Occurrence and Significance of Endophytic Fungi in the Fine Fescues

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ABSTRACT

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Acremonium-type endophytic fungi were detected in 19 of 83 seed lots of fine fescue cultivars and selections. The 19 infected seed lots were mostly of European origin and averaged 50% infected seed. Endophytes were detected in 10 of 328 fine fescue plants collected from old established turfs throughout the United States. Endophytes found in these plants and seed lots of fine fescue are, for the most part, non-choke-inducing (NCI). Of particular significance is the finding that NCI endophytes occur in blue fescue, sheeps fescue, various-leaved fescue, and *Festuca ovina* subsp. *vallesiaca*. NCI endophytic fungi were also found in three subspecies of *F. rubra*, namely, strong creeping red, slender creeping red, and Chewings fescue and in hard fescues. These endophytes appear to be associated with enhanced host plant resistance to chinch bugs, an important turfgrass pest. Breeding programs to incorporate useful NCI endophytes into leading cultivars and elite germ plasm collections of fine fescue have been initiated.

Additional key words: *Acremonium*, *Blissus leucopterus hirtus*, *Epichloë typhina*, *Festuca glauca*, *F. heterophylla*, *F. longifolia*, *F. ovina*, *F. rubra* subsp. *commutata*, *F. rubra* subsp. *litoralis*, *F. rubra* subsp. *rubra*

The occurrence of endophytic fungi (*Acremonium* spp.) in perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) and their association with insect resistance and livestock problems have been well documented (1,6,9,16,20). Some studies suggest that the endophytes in tall fescue and perennial ryegrass may also enhance growth, persistence, resistance to weed invasion, and recovery from summer stress (7,20). The occurrence and possible significance of endophytes in other grass and sedge species are also receiving considerable attention (2–5). White and Cole (21) observed endophytes in seeds of 11 species of native forage fescues but none in *F. idahoensis* Elmer, *F. ovina* L., or *F. rubra* L. They concluded that endophytes are more common than originally suspected. The presence of endophytes in fine fescue species has been known for many years, but their significance is only now being realized.

In 1933, Sampson (18) observed that the fungus, *Epichloë typhina* (Pers. ex Fr.) Tul., which incites the choke disease, systemically infects red fescue plants (*F. rubra*) and *F. ovina*. Variability in symptom expression ranged from visible external stromata on every panicle of a given plant to a total lack of external symptoms. More recently, systemic infection of grasses by *Epichloë*-like fungi that fail to produce a stromatic collar or other external symptoms was designated as non-choke-inducing (NCI) by Halisky et al (11). A study in New Zealand also revealed that *E. typhina* was found in *F. rubra* plants as an NCI endophyte (14). These studies (14,18) did not clearly indicate the species or subspecies of fine fescues studied.

The fine fescues include a number of important turfgrass species and subspecies, each one possessing unique characteristics and desirable features (13,19). Generally, they tolerate low maintenance and are useful turfgrasses with today's emphasis on reduced water, fertilizer, and pesticide use and reduced mowing frequency. The objectives of this study were to determine the presence of endophytes in seeds and plants of hard fescue (*F. longifolia* Thuill.), Chewings fescue (*F. rubra* L. subsp. *commutata* Gaud.), strong creeping red fescue (*F. rubra* L. subsp. *rubra*), slender creeping red fescue (*F. rubra* L. subsp. *litoralis* (Meyer) Auquier), sheeps fescue (*F. ovina* L.), blue fescue (*F. glauca* Lam.), various-leaved fescue (*F. heterophylla* Lam.), *F. ovina* L. subsp. *vallesiaca* Koch, *F. pseudovina*, and *F. duriscula* and to explore the possible role of endophytes in enhancing turfgrass performance.

MATERIALS AND METHODS

Endophyte status of seed lots. Eighty-three seed lots from 10 species of fine fescue, including those entered in the National Turfgrass Evaluation Program, were examined for endophyte content (Table 1). Fifty seeds were examined in endophyte-positive entries, and 25 seeds were examined in endophyte-negative entries. Seeds were stained with either trypan blue-lactic acid (10) or rose bengal (17).

Endophyte status of plant collections. Plants were selected from old established turfs throughout the United States. Individual plants from unknown sources had colonized, survived for many years, and grown to form large distinct patches. These plant selections were maintained in the greenhouse or field nurseries until screened for endophyte. One hundred nineteen strong creeping red fescues collected from the eastern United States, Texas, and Oregon; 40 Chewings fescues collected throughout the northeastern United States; and 169 blue fescues from New Jersey, New York, and Pennsylvania were examined for endophyte infection (Table 2). The inner epidermis of each of five to 10 leaf sheaths per plant selection was peeled off, stained with rose bengal, and examined under a microscope (17).

For some of the fine fescue plant selections, species identification was difficult, particularly with the blue fescues. These plants were allowed to flower, and the time of pollen shedding was observed to assist in identification. Blue fescues ($2n = 42$) shed pollen at about 12:00 noon in New Jersey, whereas hard fescues ($2n = 42$) and Chewings fescues ($2n = 42$) shed pollen at about 4:00 A.M. Strong creeping red fescues ($2n = 56$) shed pollen at about 4:00 P.M.

To determine if NCI endophyte infection has any effect on seed production, 59 hard fescue selections were planted and allowed to set seed. These selections were made from an old turf trial consisting primarily of Biljart and Scaldis germ plasm. Each selection was individually harvested for seed yield and endophyte status determinations.

Turfgrass plot evaluation. A turf evaluation trial was planted at the Soils and Crops Research Center, Adelphia, NJ, in 1978 on a sandy loam soil. Plot size was 1.0 × 1.6 m with three replicates of each of the 41 entries. The trial received high maintenance the first few years after establishment. Later, the fertility level and mowing frequency was reduced, mowing height was raised, and irrigation was discontinued to evaluate the performance of the test under low maintenance and drought stress. No

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Table 1. Seed lots of fine fescue cultivars and selections of European (E) and North American (NA) origin examined for endophyte[a]

Species	Cultivars and selections with endophyte	Infected seed (%)	Cultivars and selections without endophyte
Hard fescue	Biljart (E)	52	Scaldis (E), Waldina (E), Aurora (NA), Reliant (NA), Fo81-225 (E)
	Valda (E)	62	
	Spartan (NA)	20	
	SR3000 (NA)	95[b]	
Chewings fescue	Beauty (E)	72	HF9-3 (E), Atlanta (E), Magenta (E), Epsom (E), Waldorf (E),
	Center (E)	18	Wilma (E), Tatjana (E), Tamora (E), Checker (E), Highlight (E),
	Victory (NA)	22	Koket (E), Enjoy (E), Mary (E), Banner (NA), Jamestown (NA),
	Longfellow (NA)	98[b]	Shadow (NA)
Sheeps fescue	Saphir (E)	6	Espro (E), Azay (E), R555 (E), R568 (E), R569 (E), R570 (E),
	R571 (E)	100	R573 (E), R575 (E), R577 (E), R879 (E), Bighorn (NA)
	R572 (E)	92	
	R315 (E)	96	
Strong creeping red fescue	Pernille (E)	34	Louisa (E), Commodore (E), Ruby (E), PS430 (NA),
	Ensylva (E)	24	Flyer (NA), Boreal (NA)
	Robot (E)	5	
	Ceres (E)	5	
Slender creeping red fescue	Frt 82-50 (E)	40	LD3484 (E), Dawson (E), Merlin (E), Logro (E), Estica, (E), HF24-2 (E), Frt 401 (E), Frt 402 (E), Frt 403 (E), HF-33 (E), HF-34 (E), 30141 (E), 30143 (E), 30146 (E), 30147 (E), 30148 (E), 30149 (E), 30154 (E), Ballade (E)
Various-leaved fescue	Bar St (E)	100	...
Festuca ovina subsp. *vallesiaca*	Liwally	9	...
F. pseudovina	...	...	Vendome (E), Barfor 8 (E)
F. duriscula	...	...	Durar (NA)
Blue fescue	...	...	German lot 1380 (E), German lot 1-1985 (E), T-81 (NA), Alaska Station (NA)

[a] Fifty seeds were examined in endophyte-infected entries and 25 seeds in endophyte-free entries. Trypan blue-lactic acid or rose bengal stain was used. The endophyte status of the cultivars listed in this table applied only to the seed lots that were examined; different seed lots of a cultivar could contain different amounts of endophyte.
[b] SR3000 and Longfellow had 0% endophyte-infected seed in the National Turfgrass Evaluation Test; however, the above data are from new seed lots of these cultivars.

Table 2. Frequency of endophyte infection in fine fescue selections made in the United States

Species	States of origin	No. of selections examined[a]	No. of endophyte-infected plants
Strong creeping red fescue	New Jersey, New York, Pennsylvania, Delaware, Georgia, Kentucky, Oregon, Texas	119	2
Chewings fescue	New Jersey, New York, Pennsylvania, Maryland, Massachussets	40	3
Blue fescue	New Jersey, New York Pennsylvania	169	5

[a] Five tillers per plant selection were examined with rose bengal stain for endophyte.

insecticides or fungicides were used on the plots. The plots were evaluated several times each year for overall turf performance by a rating scale of 1–9, where 9 = best and 1 = worst.

In 1984, a chinch bug (*Blissus leucopterus hirtus* Montandon) infestation was observed in this trial, and damage was assessed by determining percent green turf. Actual chinch bug counts were made for eight cultivars showing a range of insect damage, using a water flotation technique. A round metal cylinder (31 cm in diameter) was pressed into the turf and filled with water. Chinch bugs that floated to the surface during a 5-min period were counted. Two counts were made per plot on three replicates. The endophyte status of these eight cultivars was determined by examining 30 tillers, 10 tillers per replicate, using the rose bengal staining technique (17).

RESULTS AND DISCUSSION

Endophyte status of seed lots. Four of nine hard fescue seed lots examined were infected with endophyte (Table 1). Two of these infected hard fescues were of European origin, Biljart and Valda. The other two, Spartan and SR3000, were developed in North America, but their endophyte originated from the European sources of germ plasm used in the development of these cultivars. Among the Chewings fescues, four of 20 seed lots were infected. Longfellow and Victory Chewings fescues probably contain the same source of endophyte. The parental germ plasm of both of these cultivars includes a few endophyte-containing plants collected near the Longfellow mansion in Cambridge, MA. We are unaware of any other sources of endophyte in the germ plasm of either cultivar. The other two infected Chewings fescues were of European origin. Four of the fifteen seed lots of sheeps fescue examined contained endophyte, and these seed lots were from Europe. None of the four blue fescue seed lots examined was infected. Among 10 strong creeping red fescues examined, the four infected with endophyte were of European origin. Twenty-five additional seed lots from other fine fescues were examined, and endophyte was found in three of them, also all of European origin.

When examining cultivars for endophyte content, it is important to realize that each seed lot can contain different

percentages of infection and that the percentage of infection found within a seed lot is not necessarily all viable endophyte. If seed is stored for more than 1 or 2 yr at room temperature, the endophyte may lose viability (20). The seed lots of SR3000 (experimental designation ST-2) and Longfellow used in the National Turfgrass Evaluation Program were free of endophyte because seed produced for the national program came from nurseries that had been established from old seed. In newer seed lots of these cultivars, however, the endophyte is present at a high frequency.

Endophyte status of plant collections. In our plant collection of strong creeping red fescues, only two of 119 plants examined were infected with the endophyte (Table 2). One plant was found as a contaminant in an old Kentucky bluegrass turf trial at Adelphia, NJ. The second plant was collected in an old cemetery in Portland, OR. Two of the endophyte-infected Chewings fescue plants were from Cambridge, MA, and the third was found in East Brunswick, NJ. The five blue fescues found infected, of 169 examined, were from old naturalized turfs in central New Jersey, specifically New Brunswick and Morristown.

To our knowledge, this is the first report of an NCI endophyte in blue fescue. The production of choke stroma was observed on the ornamental grass *F. glauca* in New Zealand in 1978 (12), but a strictly NCI infection has not been reported for this turfgrass species. Each of these plants was carefully examined during flowering and seed formation for external signs of the choke disease, but none were found. Seedlings from three of these blue fescues were examined for endophyte and found to be an average of 95% infected. The mycelium of the fungus found inside the blue fescue plants appeared slightly larger than that of the other fine fescue endophytes and was similar in appearance to the *Lolium* endophyte (*A. loliae*) (14), with infrequent branching. Further study is needed to identify this species of endophyte. This is also the first report of NCI endophytes in various-leaved fescue, sheeps fescue, and *F. ovina* subsp. *vallesiaca*. Previous reports of endophytes in *F. rubra* did not indicate the subspecies examined. Our study showed NCI endophytes in Chewings fescue, slender creeping red fescue, and strong creeping red fescue.

To help verify that many endophytic fungi in the fine fescue species are NCI, spaced-plant nurseries and commercial seed fields were carefully examined for choke stromata during seed formation. More than 2,000 plants of Victory Chewings fescue (22% containing endophyte) were examined during May 1983 and May 1985 near Halsey, OR, and fewer than 1% of the plants showed visible choke symptoms. On those plants with external symptoms, only a few reproductive tillers were involved. In a nursery containing more than 2,000 plants of SR3000 hard fescue near Forest Grove, OR (95% infected with endophyte), no choke symptoms were observed on 10-mo-old plants in May 1985. Three of these plants, however, developed stromatic collars on a few tillers 1 yr later. One certified and two foundation production fields of Spartan hard fescue in western Oregon (20% containing endophyte) were examined, and fewer than one in 1,000 panicles showed choke symptoms. Biljart, Spartan, and SR3000 hard fescue, Ensylva strong creeping red fescue, and Victory Chewings fescue failed to develop choke symptoms in repeated seed trials in New Jersey.

Because the choke disease adversely affects seed yield, there is concern that the NCI endophyte may also adversely affect seed yield. The results of our limited trial with 59 hard fescues failed to show significant differences in seed yield. The 29 plants with endophyte gave an average seed yield of 23.4 g, and the 30 plants free of endophyte produced an average yield of 23.2 g. Because high seed yield is an important criterion in cultivar development, cultivars containing high levels of endophyte must have adequate seed yield.

Turfgrass plot evaluation. Endophyte content and its association with turf quality in fine fescues is shown in Table 3. In 1978, 1979, and 1980, no significant differences were found between the cultivars with high endophyte and the cultivars with no or very low levels of endophyte. During the first few years of the test, when the turf was under high maintenance and very little stress, we were unable to observe any positive or negative effect of the endophyte. In 1981, SR3000 and Longfellow began to show significantly higher quality ratings and in 1982, 1983, and 1984, the differences became more pronounced. In 1984 particularly, the low endophyte plots showed very poor recovery from summer heat and drought stress. The test was receiving lower maintenance and no supplemental irrigation, which suggests that, under stress conditions, the presence of endophyte may enhance performance. Another phenomenon that may be involved here is that endophyte-free plants die off over the years because of selective pressures and the percentage of infected plants increases (5,15,16,20). The seed lots used to establish Longfellow and SR3000 were 48 and 94% endophyte-infected; 7 yr later, the percentages of infected plants had risen to 84 and 97, respectively.

In 1984, when a chinch bug infestation occurred, the two cultivars with high endophyte, SR3000 and Longfellow, had significantly more green or undamaged turf and fewer chinch bugs than the cultivars with low endophyte (Table 3). The endophyte-infected cultivars may have contained a feeding deterrent, or the chinch bugs may have just chosen where

Table 3. Relationship of endophyte content to turf quality and chinch bug infestations in fine fescue

Cultivar	Turf quality[a] 1978	1979	1980	1981	1982	1983	1984	Percent green turf (1984)	Chinch bugs per square meter (1984)	Percent endophyte infection[b] (1985)
Hard fescue										
SR3000	5.9	5.3	5.4	6.4	7.7	8.2	9.0	76.7	42.4	97.0
Tournament	5.3	4.4	4.0	3.2	3.0	5.3	3.7	35.0	103.5	10.0
Reliant	6.4	5.8	6.0	5.6	5.7	5.3	5.3	45.0	135.3	3.3
Aurora	5.9	5.4	5.6	4.9	5.3	5.7	4.7	46.7	185.7	0.0
Chewings fescue										
Longfellow	5.2	6.5	6.8	6.2	7.3	6.2	7.0	78.3	55.7	84.0
Jamestown	6.3	6.1	5.9	5.4	4.7	4.5	4.0	38.3	181.7	10.0
Shadow	6.0	5.8	6.1	5.4	4.3	3.8	2.7	26.7	132.6	6.7
Banner	6.2	6.2	6.1	5.8	4.7	4.2	3.3	41.7	132.6	3.3
LSD (5%)	1.0	0.5	0.8	1.0	1.6	1.6	1.7	20.7	103.5	18.1

[a] Average of three plots per entry. Quality scores are visual ratings from 1–9, where 1 = worst and 9 = best.

[b] Ten plants per plot were examined with rose bengal stain.

to feed on the basis of where the turf was thinned by summer stress and disease. These thinned areas provided a warm, dry environment that chinch bugs prefer. If the endophyte contributed toward a denser, healthier turf, it could have indirectly enhanced resistance to chinch bugs in SR3000 and Longfellow.

Endophyte-infected perennial ryegrasses show enhanced resistance to several lepidopterous species of sod webworm, billbugs (*Sphenophorus* spp.), and Argentine stem weevil (*Listronotus bonariensis*) (9,16). In a laboratory study, tall fescues infected with endophyte were shown to have enhanced resistance to some species of aphids (20). Endophyte-infected ryegrass and tall fescue may also show enhanced performance under some stress conditions (7). These benefits of endophyte infection now appear significant in the hard and Chewings fescues. Perhaps with further testing, strong creeping red, slender creeping red, blue and sheeps fescues will also show endophyte-enhanced performance and persistence. The fine fescues are usually used in low-maintenance situations where insecticides and other management inputs are limited, so cultivars with high levels of endophyte may perform better and persist longer. As fine fescues are not normally used for pasture or hay purposes, livestock problems associated with endophyte are of less concern (1,6); however, possible animal-related problems certainly deserve study.

Methods have been developed to incorporate endophytes into top-performing fine fescue selections (8). If these selections are commercially released, the problems of maintaining high levels of viable endophyte in the seed need to be considered.

A test, established in 1984, is now under way to compare the performance of the same hard fescue cultivars, with and without the endophyte. First-year results show that under high maintenance, as previously discussed, no significant differences were seen. The average 1985 performance score for 32 SR3000 plots with endophyte was 6.6, and the average score for 32 SR3000 plots without endophyte was 6.5; the average score for 29 A73 plots with endophyte and 30 A73 plots without the fungus was the same, 6.4. This test will be studied under stress conditions and, perhaps, with insect infestations.

Finding new sources of endophyte is an important part of this work and a major reason for screening our plant collections for endophyte. Most of the sources of endophyte in the fine fescues we have examined are of European origin (Table 1). The low incidence of endophyte in our U.S. plant collection, only 3% (Table 2), indicates that endophyte-infected fine fescue is not very common in the United States. A possible explanation for this is that the endophyte in fine fescue seed shipped over from Europe had lost viability by the time it was planted in the United States. The endophytes found in the hard fescues, red fescues, sheeps fescues, and blue fescues may differ just as the grass selections themselves differ. Some may be more effective than others in enhancing the performance of their host species.

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