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PERLOLINE, THE FORGOTTEN PLANT ALKALOID

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Abstract

Perloline, a biologically active plant alkaloid, accumulated in vegetative tissue of tall fescue during the summer months. Perloline content increased with increased available nitrogen and light. Total perloline accumulation was greatest with NO_3-N fertilizer, but greatest perloline content per unit of dry wt was measured with NH_4-N. In mature plants greatest perloline accumulation occurred in the leaves and immature inflorescences, but was not detected in the seed. Leaves of meadow fescue contained the greatest amounts of perloline, tall fescue was intermediate and giant fescue, and ryegrasses and yellow foxtail had low amounts of the alkaloid. Tryptophan and ornithine were efficient precursors of perloline biosynthesis.

Keywords: alkaloid biosynthesis, tall fescue, ryegrass, meadow fescue

Introduction

Perloline is a plant alkaloid found in many grass species and the most important alkaloid containing a diazaphenanthrene skeleton. Perloline is the principal plant alkaloid of tall fescue (*Festuca arundinacea* Schreb.) and the toxicology has been reviewed by Bush et al, 1979). Perloline does inhibit in vitro ruminal digestion of cattle and sheep. However, we know little about the biosynthesis and accumulation of this alkaloid in forage grasses. Perloline biosynthesis

is controlled primarily by a few major genes with a high degree of dominance for low perloline (Cornelius et al, 1974) and generally, accumulation reaches a maximum during late summer (Bush et al, 1979). The objective of this research was to determine environmental factors that influence accumulation and how biosynthesis and accumulation was altered during the life cycle of the plant.

Material and Methods

Seedlings were grown in a glasshouse in 10 cm deep trays containing a potting mix. Solutions of KNO_3, Ca $(NO_3)_2$ and NH_4Cl were used to provide different levels of nitrogen fertilizer. Cations were adjusted to equivalent amounts in all treatments. The different light levels were obtained by use of shade cloth. Biosynthetic precursors of perloline were measured by incorporation of ^{14}C-amino acids into the alkaloid. Amino acids were fed to roots of young seedlings. Perloline was isolated and purified from the roots and leaves. Field grown plants were in replicated space plant nursery or swards managed as pasture by clipping. Perloline was determined by the procedure of Bush et al (1970).

Results and Discussion

Perloline content of space plants varied with genotype. Leaves of meadow fescue (*F. pratensis* Huds.) had the greatest concentration, 3350 $\mu g\ g^{-1}$, of perloline. Tall fescue leaves contained approximately 800 $\mu g\ g^{-1}$. Perennial ryegrass (*Lolium perenne* L), Italian ryegrass (*L. multiflorum* Lam.) and giant fescue (*F. gigantea* (L.) Vill.) had between 200 to 600 $\mu g\ g^{-1}$ perloline in leaf tissue. Yellow foxtail (*Setaria lutescens* (Weigel) Hubb.) accumulated the least amount of perloline of the grasses tested. Some of the hybrids between the ryegrasses and

fescues contained over 10,000 μg g^{-1} perloline. During this study, perloline concentration in leaves increased significantly between 15 June and 1 July to greater than 1,500 μg g^{-1}. Levels remained high until 15 October and then decreased to levels of 400 to 700 μg g^{-1} until the next summer.

Distribution of perloline within different parts of the plant varies with plant age. In 28-day old tall fescue seedlings 70% of the perloline was found in the roots and 30% in the leaves. Whereas, in mature flowering plants the relative concentrations were 35, 25, 23, 12 and 3 % for the leaves, inflorescence (50% of spikelets at anthesis), pseudostem, flowering culm and the roots, respectively. Perloline was not detected in seeds.

The effects of nitrogen and light on perloline accumulation were investigated in glasshouse experiments. The standard nutrient solution used to fertilize seedlings contained 84 mg N L^{-1}. The high rate of N fertilization was 840 mg N L^{-1}. Shoot tissue of seedlings grown under both N treatments contained 600 μg g^{-1} 42 days after seeding. However, by day 50 the high N treatment had more than doubled to 1,300 μg g^{-1} while the control only increased 23%. By 82 days after seeding the high N fertilizer treatment had again more than doubled to 2,960 μg g^{-1} and the control had increased only 70% from the original level. Results of the comparison between NO_3-N and NH_4-N sources indicated that at both fertilizer levels, perloline accumulation was greatest with NO_3-N. The differential response was greater for NO_3-N at the low than the high fertilizer treatment. At the low treatment level the average increase of NO_3-N compared to NH_4-N was 40% whereas, for the high treatment the increased response was only one-half the size. However, the response between low and high N treatment was greatest for the NH_4-N treatment.

In these experiments dry wt of seedlings after 21 days was 30 to 50% greater for the NO_3-N treatments compared to the NH_4-N treatments. This increased dry matter production coupled with the increased perloline concentration resulted in a much greater total perloline production and accumulation in the NO_3-N treatments. At the low N and high N treatment the total increased perloline production was 60 and 55% greater, respectively, for NO_3-N compared to the NH_4-N.

To determine the effect of light on perloline production, tall fescue seedlings were grown in 14-hr photoperiod at 24°C and 10 hr darkness at 18°C with 34 and 51 W m^{-2}. irradiance. The highest light level is near light saturation for tall fescue. When the data were averaged across the N treatments there was no response to light early in seedling growth. A small positive response to light for perloline biosynthesis was measured after 21 days of light treatment. However, there were interactions between light, N source and N amount. The positive response of perloline to increased light was measured only in the low N treatments and for NO_3-N. The high N treatments and the NH_4-N source had lower perloline accumulation at the high light treatment compared with the low light treatment.

Based on the structure of perloline, a diazaphenanthrene skeleton with a dimethoxyphenyl moiety attached to the N atom in the 5 position (Fig 1), we hypothesized that tryptophan and dihydroxphenylalanine would be good amino acid precursors for biosynthesis. Several amino acids were fed to the roots of young tall fescue seedlings to measure incorporation into perloline. Perloline was isolated from root and leaf tissue, purified and incorporation of ^{14}C determined. Plants were fed equimolar amounts of each amino acid and equal amounts of ^{14}C. Tryptophan and phenylalanine had greatest incorporation into perloline in the root tissue of young tall fescue seedlings (Table 1). Other amino acids usually associated with alkaloid biosynthesis were not

efficient precursors for perloline biosynthesis. The ^{14}C in perloline in the leaf tissue was much less, except for ornithine. Translocation of perloline from root to leaf does not explain this difference and suggests translocation of ornithine to the leaf and either incorporation into a metabolically active pool of tryptophan for perloline biosynthesis or a different biosynthetic pathway in the leaf than the root.

Perloline is an important alkaloid in many forage grasses and probably affects animal performance. Many of these forage grasses have fungal endophytes and the alkaloids associated with the endophytes have received much more research attention in the recent past. Grass/endophyte associations with decreased animal toxicity are or will soon be available and the significance of perloline must be reexamined because of previously demonstrated toxicity. Greatest accumulation of perloline occurs during the season of most frequently observed decreased bovine performance. Also, some of the fescue-ryegrass hybrids have extremely high levels of perloline accumulation, well within a rumen concentration to inhibit digestion.

References

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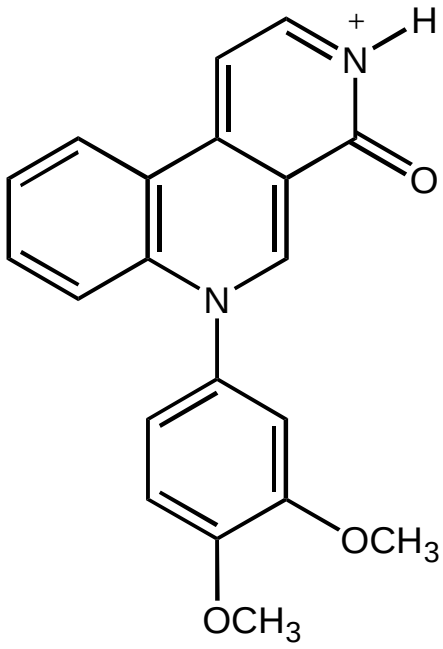

Perloline

Figure 1 – Structure of the diazaphenanthrene alkaloid perloline

Table 1 - Incorporation of ^{14}C amino acids into perloline in roots and leaves of 16 day old seedlings of tall fescue.

Amino acid	dpm mg^{-1} perloline	
	Roots	Leaves
Tryptophan–7a-^{14}C	91,700	24,900
Tryptophan-2-^{14}C	58,200	4,300
Ornithine-2-^{14}C	13,900	51,500
Phenylalanine-3-^{14}C	32,300	3,000
Dihydroxyphenylalanine-2-^{14}C	5,500	800
Lysine-2-^{14}C	5,300	3,300
Aspartic acid-UL	800	2,500