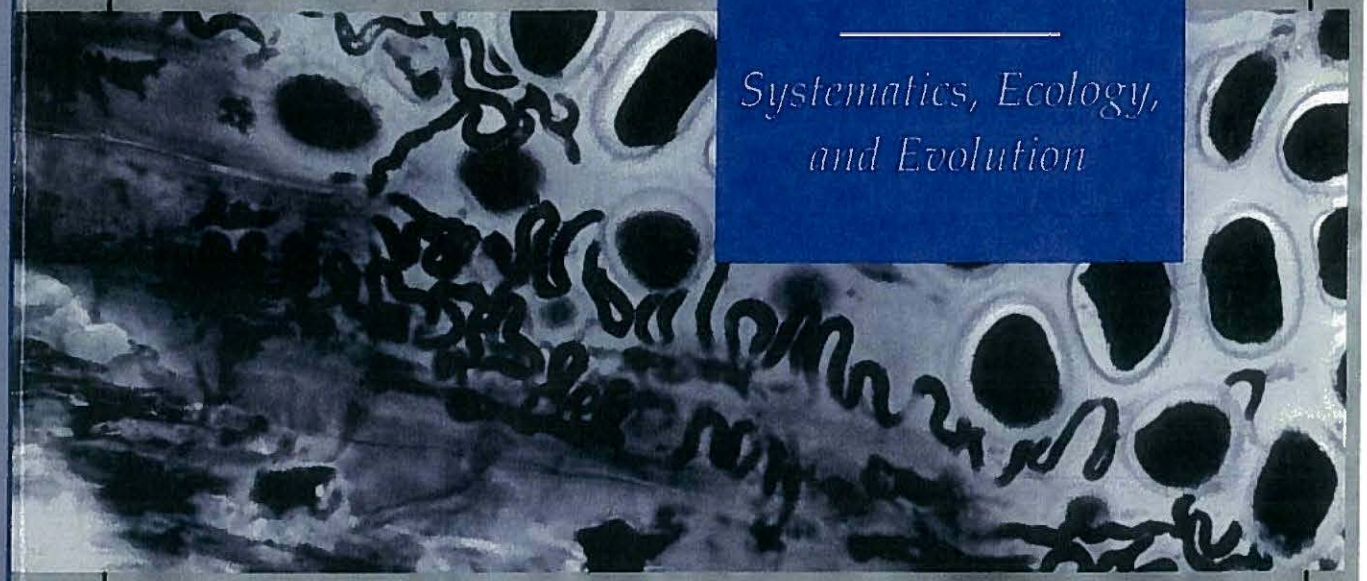
Endophytic Fungi in Grasses and Woody Plants

Systematics, Ecology, and Evolution



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CHAPTER 9

RESOURCES AND TESTING OF ENDOPHYTE-INFECTED GERMPLASM IN NATIONAL GRASS REPOSITORY COLLECTIONS

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Clavicipitaceous endophytes have been known to exist in grasses since the discovery of an endophyte in seeds of darnel (*Lolium temulentum* L.) by Vogl in 1898 (26). The oldest known specimens of darnel with endophytic mycelium were seeds retrieved from a pharoah's tomb in an Egyptian pyramid dating back to 3400 B.C. (16). Subsequent work by numerous investigators has shown that these fungi have hosts that are widely distributed in the grass family (Poaceae) and sporadically distributed in the sedge family (Cyperaceae) and rush family (Juncaceae) (3, 27). Most surveys for clavicipitaceous endophytes have concentrated on wild grasses and cultivated turfgrass and forage grass collections from Europe and North America (15, 20, 27). The extensive national collections of wild and cultivated grasses in United States repository collections have been largely ignored. The intent of this chapter is to increase general awareness of this large and valuable resource available to anyone doing research on grasses including endophyte researchers. Furthermore, this report summarizes initial grass endophyte research conducted by the author and colleagues since early 1989 on grass germplasm from these national repository collections.

Clavicipitaceous endophytes of grasses may be categorized into two groups: 1) the choke-inducing sexual forms or clavicipitaceous teleomorphic endophytes, and 2) the mutualistic asexual forms or clavicipitaceous anamorphic endophytes (29). Teleomorphic endophytes are ascomycetes (Clavicipitaceae: tribe Balansieae) that produce a sexual stage (teleomorph) on stromata and induce choke diseases which may disrupt reproduction of their hosts (8). Anamorphic endophytes are probably asexual derivatives of teleomorphic endophytes, but they lack a sexual stage, rarely sporulate in

their hosts, and form mutualistic associations with their hosts. They are imperfect fungi (Deuteromycetes) classified in the genus *Acremonium* sect. *Albo-lanosa* and in related anamorphic genera (e.g. *Gliocladium* and *Phialophora*) (13, 17). Anamorphic endophytes infect primarily cool-season grasses of the tribe Pooideae (4). The endophytic fungi considered in this paper are primarily the anamorphic forms.

A number of general characteristics *in vivo* distinguish clavicipitaceous anamorphic endophytes, particularly the *Acremonium* sp., from other endophytic fungi of grasses. Morphologically, anamorphic endophytes tend to have relatively fine hyphae (≤4 μm diameter) that are often convoluted, highly vacuolated, infrequently branched, occasionally constricted at the septa, and have septa that stain poorly or not at all. The anamorphic forms are mutualistic, obligate endosymbionts (biotrophs) producing symptomless infections of their hosts, often attributed to the absence of teleomorphic states. Most species grow entirely in the vegetative (somatic) phase throughout their life cycles *in vivo*, although some species produce conidia in their hosts (18). However, most endophytes can be cultured and induced to sporulate on common growth media. The majority of anamorphic species are slow growing *in vitro* and are characterized by their white to tan, appressed and waxy to cottony colonies that often become wrinkled, puckering the agar in older cultures. Conidiophores arise singly, or form on synnemata or branched conidiophores. The fungi are seed borne and most commonly associated with the aleurone layer and adjacent tissues of grass seeds. They grow intercellularly and systemically through grass tillers mostly parallel to the longitudinal axis of the vascular system. These fungi overwinter as dormant inoculum in the meristematic region of their host. In addition, they produce ergot and related alkaloids throughout their hosts that probably contribute most to their biological activity. Finally, some species are also known to produce the growth-promoting phytohormone indoleacetic acid (auxin) in culture (7).

The infection of grasses by clavicipitaceous endophytes may have both beneficial and deleterious consequences with considerable economic significance. Endophytes can have profound effects on host physiology, reproductive biology, selection, ecology, growth, and pest resistance. The enhanced resistance in endophyte-infected grasses to many insects and diseases are most noted (2), but these fungi also have been implicated in increasing growth and vigor, water use efficiency, tolerance to heat and drought stress, competitive ability, photosynthetic efficiency and long-term survival of their hosts (23). Endophytes have been viewed as fortuitous sources of resistance in many grasses that lack genetic pest resistance. The turfgrass industries in the U.S. and abroad have taken advantage of these traits by releasing certified endophyte-infected cultivars with resistance to pests and environmental stresses. The selection advantages afforded by endophyte infection may significantly impact the ecology of graminaceous hosts through reduction of herbivory resulting from increased resistance to

insects and ungulate, mammalian herbivores. However, the toxic effects of endophyte-infected grasses on livestock have caused large economic losses to the livestock industry. Toxicoses of livestock caused by the consumption of endophyte-infected grasses have resulted in annual losses of hundreds of millions of US dollars in lost production for both the United States and New Zealand (23).

National Plant Resources for Endophyte Research

The U.S. National Plant Germplasm System (NPGS), jointly administered by the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) and the State Agricultural Experiment Stations (SAES), maintains the largest collections of herbaceous plants in the United States. Plant materials held in NPGS repositories represent a wide diversity of ecological habitats and plant genotypes from many countries throughout the world. The NPGS presently consists of 27 repository stations strategically located throughout the United States. Eleven plant repository stations contain grass collections rich in both genetic and endophyte-based resistance to agronomic pests. The 11 repository sites holding grasses include 4 regional plant introduction stations, 3 specialized or crop-specific collections, 2 national clonal repositories, and an importation quarantine office (Fig. 1). Most germplasm held in national repository collections is available to all researchers interested in testing this material for pest resistance or agronomic traits that may be useful for eventual introductions in crop improvement applications.

The eleven national grass repository stations collectively maintain at least 235 grass genera representing over 1,600 species and more than 207,700 plant inventory (PI) accessions (Table 1). The major national cereal grass collections are located at only six repository sites (Table 2). The National Small Grains Collection (NSGC) in Aberdeen, Idaho holds the majority of the cereal grass collections including the wheat, barley, oats, rice, rye, and triticale collections. Some collections are divided between two or more sites for security. The NC-7, NSSL, and S-9 stations hold the major corn, sorghum, and millet collections. The CR-MIA station contains the sugarcane collection. Major national collections of wild grasses are less widely distributed at only two repository sites (Table 3) or at single repository sites (Table 4). Most national wild forage and turfgrass collections are located at the W-6 or Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington and the National Seed Storage Laboratory (NSSL) in Fort Collins, Colorado. Other notable major wild grass collections with an abundance of PI accessions are available at the CR-MIA, NC-7, NE-9, NSGC, and S-9 stations (Table 4).

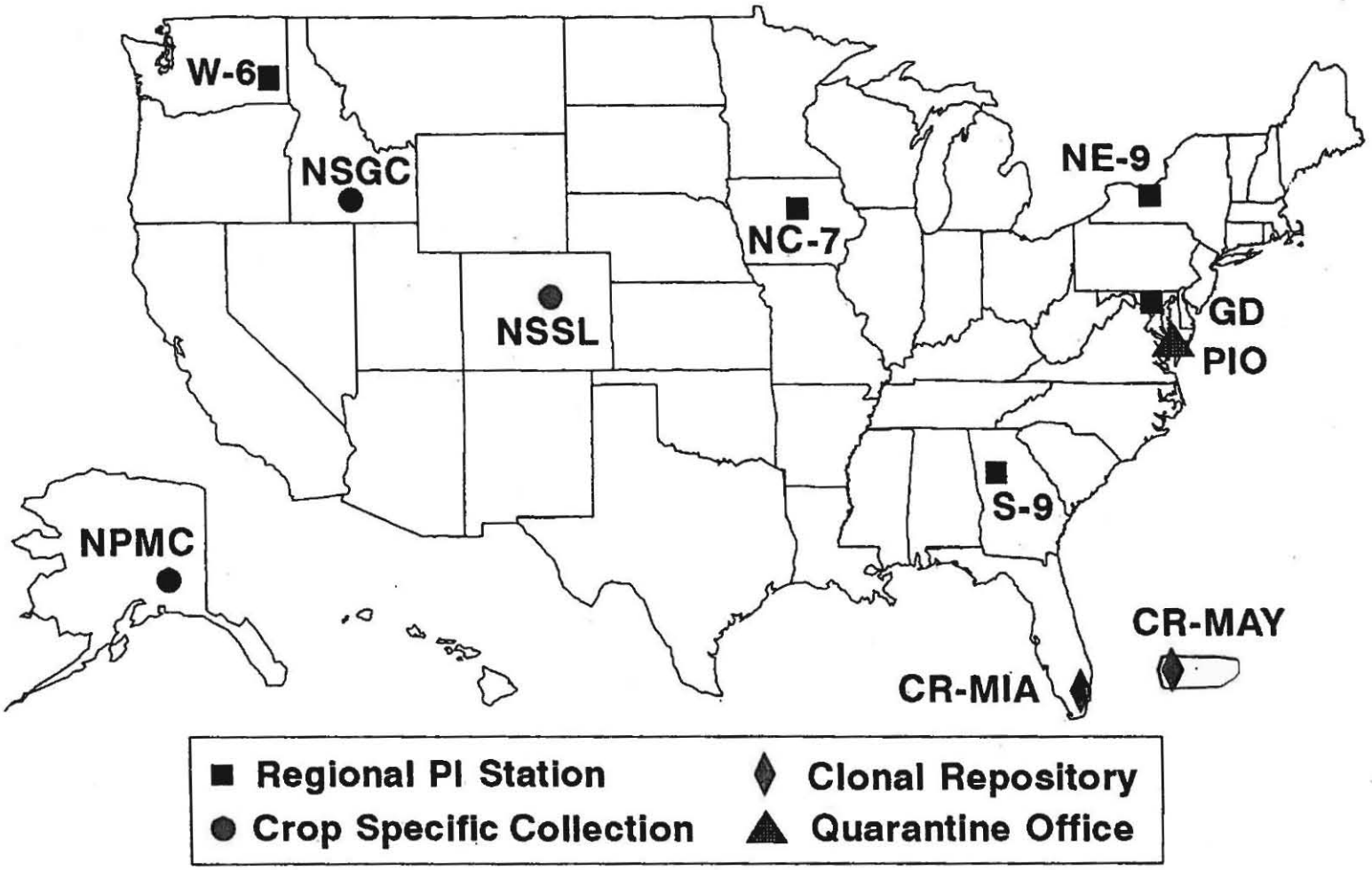

Fig. 1. U.S. grass repository stations in the national Plant Germplasm system.

Table 1. Summary of grass germplasm resources available from repository sites of the National Plant Germplasm System.[a]

Repository Sites and Locations	Station Code	Genera	Accessions
National Clonal Germplasm Repository (Mayaguez, Puerto Rico)	CR-MAY	17	97
National Clonal Germplasm Repository (Miami, Florida)	CR-MIA	19	2,510
Plant Germplasm Quarantine Office (Glenn Dale, Maryland)	GD	24	719
North Central Regional PI Station (Ames, Iowa)	NC-7	17	13,453
Northeast Regional PI Station (Geneva, New York)	NE-9	4	720
National Plant Materials Center (Palmer, Alaska)	NPMC	31	149
National Small Grains Collection (Aberdeen, Idaho)	NSGC	13	112,103
National Seed Storage Laboratory (Fort Collins, Colorado)	NSSL	62	40,019
Plant Introduction Office (Beltsville, Maryland)	PIO	32	465
Southern Regional PI Station (Griffin, Georgia)	S-9	107	25,505
Western Regional PI Station (Pullman, Washington)	W-6	74	11,984

[a]Germplasm available as of 20 May 1991. Data derived from selective searches of the Germplasm Resources Information Network (GRIN) data base.

All plant repository stations provide germplasm directly to users and research scientists worldwide. Information about specific collections is available to users through the NPGS electronic data base known as the Germplasm Resources Information Network (GRIN), a component of the Germplasm Services Laboratory at Beltsville, Maryland. The GRIN system is a public data base that is accessible to users by computer via modem and telephone links. Users can order materials electronically through the order-

Table 2. Major cereal grass collections in the National Plant Germplasm System.

Cultivated Collections	Representative Food Crops	National Plant Repository Stations[a] CR-MIA	GD	NC-7	NSGC	NSSL	S-99S
Avena	Oats	-	-	-	22,513	630	-
Eleusine	African Millet	-	1	1	-	960	774
Hordeum	Barley	-	-	-	26,271	764	-
Oryza	Rice	-	615	-	16,108	745	-
Panicum	Common Millet	-	2	2,189	-	170	701
Pennisetum	Pearl Millet	1	3	-	-	764	638
Saccharum	Sugarcane	2,051	26	-	-	6	-
Secale	Rye	-	-	-	2,593	33	-
Sorghum	Sorghum	2	-	-	-	7,777	15,983
Triticum	Wheat	-	-	-	42,506	1,622	-
Triticosecale	Triticale	-	-	-	1,078	32	-
Zea	Corn	-	-	9,073	-	-	16,384

[a]Values indicate the number of accessions of each generic collection available as of 20 May 1991.

Table 3. Major wild grass collections distributed between two repository sites.

Wild Grass Collections	Common Names	Repository Stations[a] NSSL	W-6
Agropyron	Wheatgrass	10	506
Agrostis	Bentgrass	14	213
Alopecuris	Foxtail	2	145
Bromus	Bromegrass	30	968
Dactylis	Orchardgrass	132	1,060
Elymus	Wildrye	10	1,131
Eragrostis	Lovegrass	14	1,295
Festuca	Fescue	162	1,503
Lolium	Ryegrass	117	844
Phalaris	Canarygrass	25	683
Poa	Bluegrass	93	667
Stipa	Needlegrass	1	271

[a]Values indicate the number of accessions of each generic collection available as of 20 May 1991.

Table 4. Major wild grass collections located at single Repository sites.

Wild Grass Collections	Common Names	Accessions Available	Repository Station
Aegilops	Goatgrass	1,014	NSGC
Andropogon	Beardgrass	1,066	NSSL
Bothriochloa	Bluestem	696	S-9
Cenchrus	Sandbur	826	S-9
Chloris	Fingergrass	246	S-9
Cynodon	Burmudagrass	505	S-9
Danthonia	Oatgrass	26	W-6
Digitaria	Crabgrass	661	S-9
Echinochloa	Hedgehoggrass	779	NC-7
Elytrigia	Wheatgrass	854	W-6
Erianthus	Plumegrass	209	CR-MIA
Holcus	Velvetgrass	18	W-6
Leymus	Dunegrass	346	W-6
Melica	Melicgrass	95	W-6
Oryzopsis	Ricegrass	231	W-6
Paspalum	Paspalum	1,492	S-9
Phleum	Timothy	567	NE-9
Setaria	Bristlegrass	1,325	NC-7
Sporobolus	Dropseed	118	W-6

[a]Values indicate the number of accessions of each generic collection available as of 20 May 1991.

processing system. Information of varying detail is available on approximately 370,000 items in the data base. Data on materials newly acquired from plant collectors are usually more complete.

Information in three categories may be obtained from the GRIN data base including passport, descriptors, and evaluation data (21). Passport data describe the origins and taxonomy of accessions. Descriptor data generated at the curator level provide information on the growth habit, ecology, and salient agronomic characteristics of each accession. Evaluation data are derived from performance evaluations and screening tests conducted by scientists which characterize the useful agronomic traits, pest resistance, genetics, and other unique attributes of particular accessions. For example, information on endophyte incidence in seeds of generic collections that have been surveyed can be found in the evaluation data on these collections. Data on PI accessions such as year original seed was received, number of increases since received, and year of last increase generally are not included in the GRIN data base, but such data are often available directly from

curators. Curators of individual repository stations usually keep this information for the purpose of determining the need for and planning of future collection increases.

Certain materials have restricted availability for various reasons. Some germplasm has been patented which mandates by U.S. law that the recipient must contact the owner concerning restrictions on use. Inventories of materials requested regularly often become depleted to levels requiring that these accessions be temporarily removed from availability during the time when the material is being increased in the field or greenhouse. Other materials which are noxious weeds may have restrictions for shipment into certain regions.

Testing Grass Germplasm For Endophytes

Clavicipitaceous anamorphic endophytes are well known for their systemic, symptomless infections of grasses. Consequently, the incidence of clavicipitaceous endophytes in grass germplasm may be determined primarily by diligent microscopic examination or through chemical analyses to detect their alkaloid secondary metabolites. The economic significance and potential benefits of endophyte-based pest resistance as well as the hazards associated with livestock toxicoses have increased our need to determine their incidence in major world grass collections.

Surveys for clavicipitaceous endophytes in grasses of NPGS collections began in early 1989. The survey and characterization of anamorphic endophytes in the U.S. *Lolium* collection from the W-6 station marked the beginning of surveys of our national grass collections (29). Results of the U.S. *Lolium* collection survey have shown that anamorphic endophytes occur in wild ryegrasses in many countries of the world. Besides their commonly observed presence in *Lolium* germplasm from Europe, endophytes were found in seeds from Africa, Asia, Australia, and New Zealand. The highest incidence of infection occurred in seeds from the Middle East suggesting that this region may be a center of origin of clavicipitaceous endophyte-grass associations since many wild and cultivated grasses have originated there. The overall incidence of endophyte infection among surveyed accessions in the collection was relatively low (33%) when compared with many commercial grass collections. This trend was postulated to result from nonselective maintenance procedures used by U.S. germplasm repository personnel to avoid loss of potentially valuable traits.

Morphological studies of endophytic mycelia in seeds of five *Lolium* species revealed that considerable variation can occur in endophyte morphology in different host species (29). Despite the variation in morphological characteristics, there was still considerable overlap in endophyte morphologies across host species. Controlled experiments showed that the environmental conditions under which host plants are grown

has considerable influence on hyphal diameters and the rates that endophytic hyphae of perennial ryegrass (*L. perenne* L.) enter leaf sheaths from seeds. Light intensity, soil fertility, and volume of growth media appeared to particularly influence endophyte growth and colonization of its host. The amount of mycelium in many PI accessions often was much lower than amounts observed in commercial cultivars (unpublished data). The low level of mycelium and infection rates in some accessions made detection difficult without relatively large sample sizes. In general, conditions favoring growth and vigor of the host also tended to support more vigorous growth of the endophyte. Furthermore, the systemic distribution of endophytic hyphae in host tissues depended on the particular host-endophyte association. For example, the endophytes of annual ryegrass species, unlike perennial ryegrass, poorly colonized leaf sheaths, but hyphae were found primarily in culm piths, especially in the internodes. Similar results were reported by Latch *et al.* (15).

The incidence of anamorphic endophytes in several other major grass collections including the fescue (*Festuca*), wild barley (*Hordeum*), and bluegrass (*Poa*) collections has been examined since the initial ryegrass survey (Table 5). The discovery of anamorphic endophytes in three wild *Hordeum* species demonstrated that these fungi also occur in the wild relatives of cereal grasses (30, 31, 32). The report of endophyte-infected accessions of *H. comosum* Presl. from Argentina was among the first to document the presence of anamorphic endophytes in perennial grasses from South America. The anamorphic endophytes in some accessions of *H. bogdani* Wil. and *H. brevisubulatum* ssp. *violaceum* (Trin.) Link were identified as *Acremonium* species as were the endophytes in some of the

Table 5. Summary of national grass collections surveyed for clavicipitaceous endophytes in the United States.

Generic Collection	Available[a] Sp.	Acc.	Infected Sp.	Acc.	Infection Range(%)[b]	Source Refr.
Festuca	49(36)	1,666(190)	16	41	NA	Springer(24)
Hordeum	24(17)	27,038(96)	3	17	47-100	Wilson(30)
Lolium	8(8)	961(85)	5	28	1-99	Wilson(29)
Poa	49(49)	760(51)	5	6	NA	K.Clay(pers. comm.)

[a]Values in parentheses indicate the number of species and accessions examined in the survey of each generic collection.
[b]NA, data not available.

Lolium species. A higher incidence of seed infection was found in *Hordeum* accessions from wet, uncultivated habitats than in dry, cultivated sites. Endophyte viability in *Hordeum* seeds also tended to decrease over time in storage. Similarly, seed viability appeared to decrease more rapidly over time in endophyte-infected accessions than in endophyte-free accessions. These results could suggest that endophytes may consume food reserves or accumulate toxic metabolites in seeds over long-term storage that may reduce seed viability and perhaps endophyte viability.

Morphological studies of *Acremonium* endophytes from wild *Hordeum* species showed that these fungi have very similar morphologies to *Acremonium* endophytes in forage and turf grasses. For example, an endophyte from *H. bogdani* produced an abundance of aerial synnemata in culture in the absence of host tissue. Similar synnemata were formed in culture by *A. coenophialum* Morgan-Jones & Gams on autoclaved tall fescue seedling tissue (28). The *H. bogdani* endophyte tended to gradually lose the ability to form organized synnemata as the endophyte was subcultured. Conidiophores were produced abundantly, mostly perpendicular to synnematal hyphal strands (Fig. 2). Other strains produced conidiophores singly in smooth, relatively appressed colonies. Most *Acremonium* endophytes from wild *Hordeum* species produced abundant conidia in culture. Conidia often gave rise to seed colonies around the mother colony in older cultures. Conidia were elliptical to cresent-shaped forming singly on determinant conidiophores. Some strains puckered the agar similarly to other anamorphic endophytes such as *Acremonium lolii* Latch,Christensen & Samuels.

Further testing of plant inventory accessions has differentiated genetic resistance from endophyte-associated resistance to several major cereal insect pests. Clement *et al.* (5, 6) have shown that the presence of anamorphic endophytes in grasses can significantly impact the biology of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). This aphid is an exotic pest introduced into the U.S. (Texas) from Russia probably via Mexico in 1986 (25). The aphid has subsequently spread northward throughout the Plain states and westward to the Pacific Northwest where it significantly reduces wheat and barley production. The bases of resistance associated with each endophyte-host and cereal aphid species are presented in Table 6. Most endophyte-associated resistance is due to antibiosis. However, resistance in fescue accessions to the oat birdcherry aphid (*Rhopalosiphum padi* L.) is probably due to antixenosis. The response and poor fecundity of the Russian wheat aphid on several wild grasses make it possible to use the aphid as an assay tool to detect endophytes in infected plants (29).

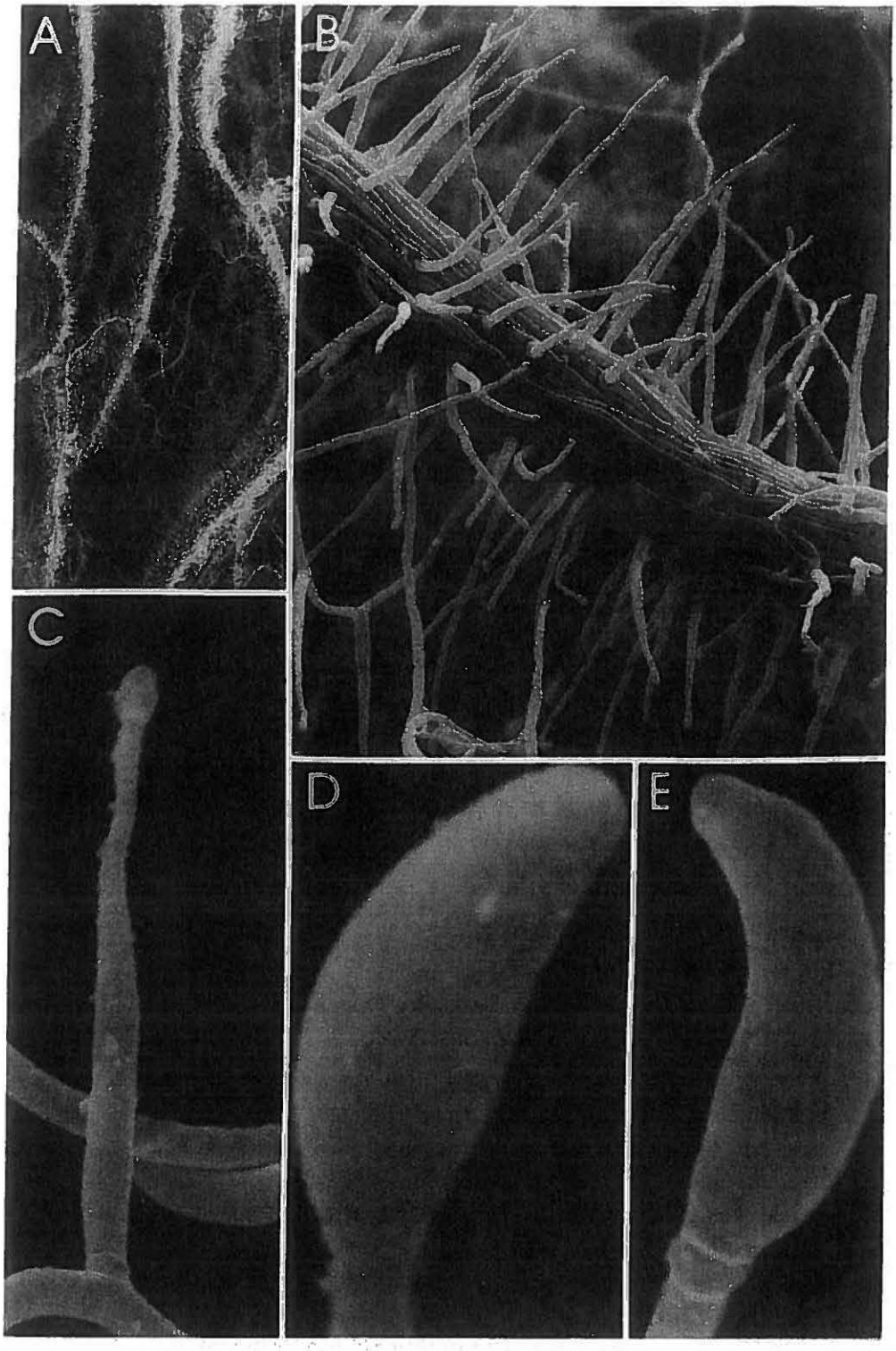

Fig. 2. Scanning electron micrographs of *Acremonium* endophyte from *H. bogdani*. (a) Synnemata, × 70 (b) Synnema, × 1170 (c) Conidiophore, × 4710 (d) Conidium, × 18480 (e) Conidium, × 11850.

Livestock Toxicoses Caused by Endophytes

Much of the initial interest in endophyte research arose with discoveries of associations between fungal endophytes of grasses and animal toxicoses. Bacon *et al.* (1) in 1977 reported the close relationship between an endophyte of tall fescue (*Festuca arundinacea* Schreb.) and incidence of fescue toxicosis (summer syndrome) of cattle in the eastern United States. Fletcher and Harvey (9) reported a similar association in 1983 between an endophyte of perennial ryegrass and ryegrass staggers of sheep in New Zealand. Further studies showed that specific alkaloids such as the tremorgenic lolitrem neurotoxins and ergot alkaloids were responsible for

Table 6. Bases of resistance in endophyte-infected national grass germplasm to cereal aphids.

Cereal Aphid Species	Host Plant Species	Endophyte Species	Resistance Mechanism	Reference
Diuraphis noxia	*Festuca arundinacea*	*A. coenophialum*	Antibiosis	Clement(5)
	Lolium perenne	*A. lolii*	Antixenosis	Clement(6), Wilson (29)
			Antibiosis	Clement(5)
	Hordeum brevisubulatum	*Acremonium* sp.	Antibiosis	UD
Rhopalosiphum padi	*Festuca arundinacea*	*A. coenophialum*	Antixenosis	UD

UD = unpublished data.

these livestock maladies (10, 11, 19, 35). The alkaloids were found to be systemically distributed in grass seeds and tillers consumed by livestock. Endophytes also were found to produce other types of alkaloids including peramine, lolines (pyrrolizidines), norharmane, halostachine, and perloline (34), some of which confer resistance to certain insect pests and some have relatively low apparent toxicities to mammalian herbivores.

Research on livestock toxicoses caused by clavicipitaceous endophytes in the U.S. has mostly concentrated on teleomorphic endophytes of the Balansieae (Ascomycetes) and fescue toxicoses caused by the anamorphic

endophyte *A. coenophialum* in the eastern states. The tall fescue endophyte causes hair loss, reduced milk production, reduced weight gain, and gangrenous dry rot of hooves (fescue foot) in cattle (22, 23). The ryegrass endophyte causes staggers in both sheep and cattle in the United States. Ryegrass staggers and fescue toxicosis likewise are frequently reported in the western states. These diseases appear more regularly in grass-seed producing areas, but livestock toxicoses have been reported throughout the west. A summary of some endophyte-associated toxicoses diagnosed by the author since 1989 are provided in Table 7. Fescue foot is probably the most observed western disease of cattle that consume endophyte-infected tall fescue.

Tests of endophyte-infected accessions in germplasm collections have permitted identification of potentially hazardous materials before distribution. Field surveys of endophyte-infected wild grasses should reveal new livestock toxicoses. A new hyperthermia syndrome of cattle apparently caused by an *Acremonium* endophyte of perennial ryegrass was recently reported in coastal pastures of Pacific Co. in Washington State (12, 33). Temperatures were elevated in cattle of all ages throughout all seasons in affected pastures, but were higher in July than in May, October, and January. Calves had higher temperatures than cows grazing on the same pasture. Cattle removed from affected pastures and fed alfalfa became normothermic within several days, suggesting that the hyperthermia was caused by a pyrogenic factor in the feed. The endophyte appeared morphologically distinct from *A. lolii* and *A. typhinum* Morgan-Jones & Gams (=*Sphacelia typhina* (Pers.) Sacc.), and grew faster in culture than most anamorphic endophytes. Other diseases caused by clavicipitaceous endophytes will no

Table 7. Examples of livestock toxicoses caused by consumption of *Acremonium* endophyte-infected forage grasses in the Pacific Northwest.

Toxicosis	Stock	Location	Endophyte	Toxin	References[a]
Ryegrass staggers	cattle	Payette Co., ID	*A. lolii*	Lolitrem neurotoxin	UD
	sheep	Benton Co., OR	*A. lolii*	Lolitrem neurotoxin	UD
Fescue Foot	cattle	Benton Co., WA	*A. coeno-phialum*	Ergot Alkaloids	UD
Ryegrass fever	cattle	Pacific Co. WA	*Acremonium* spp.	unknown	Wilson (33)

[a]UD = unpublished data.

doubt be discovered from field surveys for endophytes in forage grasses.

Conclusions

There are vast graminaceous germplasm resources in the NPGS available for surveys and testing of endophyte-based resistance to pests and useful agronomic traits. Portions of some generic grass collections have been examined for endophyte infections, but the majority of NPGS grass collections remain to be tested. Many very large collections will require extensive investigations to be adequately evaluated. The rapidly expanding germplasm resources in the already extensive NPGS inventory increase the need to test grass germplasm before introduction into NPGS inventory. This is particularly true because these resources are maintained by limited personnel with finite resources. Increasing reports of endophyte-associated livestock toxicoses in the United States and abroad are other examples where pretesting is needed before introduction and distribution of potentially toxic germplasm occurs.

Previous surveys of endophyte incidence in generic U.S. grass germplasm collections indicate that endophyte-infected accessions usually comprise a relatively small portion of any one collection (29). Endophytes are present in many generic grass collections, but they tend to occur at relatively low incidences and low rates of infection in most NPGS collections compared with commercial collections. The rarity of infected accessions in national generic grass collections and the value of this material suggest that infected accessions should be given some special care in germplasm maintenance programs to maintain endophyte viability and diversity.

The resistance that endophytes provide against graminaceous pests is independent of genetic resistance. Consequently, endophyte-infected accessions are potentially as valuable as accessions with genetic resistance. The diversity of endophyte strains among infected accessions provides a wide variety of types and amounts of alkaloids and other secondary metabolites that may be utilized in controlling specific pests or in producing desired levels of agronomic traits. The combined use of endophyte-based resistance and genetic resistance permits additional alternatives in integrated approaches to pest management. Presently, anamorphic endophytes are used primarily to suppress pests of turfgrasses and reduce populations of soilborne pathogens in certain leguminous crops via crop rotations with endophyte-infected, graminaceous trap crops. The direct use of endophytes as sources of resistance in turfgrasses might be expanded to grass crops by the creation of new endophyte-grass combinations using artificial inoculation methods developed by Latch *et al.* (14). This may be possible as indicated previously because endophytes produce some alkaloids that are toxic to pests but innocuous to mammals, perhaps including man. Endophyte-infected forage grass cultivars already are being developed in

New Zealand to provide resistance to insect pests, but with low toxicity to livestock (Latch, personal communication). Since grass crops produce the vast majority of the food consumed in the world, the utility of clavicipitaceous anamorphic endophytes as new potential sources of resistance to pests of these crops should be thoroughly explored. It is apparent that the economic importance of fungal endophytes to agriculture should encourage plant scientists to greater utilize and test these valuable national germplasm resources.

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