Occurrence of Peptide and Clavine Ergot Alkaloids in Tall Fescue Grass

PHILIP C. LYONS, RONALD D. PLATTNER, CHARLES W. BACON
Occurrence of Peptide and Clavine Ergot Alkaloids in Tall Fescue Grass

PHILIP C. LYONS, RONALD D. PLATTNER, CHARLES W. BACON

Evidence is presented that ergot alkaloids are ubiquitous in tall fescue pastures infected with the clavicipitaceous fungal endophyte Sphacelia typhina (or Acremonium coenophialum). Ergopeptide alkaloids, predominantly ergovaline, constituted 10 to 50 percent of the total ergot alkaloid concentration, which was as high as 14 milligrams per kilogram in sheaths and 1.5 milligrams per kilogram in blades. Ergot alkaloid concentrations were substantially increased by application of large amounts (10 millimoles per liter) of potassium nitrate or ammonium chloride to infected plants in the greenhouse. The results indicate that ergot alkaloids are probably responsible for the toxicity to cattle of this common pasture and lawn grass and that ergotism-like toxicoses may be caused by clavicipitaceous fungi other than Claviceps.

TALL FESCUE (Festuca arundinacea Schreb) is the predominant cool-season perennial forage grass in the United States, particularly in the transition zone of the eastern states. Unfortunately, it is frequently toxic to cattle. The most severe form of toxicity, fescue foot, is a gangrene of the animal's extremities that is strikingly similar to ergotism; but it occurs in the absence of the ergot fungus Claviceps (1-4). A less severe but economically more significant toxic manifestation of tall fescue in cattle is the so-called "summer syndrome," which is characterized by weight loss or reduced weight gain, rough hair coat, and increased temperature and respiration (5). The occurrence of these toxic syndromes has been associated with endophytic infection of the grass by another clavicipitaceous fungus, Sphacelia typhina (or Acremonium coenophialum); however, the role of this endophyte in tall fescue toxicity is not presently understood (6-8).

The similarity of fescue foot to ergotism is the basis for postulating that vasoconstrictive substances such as ergot alkaloids, synthesized by the grass or the endophytic fungus associated with it, are responsible for this disorder (9-17). We now report that ergot alkaloids, including several toxic ergopeptide species, are commonly present in all aboveground parts in infected tall fescue.

To establish whether ergot alkaloids are commonly associated with endophytic infection, we obtained samples for analyses from eight infected and two uninfected pastures in northern Georgia. All the pastures except one were sampled once between June and October 1984 (one infected pasture was sampled twice, first in December 1983 and again in June 1984 after flowering). Several of the infected pastures had recent histories of toxicity. We estimated the infection levels in the pastures by staining sheath sections (5) with p-dimethylaminobenzaldehyde (18); ergopeptide alkaloids were identified and measured by tandem mass spectrometry (MS) (Finnigan 4535/TSQ quadrupole mass spectrometer) in the negative chemical-ionization mode. This procedure separates all of the known ergopeptide alkaloids and is sensitive to the picogram level (19-21).

Ergot alkaloids were detected colorimetrically in all infected samples but not in uninfected samples (Table 1). The total ergot alkaloid concentration (micrograms of ergonovine per gram dry weight) varied among the samples from 1.0 to 14 μg/g in sheaths, where the fungus grows extensively, and from 0.4 to 1.5 μg/g in the blades, which are free of infection (Table 1). Ergot alkaloids were also present in inflorescence stems and inflorescences of the sample collected in June when the seeds were at late dough maturity. The concentrations in these tissues were comparable, respectively, to those in blades and sheaths (Table 1, sample 1-B). Both stems and inflorescences are infected by the endophyte.

Tandem MS revealed that ergopeptide alkaloids were present in all infected samples and accounted for 10 to 50 percent of the total ergot alkaloid concentration (Table 1). Five ergopeptide alkaloids were detected, of which three, ergovaline, ergosine, and ergonine, occurred in all samples in both blades and sheaths. These three alkaloids also were present in inflorescences and stems of the sample in which these parts were assayed. Ergoline and ergocornine were detected in only a few samples and in small amounts. Ergopeptide alkaloid concentrations, based on tandem MS of samples spiked with known concentrations of ergovaline, varied from 0.1 to 0.3 μg/g in blades and from 0.3 to 2.8 μg/g in sheaths (Table 1). Ergovaline was the predominant species in all the samples, accounting for 84 to 97 percent of the total ergopeptide alkaloid fraction. Ergosine and ergosine were present in about equal concentrations. All five ergopeptide alkaloids were produced (in about the same relative proportions as in the
Table 1. Concentrations of ergot alkaloids in leaf blades and sheaths of endophyte-infected tall fescue. Each pasture was randomly sampled, and the random samples were pooled to make a composite sample (>500 g). Each value is the mean of duplicate subsamples from the composite samples. The duplicate subsamples averaged ±16% and ±11% of the mean values for total ergot alkaloids and ergopeptide alkaloids, respectively.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Infection (%)</th>
<th>Total ergot alkaloids (micrograms of ergonovine per gram of weight)</th>
<th>Ergopeptide alkaloids (micrograms of ergonovine per gram of dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blade</td>
<td>Sheath</td>
</tr>
<tr>
<td>1-A*</td>
<td>98</td>
<td>1.0 2.8</td>
<td>0.2 1.1</td>
</tr>
<tr>
<td>1-B*</td>
<td>98</td>
<td>0.7 2.1</td>
<td>0.1 1.1</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>0.4 0.7</td>
<td>&lt;0.1 0.3</td>
</tr>
<tr>
<td>3</td>
<td>98</td>
<td>1.2 1.7</td>
<td>0.1 1.0</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0.7 1.3</td>
<td>0.1 0.6</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>0.6 2.3</td>
<td>0.1 0.8</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>7</td>
<td>93</td>
<td>1.4 8.8</td>
<td>0.1 1.0</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
<td>1.5 13.8</td>
<td>0.3 2.6</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>1.1 1.7</td>
<td>0.1 0.3</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

*Samples 1-A and 1-B were collected from the same pasture in December and June, respectively.

It is logical to assume the alkaloids are synthesized by the fungus, not by the grass, since they are produced in vitro by the fungus (16, 24) and are absent from unin­fected samples. The biosynthesis of ergot alkaloids, according to present knowledge, is restricted to fungi with the exception of two plant genera in the Convulvulaceae (25), and the tall fescue endophyte is different from Claviceps and other fungi in its synthesis of ergovaline as the major ergopeptide alkaloid (24). Ergot alkaloids are present in all aboveground parts of tall fescue; the blade is the major part consumed by grazing animals, although at various times all parts are ingested in considerable quantities. The ruminal is very efficient at extracting ergot alkaloids from plant tissue (12). Long-term ingestion of smaller amounts of ergopeptide alkaloids causes less acute symptoms, which become severe in cold, damp weather. Limited experimental data on laboratory animals indicate that ergovaline, which is structurally similar to the vasoconstrictive parent compound ergotamine, is generally more active.

REFERENCES AND NOTES
18. Samples (2 mg) of hyphalized, ground leaf blatt, stem, or leaf sheath, and samples (4 g) of leaf blade were extracted twice with ammonium methan­ol (50 ml of concentrated NH₄OH per 1000 ml of 80% methanol; 25 ml dry weight) and then dried under vacuum. Samples were prepared for colorimetry by the following procedure: the extract was dissolved in 2% tartaric acid (TA) (50 ml), and the solution was transferred to a separatory funnel where it was extracted with x-hexane (20 ml), the hexane solution was then extracted with TA (10 ml), and the combined aqueous TA fractions were adjusted to pH 9; this aqueous solution was...
extracted three times with CHCl₃ (40 ml); the combined CHCl₃ fractions were reduced to about 12 ml under vacuum, and the residue was transferred to a separatory funnel and extracted three times with TA (20 ml); the resulting aqueous fraction was centrifuged to remove any emulsion, adjusted to pH 9, and extracted three times with CHCl₃ (60 ml); the CHCl₃ was removed under vacuum, and the residue was transferred in water (2 ml) to a carboxymethyl-cellulose column (1 cm by 5 cm); the column was rinsed with water (10 ml), and then the alkaoids were eluted with 4M NH₄Cl (15 ml); the eluate was extracted three times with CHCl₃ (15 ml), the combined CHCl₃ extracts were dehydrated with Na₂SO₄, and then the solvent was removed under vacuum. The residue was assayed for total ergot alkaoids with p-dimethylaminobenzaldehyde (PDAB), according to the procedure of L. E. Michelson and W. J. Kelleher [Lloydia 26, 192 (1963)], except that it was dissolved in TA (0.5 ml) and treated first with PDAB (0.5 ml) and then, after 10 minutes, with NaNO₂ (0.1%, 0.1 ml). Ergonovine maleate was used as a standard.

20. R. D. Plattner, S. G. Yates, J. K. Porter, J. Agric. Food Chem. 31, 785 (1983); for tandem MS, an aliquot was removed from the sample just before the column purification step described in (18).


25 May 1985; accepted 19 February 1986