the dominant turf *Poa*, is not known to harbor endophytic fungi. Increased utilization of infected *Festuca* and *Lolium* could cause problems for other grass species by shifting the insect's preferences to other food plants. In greenhouse experiments, fall armyworm larvae were released in pots containing mixtures of *P. pratensis* and *L. perenne*. When the ryegrass was endophyte-free, ryegrass sustained virtually all of the insect damage but if ryegrass was endophyte-infected then increased damage to *Poa* resulted (Clay, unpubl.).

There are other economic considerations in establishing endophyte-infected stands of grass. In addition to the efforts devoted to producing endophyte-infected seed stocks, there are storage requirements not necessary for ordinary grass seed. Welty, Azevedo & Cooper (1987) have demonstrated that endophyte viability in tall fescue and perennial ryegrass seeds declines more rapidly than seed viability itself. Under conditions of ambient temperature and humidity, the endophyte in most infected seed will be inviable after a year. Storage at low humidity and temperature can greatly increase the effective life of endophyte-infected seeds, although increasing the potential costs to the producer. Establishing endophyte-infected turfs also represents a potential cost. The removal or destruction of existing turf and replacement with infected material can be expensive. For grasses sterilized by infection, establishment from seed is impossible so that sprigs or plugs must be planted. However, these costs may be less than it would first appear because of the competitiveness of infected plants. Bradshaw (1959), Kelley & Clay (1987) and others have documented the proportional increase of infected plants over time in mixed stands with uninfected plants. Thus, complete replacement is not necessary; introduction of some small proportion of infected plants may be sufficient to produce a highly infected stand within several years.

CONCLUSIONS

Recent research on the relationship between clavicipitaceous endophytes and their host grasses has indicated that infected plants exhibit increased resistance to insect pests compared to uninfected conspecifics. Endophyte-infected grass varieties

can be developed by several techniques to take advantage of this property. The use of infected grasses as biocontrol agents of insect pests has great potential with few of the problems traditionally associated with biocontrol or with chemical insecticides. Several infected grass varieties have already been developed and more will undoubtedly appear soon.

The success of this approach will depend on the results of research in three areas. Large-scale surveys of native grasses are needed to document the extent and frequency of endophyte infection in nature. Isolation and culture of these endophytes will provide an array of fungal strains, each with their own unique traits, to be characterized, modified and used for inoculations of grasses. Investigations devoted to perfecting inoculation techniques and elucidating the factors determining host range will define the extent to which endophytes can be introduced into diverse grasses. Finally, genetic and biochemical research on the endophytes themselves will provide the opportunity for genetic transformation of endophyte strains that can then be used to produce grass strains with unique combinations of characters.

The evolution of the relationship between grasses and clavicipitaceous endophytes has been characterized by the development of mutualistic interactions from pathogenic ones with concomitant changes in the reproductive systems of both partners (Clay, 1988 b). However, the anti-insect properties of infected grasses appear to occur regardless of the nature of the relationship. The derived, mutualistic associations appear to offer the greatest potential for biocontrol since the fungi are integrated into the host's reproductive system and are passed down through the seed. Unlike other biocontrol agents, which are based on antagonistic interactions, the use of endophytes as biocontrol agents is based on a naturally occurring mutualistic relationship. However, in common with other biocontrol agents, utilization of endophytes exploits naturally occurring ecological associations that have been shaped by natural selection acting for thousands of generations.

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