

INTERACTION OF SYMBIONT ALKALOID ACCUMULATION AND GROWTH

L.P. Bush¹, M.A. do Valle Ribeiro², J. Hogan² and P.B. Burrus, II¹

¹Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091, USA

²Teagasc, Oak Park Research Centre, Carlow Ireland

ABSTRACT

Several classes of alkaloids are present in tall fescue (*Festuca arundinacea* Schreb.) and the tall fescue-endophyte (Neotyphodium [=Acremonium] coenophialum [Morgan-Jones and Gams] Glenn, Bacon and Hanlin comb.nov.) association. Experiments were conducted to determine the effect of the endophyte on seed germination and seedling growth and to measure the effect of host perloine concentration on endophyte development and loline accumulation. Seed germination was not altered by the presence of the endophyte. In non-stress field conditions of Ireland emergence of first leaf occurred sooner and dry weight was greater in endophyte infected seedlings than endophyte free seedlings, whereas no significant differences were observed for days to emergence, tiller number, shoot length or number of leaves between the endophyte treatments. The numbers of mycelial strands in leaf sheaths were positively associated with loline accumulation, but perloine content had no effect on the number of mycelial strands in leaf sheaths or on loline accumulation.

KEYWORDS

Tall fescue, Acremonium, Neotyphodium, Festuca, lolines, perloine

INTRODUCTION

The presence of the endophytic fungus (*N. coenophialum*) in tall fescue has often been related to enhanced host production and insect, nematode and mammalian toxicity (Siegel and Bush, 1994). Desirable properties of endophyte infected grasses have been characterized as improved plant growth, i.e., increased tiller number, dry weight accumulation, tolerance to water and temperature stress, tolerance to over-grazing by herbivores, and tolerance to attack by insects. Alkaloids in the symbiotum have been characterized generally as the causal agents for animal toxicity (Porter, 1995; Siegel and Bush, 1994). Saturated N-amino pyrrolizidine alkaloids (lolines) are found in the symbiotum in greatest amounts and have been shown to have insecticidal activity (Riedell et al., 1991). Perloine is an alkaloid produced by tall fescue and accumulates under genetic control of the plant (Buckner et al., 1973). Perloine has been shown to inhibit growth of many bacteria, fungi and other lower organisms in vitro. However, the relationship between the presence of perloine and the development of the endophyte in the grass and the accumulation of lolines has not been elucidated.

The objectives of this research, in the relatively non-stress environment of Ireland, were (1) to determine the effect of the endophyte and seed quality on tall fescue seed germination and plant development and (2) to measure the effect of perloine concentration on endophyte development.

MATERIALS AND METHODS

Endophyte free and infected seeds of tall fescue "KY 31" were produced at Lexington, KY during the growing season of 1986. The two groups of seeds were each separated into 3 lots based on seed weight. Endophyte-free seeds were divided into lots with seed weights of 2.10, 1.80, and 1.67 g 1000 seed⁻¹. Endophyte infected seeds were divided into lots with seed weights of 2.02, 1.72, and 1.50 g 1000 seed⁻¹.

Germination of each of the six seed lots was determined at 8, 18 or 28°C in the dark. Six replications with 50 seeds in each replication of each seed lot were placed in each temperature. Germination was

determined to have occurred if a normal root had protruded from the seed and the coleoptile was 1 cm long. The 18 and 28°C tests were completed in 14 days and the 8°C test was terminated after 122 days.

Growth studies were conducted in 4x4x6 cm pots placed on a cement platform outdoors at the Oak Park Research Centre in Carlow, Ireland from 15 June 1987 to 8 October 1987. Six replications with 40 individual pots per replication of each seed lot were placed in a RCB design. Days to coleoptile, first leaf, and first tiller emergence were recorded. At 4, 5, 8, 10, 12, and 16 weeks after planting the shoot length, number of collared leaves and number of tillers per plant were measured. Dry weight per plant was determined after 16 weeks. There were 240 plants for each seed lot and 720 plants for the endophyte absent and endophyte present comparison.

Twenty-five plants from each of 6 crosses segregating for perloine content were grown in the field at the Oak Park Research Centre. Leaf sheaths from 5 tillers from each plant were sampled to determine the number of mycelial strands per mm transverse-distance of sheath. Numbers of mycelial strands in two adaxil epidermal peels were counted from each sheath after staining with lactophenol-cotton blue. Additional leaf sheaths (pseudostems) from each plant were taken for perloine and N-formyl- and N-acetyl- loline determinations.

RESULTS AND DISCUSSION

The three temperatures chosen for the germination studies represented a low temperature stress, a nearly ideal temperature, and a high temperature stress for germination of tall fescue seeds. Temperature did affect the rate of germination and the heavier seed lots germinated faster but there was no difference between seeds with or without the endophyte (Fig. 1). Seeds with the endophyte absent had a greater percentage of germination during the later days of the 18 and 28°C tests. Seeds that had not germinated during the 122 day trial at 8°C did germinate greater than 90% within 11 days after being placed at 20°C.

Growth and development of endophyte infected seedlings and seedlings with the endophyte absent were not greatly different in the minimum stress environment at Carlow, Ireland. The high temperature never exceeded 26°C and the lowest minimum temperature was 1°C. Maximum sunlight was 15.3 h and greatest evaporation was 10.5 mm day⁻¹.

There were the expected differences between seed lots with different seed weight as the heavier seeds had greater seedling vigor. However, few significant differences were found between plants with and without the endophyte (Table 1). Seedlings with the endophyte present tended to have greater leaf and tiller numbers than endophyte free seedlings that was expressed in significantly greater shoot dry weight per plant at the termination of the experiment.

Perloine content of the plants examined for mycelial strands ranged from 0 to 3195 (g g⁻¹ dw with an average of 762 (g g⁻¹. Mycelial stands ranged from 0 to 32.4 mm⁻¹ with an average of 14.4. N-acetylloine ranged from 0 to 195 (g g⁻¹ with an average of 47 (g g⁻¹ and N-formylloine ranged from 0 to 1277 (g g⁻¹ with an average of 338 (g g⁻¹.

The correlation between perloine content and numbers of mycelial strands was not significant. The correlations between mycelial strands and the pyrrolizidine alkaloids were positive and significant. The *r* values were low - 0.44, 0.49, and 0.52 - for the correlations between mycelial strands and N-acetylloine, N-formylloine and their sum, respectively. The predictive equations for ug g-1 lolines from the number of mycelial strands per mm leaf sheath were: N-acetylloine = -5.87 + 3.699 x number of mycelial strands; N-formylloine = 62.73 + 19.42 x number of mycelial strands; N-formyl- + N-acetyl- loline = 56.86 + 23.12 x mycelial strands mm-1 leaf sheath.z

REFERENCES

Buckner, R.C., L.P. Bush and P.B. Burrus, II. 1973. Variability and heritability of perloine in *Festuca* sp., *Lolium* sp., and *Lolium-Festuca* hybrids. *Crop Sci.* **12**: 666-669.

Porter, J.K. 1995. Analysis of endophyte toxins: Fescue and other grasses toxic to livestock. *J. Anim. Sci.* **73**: 871-880.

Riedell, W.E., R.E. Kieckhefer, R.J. Petroski and R.G. Powell. 1991. Naturally-occurring and synthetic loline alkaloids: insect feeding behavior modification and toxicity. *J. Entomol. Sci.* **26**: 122-129.

Siegel M.R. and L.P. Bush. 1994. Importance of endophytes in forage grasses, a statement of problems and selection of endophytes. Pages 135-150 in C.W. Bacon and J.F. White, Jr. ed. *Biotechnology of endophytic fungi of grasses*, CRC Press, Boca Raton, FL.

Table 1
Comparison of growth paramenters of KY 31 tall fescue seedlings with *N. coenophialum* absent or present grown in small pots (field environment) at Oak Park Research Centre, Carlow, Ireland

		<u>Endophyte</u>	
		Absent	Present
Days to Emergence ^z		14.8	13.9
Days to emergence of 1st Leaf		22.5	20.9
Number of leaves at	10 week	7.2	7.6
	12 weeks	7.8	8.5*
	16 weeks	9.8	10.3
Tiller number at	10 weeks	4.3	4.5
	12 weeks	4.5	4.7
	16 weeks	4.9	5.3*
Shoot length (cm) at	10 weeks	14.8	14.7
	12 weeks	15.7	15.6
	16 weeks	16.5	16.4
Shoot dry wt per plant (g) at	16 weeks	.55	.61*

^zSeeded by June 1987

*Values on the same line are significantly different at P < 0.005

Figure 1

Percent germination of KY 31 tall fescue seed averaged across the three different seed weight lots within each of the germination temperature of 8, 18, and 28°C. Seedlots with the endophyte are represented by — and without the endophyte by - - - - -.

