APPEARANCE AND MOVEMENT OF FUNGAL ENDOPHYTE IN ANNUAL RYEGRASS

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ABSTRACT
In this study we investigated the efficiency of fungal endophyte (*Acremonium loliii*) transmission from plant to seed in annual ryegrass (*Lolium multiflorum*). Results indicated that the endophyte begins to move up or grow up the plant at about growth stage 30 of Zodoks scale, when day temperatures were near 25° C. In our study, we found that the endophyte was not transmitted to all seed for two reasons. Most importantly, the endophyte was not transmitted into some seedheads even though the plant was infected, indicating the tiller was not infected. Secondly in some infected seedheads, a very small number of seed were not infected, indicating that the endophyte was not transmitted into a seed.

KEYWORDS
Acremonium lolii, *Lolium multiflorum*, annual rye grass

INTRODUCTION
Several cultivars of annual ryegrass (*Lolium multiflorum*) are reported (Nelson and Ward, 1990) to be partially infected with the fungal endophyte (*Acremonium lolii*). This endophyte is found in both annual and perennial ryegrass, however, apparently different races of the endophyte occur in annual, than are found in perennial ryegrass. Nelson, *et al.*, 1995, also reported significant forage yield increases in E+ ryegrass, compared to E- breeding lines. Nelson, et al., 1993, reported a significant reduction in greenbug injury in endophyte-infected (E+) annual ryegrass compared to uninfected ryegrass (E-). The presence of the endophyte can be determined in plants by microscopic observation and identification of mycelium in certain plant tissue such as leaf sheath, nodes, etc. and also in the seed. Little information is available with annual ryegrass in regard to which stage of maturity the endophyte can be observed in plant tissue. We have also selected endophyte infected seed from spaced plants, and uninfected seed from other plants to develop E+ and E- populations. During this identification process, we noted that it was normal for some plants to produce both E+ and E- seed. Because there was little information available on the efficiency of the endophyte plant-to-seed transmission, the following study was conducted. The objectives were to determine at which stage of maturity of the ryegrass plant can the endophyte be observed and identified as fungal mycelium in the leaf sheath, and second, to determine the efficiency of fungal transmission of field grown plants to individual tillers and seed

MATERIALS AND METHODS
An annual ryegrass breeding population designated TXR95-1 was selected for this study. Through several cycles of selecting seed from infected plants, TXR95-1 was thought to be 100% infected. Seed were germinated in petri dishes in October of 1995, and planted into soil in peat cups in the greenhouse. We would normally transplant these plants after about 1 month in the greenhouse into the field. Very dry growing condition in 1995 resulted in delaying transplanting until 1 December. Drought stress continued for the next 3 months; however, sufficient plants survived to test these plants for endophyte infection. December through February temperatures are quite variable at our location with day temperatures averaging near 15°C and night time temperatures near 0°C. The first plant endophyte screening procedure was conducted on 4 April, 1996. Day-time temperatures had been near 25°C for several days and plants were actively growing. Plants had been tillering throughout the winter, with most plants having from 10 to 30 tillers. The average growth stage was about 30 as measured by the Zodoks scale. We had waited until this growth stage because earlier research had indicated that presence of the endophyte could not be determined during early growth stages in our environment. Two tillers (one from center of crown, and the other from the outside) were removed from 15 plants. These tillers were taken to the laboratory and two or more epidermal peels from the leaf sheath of each tiller was examined with a microscope. This procedure is similar to that reported by Saha *et al.*, 1988. On 22 April, a second set of tillers were collected. Plant maturity was approximately at stage 32 to 34. At plant maturity or growth stage 69 to 90, all heads were harvested with a hand sickle, placed in a paper sack and stored for later testing. Ten spikes from each plant were hand threshed, but kept separate, and ten seed were tested as followed. Seed were placed in 5% NaOH for 15 hr, washed for 1 to 2 minutes, or less, stained in .25% Rose Bengal stain, and then placed on a slide, squashed and tissue observed for presence of mycelium near starch granules.

RESULTS AND DISCUSSION
The dry growing conditions delayed transplanting of plants which may have affected development of the endophyte in the ryegrass plants. Two plants (4 & 9) were lost during the study and data were discarded. The first sampling date was 4 April (Table 1). At this date we detected endophyte mycelium in 6 of 13 plants tested. During the screening procedure, several epidermal peels were required to find any endophyte mycelium and as shown in plant 11 and 13, we could not find mycelium in some tillers. With several other plants, no endophyte could be observed. This indicates that the endophyte was just beginning to grow up the plant during growth stage 30. This was when the growing point was about 1 cm above the soil surface. On the second screening date, we observed endophyte in all plants which as it turned out were E+ (Table 1). Plant 2 was the only plant in which we did not find the presence of the endophyte in one tiller.

The efficiency of endophyte transmission from plant to seed is shown in Table 2. If a seed sample of bulked seed had been taken, the percent infected seed would have been approximately 87% as the data shows. The bulk had been estimated at 100% E+, however we know that some E- seed almost always are found in all bulks. Data indicated that one plant, number 3, was not infected. This resulted in a significant number of seed (13%) which were E-. E- seed were produced by E+ plants, and this occurred for two reasons. First, for some reason the endophyte was not transmitted or did not develop in some tillers, and therefore no E+ seed were produced by those seed heads. Note plant 13 and 14, which had three and one seed head respectively which had no infected seed (tillers) in them. This resulted in a fairly large number of E- seed being produced. The second method E- seed were produced by E+ plants was when the endophyte was not transmitted into a seed from an infected seed head. This resulted in only a few E- seed being produced. Therefore our data indicated that the endophyte may not be efficient in transmission through the plant into developing seed. The most likely cause of this was that in some tillers, the endophyte does not move up the tiller to the seed head.

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Table 1
Date endophyte mycelium was detected in leaf sheath tissue and seed of 13 plants.

Plant Number	April 4 Tiller Location		April 22 Tiller Location		May 24 In Seed
	Middle	Outside	Middle	Outside	
1	-[z]	-	+	+	+
2	-	-	+	-	+
3	-	-	-	-	-
5	-	-	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
10	+	+	+	+	+
11	-	+	+	+	+
12	-	-	+	+	+
13	+	-	+	+	+
14	-	-	+	+	+
15	-	-	+	+	+

[z]+ indicates presence of endophyte and - indicates no endophyte was observed.

Table 2
Endophyte detected in seed of seed heads from 13 annual ryegrass plants.

Plant Number	% E+ Seed	Number E+ Seed Heads	Number E- Seed per Seed Head
1	98	10/10W	1; 1[x]
2	91	10/10	1; 2; 3; 3[x]
3	0	0/10	-[y]
5	100	10/10	-[z]
6	100	10/10	-[z]
7	99	10/10	1[x]
8	99	10/10	1[x]
10	99	10/10	1[x]
11	96	8/8	1; 2[x]
12	98	9/9	2[x]
13	67	6/9	10; 10; 10[x]
14	90	9/10	10[x]
15	100	10/10	-[z]
Mean	87%		

[w] Indicates number of infected seed heads/total seed heads examined per plant.
[x] Indicates number of seed not infected out of 10 seed inspected per head. All other seed were 100% infected.
[y] All seed were negative for endophyte for this plant.
[z] All seed were infected with endophyte for this plant.