

Widespread Distribution of Endophytes in the Poaceae

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ABSTRACT

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Herbarium collections representing about 100 genera of grasses were assessed for the presence of fungal endophytes. Twenty-two species in nine grass genera were found to contain typical endophytic mycelium. Two of these species, *Melica decumbens* and *Stipa robusta*, are reported as being toxic to cattle. Field studies suggest that both choke-inducing and non-choke-inducing forms are common in grasses. Most isolates are referable to *Acremonium* section *albo-lanosa*; however, sterility of the *S. robusta* isolate prevents definite classification.

Since Vogl (31) first determined the presence of endophytic mycelium in seeds of darnel (*Lolium temulentum* L.), our knowledge about the distribution of endophytes in grasses has increased slowly and sporadically, with research on endophytes centering around associations in the genera *Lolium* L. and *Festuca* L. (8,23,24,27,29). The discovery that endophytes in fescue and ryegrass cause toxic syndromes in cattle (1,7) and impart resistance to certain insects (3,9) has given considerable impetus to studies concerning these fungi and underlined the need for a greater understanding of basic as well as applied aspects of these associations.

Our present knowledge of these fungi suggests that they are vegetative phases of fungi that are, or have evolved from, species of the Ascomycetous genus *Epichloë* (Fr.) Tul. (5,16,18,22). Many of these endophytic forms rarely or never produce an external phase, some apparently having completely lost the ability to sporulate (30). Typically, endophytic mycelium is present intercellularly in ground tissue of stems and leaves. The endophyte is propagated vegetatively by growing into the nucellus of the ovule and penetrating the embryo within the seed (8,30).

The purpose of this article is to report the presence of endophytes in grasses other than *Lolium* and *Festuca*.

MATERIALS AND METHODS

Numerous specimens from the plant herbaria at the University of Texas, Texas A&M University, and Sul Ross State University were examined for the presence of typical endophytic mycelium

(11,27,29) (Fig. 1) by the pith-scraping procedure (6). This is briefly described as follows: A longitudinal section of stem about 2 cm long was removed from the specimen. Aniline-blue stain (1%, aqueous) was then applied to the exposed ground tissue, which was scraped onto a clean glass slide. A drop of water and coverslip were applied to the tissue, which was then examined with a compound microscope. Species from about 100 genera of the Poaceae were assessed for endophytes. In grass species where endophytic mycelium was not observed, four specimens were examined; however, if an endophyte was found, numerous additional specimens were examined and the results recorded (Table 1). After examination, an annotation was affixed to each collection indicating the presence or absence of endophytic mycelium. Examination of field populations for external signs of *Epichloë* were made at the time of flowering for *Agrostis hiemalis* (Walt.) B.S.P., *Elymus canadensis* L., *Poa autumnalis* Muhl., *Stipa eminens* Cav., and *S. robusta* (Vasey) Scribner. Isolations were made from leaf sheaths, stems, and seeds of grasses following the procedures of Clark et al (6). Isolates were grown on potato-dextrose agar (PDA, Difco) for about 5 wk at 22 C, then microscopically examined. Unfortunately, because of the broad distribution of many of these grasses, field examinations and isolations from all host species (Table 1) were not practical.

RESULTS AND DISCUSSION

Certain species (e.g., *Festuca arundinacea* Schreb., *Lolium perenne* L., and *L. temulentum* L.) have long been known to harbor seed-transmitted fungal endophytes distributed in leaves, stems, and seeds (8,23,25). Using a technique to examine grass stems for the presence of fungal elements, I have demonstrated that a significant number of additional grass species contain similar fungal endophytes (Table 1) (29). All six

subfamilies of the Poaceae (10) were represented in this study; however, endophytes were most prevalent in subfamily Festucoideae. The presence of an endophyte in *Digitaria insularis* (L.) Mez. (subfamily Panicoideae) demonstrates that these associations are not limited to festucoid grasses. *Epichloë typhina* (Fr.) Tul. shows a similar pattern of distribution, usually associating with members of the Festucoideae and only occasionally occurring in other subfamilies (28).

Symptoms of "choke disease" caused by *Epichloë* spp. (5,16) were occasionally observed on culms of *A. hiemalis* in Brazoria County and *E. canadensis* in Travis County, Texas. These symptoms include a white conidial stroma of variable length that occurs on the sheath of the flag leaf, preventing the emergence of the inflorescence from the elongating culm. Later, this stroma became orange as perithecia were produced. Microscopic examination of conidial and perithecial stromata demonstrated conidia and



Fig. 1. Typical endophytic hypha in pith-scraping preparation of *Melica decumbens* showing characteristic convoluted pattern of growth.

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ascospores typical of *E. typhina* (5,16, 18,22). In contrast, examination of several populations of *S. eminens* in Brewster County, Texas, *S. robusta* in Otero County, New Mexico, and those of *P. autumnalis* in Brazoria County, Texas, did not reveal any external signs of the endophytes. It is evident that both choke-inducing and non-choke-inducing forms (11) are common in grasses.

Isolations of endophytic fungi present in many of the grasses (Table 1) have been made, and in each case affinity with *Acremonium* section *albo-lanosa* M.-J. & G. was apparent (18,22,30). All isolates were slow growing, frequently no more than 1-3 mm of diametric growth per week. Where conidia were produced, only one or two were observed associated with the apex of a conidiogenous cell. Conidiogenous cells, where present, were consistently of the *Acremonium*-type but lacking the apical wall thickening typical of other species of *Acremonium* Link (18). Considerable variability was encountered in examination of cultures of endophyte isolates. It is evident that the endophytes are a complex of related fungi, within which the delimitation of species taxa will come with some difficulty. Contributing to difficulties of assigning names to these fungi is the occurrence of forms that lack conidiation (30). Although bearing gross cultural resemblance to those species classified in *Acremonium* section *albo-lanosa*, they have apparently lost the capacity to produce disseminating propagules and appear to rely exclusively on vegetative growth. The endophyte from *S. robusta* appears to be one of these sterile forms.

It is now firmly established that endophytic fungi cause toxicosis in grazing cattle (1,15,19). From this perspective, certain of the grasses (Table 1) are notable. Species to which toxic qualities have historically been assigned include *Melica decumbens* Thunb. and *S. robusta*. *M. decumbens* occurs only in South Africa, where it is known as "dronk gras" because of the severe staggers condition experienced by cattle ingesting a sufficient quantity (12,13, 21,26). *S. robusta* occurs in parts of North America and is known as "sleepy grass" because of the temporary narcosis that mammals, especially horses, encounter on ingesting as little as 1% of their body weight of the grass (2,17,20). Other *Stipa* species referred to as sleepy grasses, which I have not examined, include *S. inebrians* Hance and *S. sibirica* Lam., both of which occur in Asia (4). In addition to toxicosis, it seems probable that unpalatability of grasses such as *D. insularis*, known as "sour grass" (14), may be attributed to endophytes that are present in most collections of this species (Table 1). Further studies are necessary to confirm the role of endophytes in unpalatability and toxicity of these grasses.

This brief assessment of endophytes in

Table 1. Grasses found to contain endophytic mycelium by culm examination of herbarium collections

Host grass	Source of collections	No. with endophyte/total examined
<i>Agrostis hiemalis</i> (W.) B.S.P.* ^a	Eastern United States	41/92
<i>A. perennans</i> (W.) Tuckerm.	Eastern United States	21/35
<i>Bromus anomalus</i> Rupr.*	Western United States	13/72
<i>Cinna arundinacea</i> L.	Eastern United States	20/28
<i>C. latifolia</i> (Trevir.) Griseb.	United States	11/24
<i>Digitaria insularis</i> (L.) Mez.	North and South America	45/61
<i>Elymus canadensis</i> L.*	United States	38/62
<i>E. virginicus</i> L.	United States	21/45
<i>E. europaeus</i> L.	Europe	3/3
<i>Melica ciliata</i> L.	Europe	8/10
<i>M. decumbens</i> Thunb.	South Africa	4/4
<i>M. picta</i> L.	Europe	3/3
<i>M. scaberrima</i> (Nees) Hook.	India	1/1
<i>M. transilvanica</i> Schur.	Germany	1/1
<i>Poa autumnalis</i> Muhl.*	Eastern United States	14/14
<i>P. palustris</i> L.	Northern United States	24/45
<i>P. paucispicula</i> Scribn. & Merr.	Northwest United States	1/1
<i>Stanion longifolium</i> J.G. Sm.*	Texas	47/55
<i>Stipa eminens</i> Cav.*	West Texas and Mexico	19/19
<i>S. lobata</i> Swall.*	Western United States	6/17
<i>S. robusta</i> (Vasey) Scribn.*	Western United States	8/8
<i>S. viridula</i> Trin.	Western United States	2/9

* Fresh collections were examined and endophytic fungi studied in vitro.

the Poaceae is far from exhaustive; however, it does suggest that the impact of endophytic fungi on world rangelands may be considerable. Continued research from a range of disciplines is necessary before we can fully appreciate the extent to which these cryptic fungi affect agriculture.

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