Endophyte Content of Seed Harvested from Endophyte-infected and Endophyte-free Tall Fescue

Ronald E. Welty[1,3] and Mark D. Azevedo[2]

ABSTRACT

Endophyte-infected (E+) and endophyte-free (E-) tall fescue (*Festuca arundinacea* Schreb.) was space-planted in field plots at seven locations in Oregon, USA. Seeds were harvested in each of four years (1984-1987) and examined for endophyte hyphae by seed-staining procedures. Endophyte infection in seeds of E+ cv. G1-307 ranged from 83 to 99%; seeds of E- cv. Forager remained free of endophyte hyphae. In another experiment, dry matter (leaves, reproductive stems, and crowns) was burned or mechanically removed from E+ plants following seed harvest. Seed harvested the following year from burned plants had similar amounts of endophyte-infected seed as non-burned plants. No evidence was found for natural transmission of *Acremonium coenophialum* from E+ plants to E- plants; burning dormant plants did not affect endophyte content of seed harvested the following year.

Additional index words: **seed production, seed-borne fungi, cool-season grasses, fescue toxicity, fescue foot, fat necrosis.**

INTRODUCTION

Acremonium coenophialum Morgan-Jones and Gams is a symbiotic endophyte of tall fescue (*Festuca arundinacea* Schreb.). The fungus is commonly described as an endophyte because it grows entirely within the grass host without external signs of infection. Fungal hyphae are easily detected in seeds, seedlings, meristems, leaf sheaths, and seed culms by staining (Clark, White and Patterson, 1983; Saha, Jackson and Johnson-Cicalese, 1988; Siegel, Latch and Johnson, 1985; Welty, Azevedo and Cook, 1986), ELISA (Johnson, Pirone, Siegel and Varney, 1982; Welty, Milbrath, Faulkenberry, Azevedo, Meek and Hall, 1986) and immuno-blot (Gwinn, Collins-Shepherd and Reddick, 1991).

When endophyte-infected (E+) tall fescue is grazed in pastures or fed to livestock as hay, animal toxicity has been observed. In cattle, specific disorders include fescue foot (Garner and Cornell, 1978), fescue toxicosis (Hemken, Jackson and Boling, 1984), fat necrosis (Bush, Bowling and Yates, 1979; Stuedemann, Wilkinson, Williams, Jackson and Jones, 1973), and reduced milk production (Hemken *et al.* 1984; Wallner, Booth, Robbins, Bacon, Porter, Kiser, Wilson and Johnson, 1983). Agalactia has been associated with postparturient horses (Heinmann, Garrett, Lock, Morris and Pfander, 1981).

Endophyte-infection in tall fescue used as turfgrass is beneficial because it reduces or deters feeding by insects (Siegel, Latch and Johnson, 1987) and reduces populations of some species of plant-parasitic nematodes (Pedersen, Rodriguez-Kabana and Shelby, 1986). Generalisations about beneficial aspects of increased resistance to plant pathogens of E+ cool-season grasses should be provisional because not all endophytes induce resistance to other plant pathogens (Cook, Lewis and Mizen, 1991; Cromey and Cole, 1984; Hurley, 1991; Welty, Barker and Azevedo, 1992). Endophyte infection also alters host morphology (Hill, Stringer, Rottinghaus, Belesky, Parrott and Pope, 1990) and increases drought tolerance (Bacon and Siegel, 1988).

Transmission of endophytic fungi growing *in vitro* to E- cool-season grass hosts has been tried with several grasses and endophyte species. Successful infection in field-grown plants of orchardgrass (*Dactylis glomerata* L.) occurred when freshly cut stubble was inoculated with germinated conidia and ascospores of *Epichloe typhina* (Pers.) Tul (Western and Cavett, 1959), but this work has not been confirmed. Repeated inoculations of perennial ryegrass (*Lolium perenne* L.) with *E. typhina* (Neill, 1941) and tall fescue with *A. coenophialum* were unsuccessful in establishing endophyte-infected plants (Siegel, Johnson, Varney, Nesmith, Buckner, Bush, Burrus, Jones and Boling, 1984). Likewise, attempts to infect *Phleum pratense* L. (timothy) in the field with conidia or ascospores, or fungus-infested soil, were unsuccessful (Muhle and Frauenstein, 1970). Successful inoculations have been achieved in the laboratory with *A. coenophialum* in tall fescue (Johnson, Bush and Siegel, 1986), *A. lolii* in perennial ryegrass (Latch and Christensen, 1985) and *E. typhina* in *P. pratense* (Muhle and Frauenstein, 1970). The only known mode of natural spread of *A. coenophialum* in tall fescue is by sowing E+ seed (Siegel *et al.*, 1984; Siegel *et al.*, 1985; Siegel *et al.*, 1987).

Renovating pastures with tall fescue seed free of *A. coenophialum* or seed of a cultivar with a low percentage of endophyte-infection (usually less than 5%) is an inexpensive method for controlling the toxicity disorder in animals. Voluntary testing of seed lots for endophyte-infected seed has been established in some states in the US. In 1983, the

[1] USDA Agricultural Research Service, National Forage Seed Production Research Centre; [2] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-7102, USA. Oregon State Agricultural Experiment Station Technical Paper No. 9986; [3] Corresponding author. Accepted for publication 11 January 1993.

Oregon Department of Agriculture began a voluntary endophyte testing programme for tall fescue. Tags are issued for attachment to bags for those seedlots produced in Oregon that contain 5% or less endophyte. Since the programme began in 1983, 2787 lots of seed of tall fescue (representing 44.2 million kg of seed) were tested for endophyte, and 95% of the lots contained less than 5% endophyte-infection (Milbrath, pers. comm.).

Open field burning for grass seed fields after seed harvest has been used as a cultural practice in Oregon since the 1940s (Conklin, Young and Youngberg, 1989). Benefits include improved disease, weed, and insect control, stimulation of seed yield, and removal of unwanted plant residues following seed harvest.

This study was done to determine if E- tall fescue plants would remain E- when grown in the field in proximity to E+ tall fescue plants (Experiments 1 and 2). Another experiment determined the effect of burning E+ plants on endophyte survival and infection percentages in seed harvested in the year following burning (Experiment 3).

MATERIALS AND METHODS

Experiment 1

To provide plants for the experiment, seeds of E- cv. Forager and E+ cv. G1-307 were sown in pasteurised soil in 100 cm^2 pots and grown at 16-18°C (±2°C) in a greenhouse. After 10 weeks, 5 leaf sheaths from each plant were stained and examined at x100 to x400 for fungal hyphae typical of *A. coenophialum* (Siegel *et al.*, 1984; Siegel *et al.*, 1985; Welty *et al.*, 1986). Only plants examined for endophyte were used in the experiment.

In September 1983, 30 plants were transplanted in five rows in six columns on 0.9 m centres, 18 E- plants of Forager in the outer border; 10 E+ plants of G1-307 inside these toward the centre; and two E- plants of Forager in the centre of the planting. Field plot design was similar at each location.

Planting sites were selected for their diversity in environmental conditions to determine if some climatic factors influenced infection rating. Field plots were established in Oregon at the Central Oregon Experiment Station at Redmond (lat. 44°C 267' N, long. 121° 167' W, elevation 906 m), Columbia Basin Agricultural Research Centre at Pendleton (lat. 45° 72' N, long. 118° 63' W, elevation 447 m), Eastern Oregon Agricultural Research Centre at Burns (lat. 43° 58' N, long. 119° 05' W, elevation 1242 m), Hyslop Crop and Soil Science Field Research laboratory at Corvallis (lat. 44° 63' N, long. 123° 20' W, elevation 69 m), Klamath Experiment Station at Klamath Falls (lat 42° 17' N, long. 121° 75' W, elevation 1227 m), Mid-Columbia Agricultural Research and Extension Centre at Hood River (lat. 45° 68' N, long. 121° 52' W, elevation 150 m) and Southern Oregon Experiment Station at Medford (lat. 42° 30' N, long. 122° 87' W, elevation 438 m).

Seeds from plants at each location were harvested when mature, dried to about 12% moisture content (wet-weight basis), threshed, cleaned, and stored at about 20-23°C until examined. In 1984, 1985, and 1986, seeds from 10 plants of E+ G1-307 were blended, 2 g of seed stained, and 100-200 seeds examined for endophyte by a staining procedure previously described (Welty *et al.*, 1986). In 1984 and 1985, seeds from 20 plants of E- Forager were blended, 2 g of seed stained, and 100-200 seeds examined for endophyte. Also in 1985, seeds from the 20 plants of Forager at the Columbia Basin Agricultural Research Centre (CBARC) were harvested, stored separately, and 50 seeds from each plant examined for endophyte. In 1985, 1986, and 1987, seeds from 19 plants of E- Forager were blended, stained, and 200 seeds examined for endophyte. Also in 1985, 50 seeds from an E+ plant of Forager were stained and examined for endophyte.

Experiment 2

Three tests were started in July 1986 at CBARC and identified as CBARC-1, CBARC-2 or CBARC-3. A different planting design was used for each test. CBARC-1 was arranged as a square with one E+ G1-307 plant transplanted in the centre of a three row x three-column design. Eight E- plants of G1-307 were transplanted at right angles 30 cm from the E+ centre plant. The test was repeated six times.

CBARC-2 was similar to CBARC-1 except one E+ Forager plant was transplanted in the centre and eight E- plants of Forager were transplanted at right angles, 30 cm from the centre. The test had six replications.

CBARC-3 was arranged in "+" design with one E+ plant of G1-307 in the centre of the plot surrounded by a barrier of fibreglass buried 90 cm in the soil that extended 10 cm above ground. The barrier was 15 cm from the centre plant. Four E- plants of G1-307 were transplanted at right angles to the centre plant on 30 cm centres (ie 15 cm from the fibreglass barrier). A 900 cm^2 fibreglass box surrounded the E+ G1-307 plant. The experiment was replicated six times.

Leaf sheath tissues of plants used for CBARC-1, CBARC-2 and CBARC-3 in Experiment 2 were examined for endophyte by methods used in Experiment 1. For CBARC-1 and CBARC-3, seeds of E+ G1-307 were freed from endophyte by a method previously reported (Azevedo and Welty, 1990). For CBARC-2, E- and E+ seeds of Forager were harvested from E- and E+ plants growing at CBARC in Experiment 1.

Experiment 3

In the first test (Oregon State University, Hyslop Crop and Soil Science Field Research Laboratory), one plant of E+ G1-307 was burned and one plant was not burned in the fall (autumn) of 1986. In 1987, seeds were harvested from each plant, and 200 seeds were examined for endophyte.

In the second test, 10 rows (15 m) of tall fescue plants

were established in a field near Corvallis, OR (Oregon State University, Botany and Plant Pathology Field Laboratory). Each row contained varying numbers of E+ plants. In late summer of 1985, five rows were burned with a kerosene torch-burner and five rows remained non-burned. In 1986, seeds from each row were hand-harvested, bulked, dried, cleaned and threshed. Two hundred and fifty seeds for each treatment were stained and examined for endophyte as described previously.

RESULTS AND DISCUSSION

Experiment 1

In 1984, seeds were harvested at Corvallis, Hood River, Medford and Pendleton. Seeds failed to develop at Redmond, Klamath Falls, and Burns during the first year because transplanting occurred too late for vernalisation of the plants. Seeds were subsequently harvested at all seven locations in 1985 and 1986.

In 1984, endophyte infection ranged from 89-90% in seeds harvested from E+ G1-307 grown at four locations (Table 1). In 1985 and 1986, endophyte infection ranged from 83-98% in seeds harvested from E+ G1-307 at seven locations. These results indicate that endophyte-infected plants produce endophyte-infected seeds in a variety of growing conditions and at elevations that range from 69 to 1242 m.

The percentage of E+ seeds produced on E+ plants of G1-307 growing at Corvallis and Pendleton-1 was found to decrease over the 5 years of seed collection. Although this study was not designed to evaluate the ability of E+ plants to produce E+ seeds over time, subsequent studies (Welty, unpublished data) indicate that variation among E+ plants exists in their ability to produce E+ seeds. This variability was observed in E+ plants within the same cultivar in different years of production. The commercial importance of this variation is that repeated harvests of seed from the same plant or field of the same cultivar may not result in a consistent percentage of E+ seeds. If there becomes a gradual decline in the percentage of E+ seeds from an E+ cultivar as the age of the field increases, sowing seeds harvested from this field could result in a gradual lowering of the E+ seed content in a cultivar. This would be of special interest to seed production of turf-type cultivars where high amounts of E+ seed are desirable. If the E- plants do not survive as well as E+ plants, as has been reported (Bacon and Siegel, 1988), the situation is self-correcting.

Table 1. The percentage of endophyte-infected seeds harvested from plants of cv. G1-307 and Forager grown at seven Agriculture Research and Experiment Stations in Oregon, 1984-1987.

	Endophyte-infected seed cv. G1-307[1]				Endophyte-infected seed cv Forager[2]			
Experiment Station	**1984**	**1985**	**1986**	**1987**	**1984**	**1985**	**1986**	**1987**
Corvallis	98	88	83	82	0	0	0	0
Pendleton-1	91	92	92	81	8	3	-[3]	-
Pendleton-2	-[4]	-	-	-	-	0[4]	0[4]	0[4]
Hood River	89	95	98	-	0	0	0	-
Redmond	-	96	95	-	-	0	0	-
Medford	95	94	94	-	0	0	0	-
Klamath Falls	-	95	96	-	-	0	0	-
Union	-	95	99	-	-	0	0	-

[1] Seeds from 10 plants were combined and 2 g of seed sampled; 100-200 seeds were examined for endophyte.
[2] Seeds from 20 plants (except Pendleton-2) were combined and 2 g of seed sampled; 100-200 seeds were examined for endophyte.
[3] Seeds not tested.
[4] Seeds from 19 plants of E- Forager at Pendleton-2 were blended and 2 g of seed stained; 200 seeds were examined for endophyte. In 1985, seeds from the E+ Forager plant were stained and examined; 47 of 50 seeds contained endophyte.

In 1984-1987, endophyte infection in seed harvested from E- Forager was 0% at all locations except Pendleton. At Pendleton in 1984, endophyte infection in the blended bulk of seeds from E- Forager was 8%. In 1985, seeds from each of the 20 plants of E- Forager were harvested, kept separate, and 50 seeds from each plant examined for endophyte. Seeds from 19 plants of E- Forager were E-; one plant of Forager was E+, and 47 out of 50 seeds from this plant were E+. In 1985, 1986 and 1987, seeds from the 19 E- plants of Forager were harvested, bulked and blended, threshed and cleaned, and

examined for endophyte. Two hundred seeds from the E- plants were examined and found free of endophyte. Seeds from the E+ plant of Forager were endophyte-infected. Also in 1985, a portion of seeds from the 20 plants of Forager were bulked and blended, and 200 seeds examined for endophyte. Endophyte infection in seeds from this 20-plant mix was 3%. We concluded the single E+ plant of Forager was initially infected but was not detected in the endophyte-staining routine screening before transplanting. During endophyte testing by the Oregon Department of Agriculture, seed lots of Forager have been found to contain 2-4% E+ seeds (Milbrath, pers. comm.).

These results indicate that E- plants of Forager remained free of endophyte when planted 0.9 m from E+ plants of G1-307. The results were consistent for three years at seven locations. Forager and G1-307 had different growth responses in the conditions of this test. Most noticeably, anthesis and pollination dates in G1-307 were 12-15 days later than Forager. To test this anthesis-pollination maturation effect, three tests were done in Experiment 2 using E+ and E- plants of the same cultivar.

Experiment 2

Three tests (CBARC-1, 2 and 3) were done at CBARC with E+ plants of G1-307 and E- plants of Forager. E+ plants produced E+ seeds and E- plants produced E- seeds, regardless of planting design (Tables 2 and 3). No transmission of endophyte occurred from E+ plants to E- plants for either cultivar. CBARC-3 was done to evaluate above ground v. below ground transmission, if endophyte transmission had occurred in either CBARC-1 or CBARC-2. No endophyte transmission from E+ G1-307 to E- G1-307 occurred in CBARC-3, supporting results from CBARC-1 and CBARC-2.

Seed harvested from the E+ plant of G1-307 and Forager was 76% and 20% E+, respectively (Table 2). Plant-to-plant variation in the capacity of an E+ plant to produce E+ seed was also observed in this experiment. Reasons for this variation are unclear. Perhaps as shoots in the apical meristem differentiate into bud primordia and florets develop, not all ovaries become invaded by endophyte hyphae. This variation may occur among plants of the same or different cultivars.

Table 2. Endophyte-infection in seeds harvested from one endophyte-infected (E+) plant and eight endophyte-free (E-) plants transplanted on 30 cm centres[1].

Cultivar and plant location		Seeds infected/ seeds examined	Cultivar and plant location		Seeds infected/ seeds examined
G1-307 E +:	centre	38/50[2]	Forager E +:	centre	10/50
G1-307 E -:	north	0/50	Forager E -:	north	0/50
	northeast	0/50		northeast	0/50
	east	0/50		east	0/50
	southeast	0/50		southeast	0/50
	south	0/50		south	0/50
	southwest	0/50		southwest	0/50
	west	0/50		west	0/50
	northwest	0/50		northwest	0/50

[1] Endophyte-infected plant was in the centre of a three-row and three-column planting.
[2] Seeds were harvested from each location (E+ or E- plant) in six replications and combined.

Table 3 Endophyte infection in seeds harvested from endophyte-infected (E+) and endophyte-free (E-) plants transplanted on 30 cm centres[1].

Cultivar	Location	Seeds infected/ seeds examined[2]
G1-307 E+	Centre	26/50
G1-307 E-	North	0/50
	East	0/50
	South	0/50
	West	0/50

[1] The E+ plant was separated from E- plants by a fibreglass barrier.
[2] Seeds were harvested from each location (E+ or E- plant) in six replications and combined.

Data from Experiments 1 and 2 support the conclusion that under the conditions in these studies, *A. coenophialum* does not spread from infected plants to non-infected plants. These results confirm other reports concerning the lack of field transmission of endophytes from E+ plants to E- plants (Neill, 1940; Siegel *et al.*, 1984; Siegel, Varney, Johnson, Nesmith, Buckner, Bush, Burrus and Hardison, 1984).

Experiment 3

In the first test of field-burning v. endophyte content (Hyslop Crop and Soil Science Field Research Laboratory), 187 of 200 (94%) seeds of burned E+ G1-307 contained endophyte, and 164 of 200 (82%) seeds of non-burned E+ G1-307 contained endophyte. In the second test, 102 of 250 seeds (41%) from burned plants and 119 of 250 seeds (48%) from non-burned plants contained endophyte, respectively (Table 4). These results indicate burning plants did not reduce the percentage of endophyte-infected seeds produced by an E+ plant. Other work (Welty *et al.*, 1986) describes endophyte hyphae in crown meristems of tall fescue. It seems likely that unregulated fires (ie temperatures not controlled) that kill endophyte hyphae in crown meristems would also kill the meristem. Use of fire for removal of post-harvest crop residue does not kill the meristem and is unlikely to kill the endophyte.

Table 4. Number of endophyte-infected seeds harvested from five rows of burned and five rows of non-burned *Festuca arundinacea*.

Dry matter residue	Row number					
	1	2	3	4	5	Total
Burned	38/50[1]	16/50	3/50	1/50	44/50	102/250
Non-burned	37/50	30/50	1/50	5/50	46/50	119/250

[1] Number of seeds with endophyte/number of seeds examined.

The percentages of E+ seed harvested from burned and non-burned E+ plants were lower than the percentage of E+ seed harvested from E+ plants reported in Table 1. This is most likely due to the different amounts of E+ seeds in the seed lots used to establish the rows. The identity of the original seed lots used is unknown; however, rows 3 and 4 were of a low E+ cultivar, row 2 was a low-moderate E+ cultivar, row 1 was of a high-moderate E+ cultivar, and row 5 was a high E+ cultivar. When the five rows were used as replications to compare the effect of burning v non-burning, there was no significant ($P = 0.299$) difference between the E+ seed means from burned (41%) v. non-burned (48%) plants.

We conclude from this study that *A. coenophialum* is not transmitted from E+ to E- plants grown in the field in these conditions; and in a limited study, field-burning of tall fescue for residue management has no effect on the amount of endophyte in seed harvested the following year.

REFERENCES

1. Azevedo, M.D. and Welty, R.E. 1990. Use of propiconazole for removal of *Acremonium coenophialum* from germinating tall fescue seedlings and its effects on plant growth. *Proceedings of the First International Symposium on Acremonium/Grass Interactions*, New Orleans, Louisiana, np.

2. Bacon, C.W. and Siegel, M.R. 1988. Endophyte parasitism of tall fescue. *Journal of Production Agriculture* 1: 45-55.

3. Bush, L.P., Boling, J.A. and Yates, S.G. 1979. Animal Disorders. In: Tall Fescue (ed. T.C. Buckner and L.P. Bush), 247-292, American Society of Agronomy, Madison, Wisconsin.

4. Clark, E.M., White, J.F. and Patterson, R.M. 1983. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of *in vitro* culture of the fungus. *Journal of Microbiological Methods* 1: 149-155.

5. Conklin, F.S., Young, W.C. III, and Youngberg, H.W. 1989. Burning grass seed fields in Oregon's Willamette Valley: The search for solutions. Oregon State University Extension Service, Oregon Agricultural Experiment Station, Corvallis. 68 pp.

6. Cook, R., Lewis, G.C. and Mizen, K.A. 1991. Effects on plant-parasitic nematodes of infection of perennial ryegrass, *Lolium perenne*, by the endophytic fungus, *Acremonium lolii*. *Crop Protection* 10: 403-407.

7. Cromey, M.G. and Cole, A.L.J. 1984. An association

between a *Lolium* endophyte and *Drechslera dictyoides*. *Transactions of the British Mycological Society* 83: 159-161.

8. Gardner, G.B. and Cornell, C.N. 1978. Fescue foot in cattle. In: Mycotoxic fungi, mycotoxins, mycotoxicoses: an encyclopedic handbook. Vol. 2. (ed T.D. Wyllie, L.G. Morehouse), 45-62, Dekker, New York.

9. Gwinn, K.D., Collins-Shepard, M.H. and Reddick, B.B. 1991. Tissue print-immunoblot, an accurate method for the detection of *Acremonium coenophialum* in tall fescue. *Phytopathology* 81: 747-748.

10. Heinmann, E.D., Garrett, L.W., Lock, W.E., Morris, J.S. and Pfander, W.H. 1981. Selenium and reproductive abnormalities in pregnant pony mares grazing fescue pastures. *Proceedings of the 7th Equine Nutrition Physiology Symposium*. 247 pp. Warrenton, Virginia.

11. Hemkin, R.W., Jackson, J.A. Jnr and Boling, J.A. 1984. Toxic factors in tall fescue. *Journal of Animal Science* 58: 1011-1016.

12. Hill, N.S., Stringer, W.C., Rottinghaus, G.E., Belesky, D.P., Parrott, W.A. and Pope, D.D. 1990. Growth, morphological, and chemical component responses of tall fescue to *Acremonium coenophialum*. *Crop Science* 30: 156-161.

13. Hurley, R. 1991. Endophytes: insurance against insects. *Landscape Management* 30: 26-28.

14. Johnson, M.C., Bush, L.P. and Siegel, M.R. 1986. Infection of tall fescue with *Acremonium coenophialum* by means of callus culture. *Plant Disease* 70: 380-382.

15. Johnson, M.C., Pirone, T.P., Siegel, M.R. and Varney, D.R. 1982. Detection of *Epichloe typhina* in tall fescue by means of enzyme-linked immunosorbent assay. *Phytopathology* 72: 647-650.

16. Latch, G.C.M. and Christensen, M.J. 1985. Artificial infection of grasses with endophytes. *Annals of Applied Biology* 107: 17-24.

17. Muhle, E. and Frauenstein, K. 1970. Observations dealing with the occurrence of the choking fungus, *Epichloe typhina* (Per.) Tul on forages. (In German) *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* 77: 177-185.

18. Neill, J.C. 1940. The endophyte of ryegrass *Lolium perenne*. *New Zealand Journal of Science and Technology* Sect. A 21: 280-291.

19. Pedersen, J.F., Rodriguez-Kabana, R. and Shelby, R.A. 1988. Ryegrass cultivars and endophyte in tall fescue affect nematodes in grass and succeeding soybean. *Agronomy Journal* 80: 811-814.

20. Saha, D.C., Jackson, M.A. and Johnson-Cicalese, J.M. 1988. A rapid staining method for detection of endophytic fungi in turf and forage grasses. *Phytopathology* 78: 237-239.

21. Siegel, M.R., Johnson, M.C., Varney, D.R., Nesmith, W.C., Buckner, R.C., Bush, L.P., Burrus, P.B.II., Jones, T.A. and Boling, J.A. 1984. A fungal endophyte in tall fescue: Incidence and dissemination. *Phytopathology* 74: 932-937.

22. Siegel, M.R., Latch, G.C.M. and Johnson, M.C. 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: Significance and control. *Plant Disease* 69: 179-183.

23. Siegel, M.R., Latch, G.C.M. and Johnson, M.C. 1987. Fungal endophytes of grasses. *Annual Review of Phytopathology* 25: 293-315.

24. Siegel, M.R., Varney, D.R., Johnson, M.C., Nesmith, W.C., Buckner, R.C., Bush, L.P., Burrus, P.B. II. and Hardison, J.R. 1984. A fungal endophyte of tall fescue: Evaluation of control methods. *Phytopathology* 74: 937-941.

25. Stuedemann, J.A., Wilkinson, S.T., Williams, D.J., Jackson, W.A. and Jones, J.B. 1973. The association of fat necrosis in beef cattle with heavily fertilized fescue pastures. *Proceedings of the Fescue Toxicity Conference*, Lexington, Kentucky, 9-23.

26. Wallner, B.M., Booth, N.H., Robbins, J.D., Bacon, C.W., Porter, J.K., Kiser, T.E., Wilson, R. and Johnson B. 1983. Effect of an endophytic fungus isolated from toxic pasture grass on serum prolactin concentrations in the lactating cow. *American Journal of Veterinary Research* 44: 1317-1322.

27. Welty, R.E., Azevedo, M.D. and Cook, K.L. 1986. Detecting viable *Acremonium* endophytes in leaf sheaths and meristems of tall fescue and perennial ryegrass. *Plant Disease* 70: 431-435.

28. Welty, R.E., Barker, R.E. and Azevedo, M.D. 1991. Reaction of tall fescue infected and non-infected by

Acremonium coenophialum to *Puccinia graminis* subsp. *graminicola*. *Plant Disease* 75: 883-886.

29. Welty, R.E., Milbrath, G.M., Faulkenberry, D., Azevedo, M.D., Meek, L. and Hall, K. 1986. Endophyte detection in tall fescue seed by staining and ELISA. *Seed Science and Technology* 14: 105-116.

30. Western, J.H. and Cavett, J.J. 1959. The choke disease of cocksfoot (*Dactylis glomerata*) caused by *Epichloe typhina* (Fr.) Tul. *Transactions of the British Mycological Society* 42: 298-307.