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Occurrence of Peptide and Clavine Ergot Alkaloids in Tall Fescue Grass

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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 toxicosis, 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 endophyte 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 from 40 randomly chosen tillers with aniline blue and examining them microscopically for the fungus. Total concentrations of ergot alkaloids were measured colorimetrically on the basis of the formation of a blue complex with *p*-dimethylaminobenzaldehyde (*18*); ergopeptide alkaloids were identified and measured by tandem mass spectrometry (MS) (Finnigan 4535/TSQ quadropole 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. Ergoptine 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. Ergonine and ergosine were present in about equal concentrations. All five ergopeptide alkaloids were produced (in about the same relative proportions as in the

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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.

Sample	Infection (%)	Total ergot alkaloids (micrograms of ergonovine per gram of weight)		Ergopeptide alkaloids (micrograms of ergovaline per gram of dry weight)	
		Blade	Sheath	Blade	Sheath
1-A*	98	1.0	4.8	0.2	2.8
1-B*	98	0.7	2.1	0.1	1.1
2	60	0.4	0.7	<0.1	0.3
3	98	1.2	1.7	0.1	1.0
4	100	0.7	1.3	0.1	0.6
5	93	0.6	2.3	0.1	0.8
6	0	0	0	0	0
7	93	1.4	8.8	0.1	1.0
8	90	1.5	13.8	0.3	2.6
9	100	1.1	1.7	0.1	0.3
10	0	0	0	0	0

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

grass) by culturing an isolate of the endophyte from sample 1-A. Thin-layer chromatography on silica gel of extracts of an infected sample (Table 1, sample 1-A), with chloroform and methanol (4:1) used as developing solvent, indicated that, in addition to the ergopeptide alkaloids, several ergot alkaloids occurred at R_f values reported for clavine ergot alkaloids (chanoclavines, penniclavine, and agroclavine) (*16*).

The occurrence of ergot alkaloids in all the infected pasture samples indicated that these compounds are commonly present in endophyte-infected tall fescue and that their synthesis in the grass does not require unusual environmental or host conditions. However, among the samples there was more than a tenfold variation in the range of alkaloid concentrations in each plant part, despite the fact that the infection incidence exceeded 90 percent in all but one case (Table 1). This suggested that growth conditions, among other factors, might affect ergot alkaloid accumulation in the infected plant. Since high rates of nitrogen fertilization are conducive to tall fescue toxicity (*5, 22, 23*), we conducted a study in the greenhouse to determine if ergot alkaloid concentrations are affected by the rate of application or form of nitrogen on which endophyte-infected tall fescue is grown.

Analysis after 6 months of 16 infected plants that had received nitrogen fertilization indicated that all contained ergot alkaloids whereas uninfected plants did not. Total ergot alkaloid and ergopeptide alkaloid concentrations in sheaths of infected plants grown at the low rates of nitrogen fertilization were within the range of concentrations that occurred in sheaths of the pasture samples (Table 2). However, analysis of variance indicated that plants grown at the high rates of nitrogen fertilization had significantly higher total concentrations of ergot alkaloids and ergopeptide alkaloids than plants grown at the low nitrogen fertilization rates, although the form of nitrogen, KNO_3 or $(NH_4)_2SO_4$, had no effect. The results suggest that ergot alkaloid concentrations may increase significantly when conditions in the host favor synthesis of these compounds by the fungus.

Table 2. Concentrations of ergot alkaloids in sheaths of endophyte-infected tall fescue grown at low or high rates of fertilization with KNO_3 or $(NH_4)_2SO_4$. Plants were fertilized twice weekly with complete nutrient solution adjusted to 0.5 m*M* (low rate) or 10 m*M* (high rate) nitrogen. Values are means (± SD) of four replicates for total ergot alkaloids and three replicates for ergopeptide alkaloids.

Nitrogen treatment	Ergot alkaloid concentration (μg/g)			
	Total		Ergopeptide	
	Sheath	Blade	Sheath	Blade
Low KNO_3	8.9 ± 2.3	2.5 ± 0.9	1.3 ± 0.9	0.17 ± 0.10
High KNO_3	22.2 ± 5.3	3.5 ± 1.1	2.8 ± 0.2	0.14 ± 0.07
Low $(NH_4)_2SO_4$	9.5 ± 1.9	3.1 ± 0.4	1.3 ± 0.6	0.07 ± 0.01
High $(NH_4)_2SO_4$	17.4 ± 2.8	3.1 ± 0.2	4.0 ± 0.7	0.47 ± 0.07

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 Convolvulaceae (*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 ruminant 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.

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18. Samples (2 g) of lyophilized, ground leaf sheath, stem, or inflorescence, and samples (4 g) of leaf blade were extracted twice with ammoniacal methanol (50 ml of concentrated NH_4OH per 1000 ml of 80% methanol; 25 ml dry weight) and taken to dryness under vacuum. Samples were prepared for colorimetry by the following procedure: the extract was dissolved in 2% tartaric acid (TA) (20 ml), and the solution was transferred to a separatory funnel where it was extracted with *n*-hexane (20 ml); the hexane solution was then extracted twice with TA (10 ml), and the combined aqueous TA fractions were adjusted to *p*H 9; this aqueous solution was

extracted three times with $CHCl_3$ (40 ml); the combined $CHCl_3$ 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 *p*H 9, and extracted three times with $CHCl_3$ (60 ml); the $CHCl_3$ 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 alkaloids were eluted with 4*M* NH_4Cl (15 ml); the eluate was extracted three times with $CHCl_3$ (15 ml), the combined $CHCl_3$ extracts were dehydrated with Na_2SO_4, and then the solvent was removed under vacuum. The residue was assayed for total ergot alkaloids with *p*-dimethylaminobenzaldehyde (PDAB), according to the procedure of L. E. Michelon 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 $NaNO_2$ (0.1%, 0.1 ml). Ergonovine maleate was used as a standard.

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De Gustibus Non Est Disputandem: A Spiral Center for Taste in the Brain of the Teleost Fish, *Heterotis niloticus*

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The teleost fish, *Heterotis niloticus*, has elaborate paired, spiraled pharyngeal structures that aid in concentrating and swallowing food. These epibranchial organs are lined by an epithelium rich in taste buds. Both the taste buds and the muscles of the epibranchial organs are innervated by components of the vagal nerve. Horseradish peroxidase neuronal tracing experiments show that these nerve fibers are connected centrally to an enormous epibranchial portion of the vagal lobes—a special visceral sensory and motor region of the medulla. The epibranchial portion of the vagal lobe is among the most remarkable structures found in the brains of vertebrates, for it is itself a spiral.

A NUMBER OF SPECIES OF TELEOSTEan fishes have paired structures at the posterior end of the pharynx that function as accessory digestive organs aiding in both concentrating and swallowing food particles. These pharyngeal structures, generally known as epibranchial organs (*1–3*), are secondary upgrowths of the walls of the most posterior gill pouch and are intimately associated with the epibranchial portions of the fourth and fifth gill arches. Epibranchial organs occur in some members of four groups of teleosts: Osteoglossiformes (one species), Clupeiformes (many), Gonorynchiformes (possibly all), and Characiformes (some). On the basis of their distribution and their various morphologies, epibranchial organs are believed to have evolved independently in each of these major groups in relation to the repeated evolution of microphagous habits (*1*).

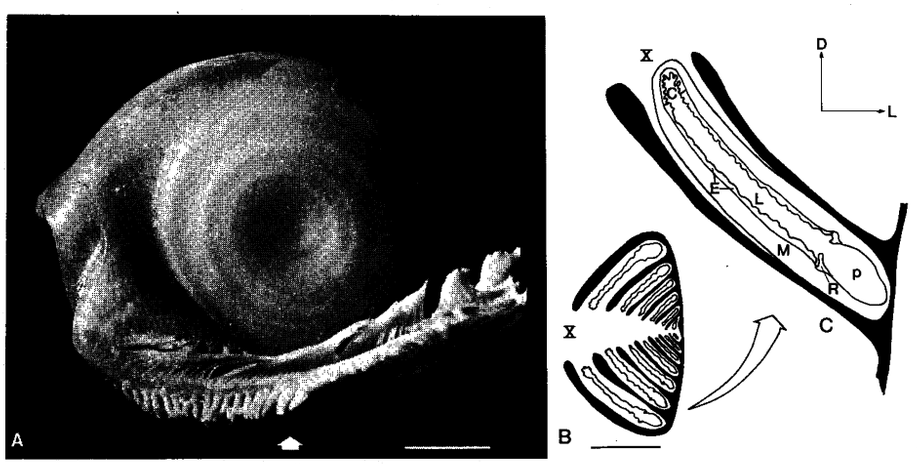

Fig. 1. Epibranchial organ of *Heterotis*. (A) Lateral view of right epibranchial organ and adjoining gill arch from a 27-cm specimen. Scale bar, 0.5 cm. (B) Line drawing of a transverse paraffin section through the epibranchial organ from a slightly smaller specimen. The white arrow in (A) indicates the level of this section. Scale bar, 0.5 cm. (C) Line drawing of a section through a single turn (enlarged) of the epibranchial organ shown in (B). In both (B) and (C) the cartilaginous capsule of the epibranchial organ is shown in solid black. Arrows in the upper right indicate dorsal and lateral directions for both (B) and (C). Abbreviations: C, central portion of the lumen; E, epithelium rich in taste buds and mucous glands; L, lumen; M, striated muscles; p, peripheral portion of lumen; R, modified gill rakers; X, portion of the vagal nerve that innervates the epibranchial organ.

Among the most elaborate of the known epibranchial organs are those of the freshwater African species, *Heterotis niloticus* (family Osteoglossidae). In *Heterotis*, each epibranchial organ consists of a flattened, blind tube that is coiled concentrically on itself, forming a spiral (Fig. 1). The number of turns of the spiral increases with the age and size of the fish, and organs with as many as seven complete revolutions have been reported in large specimens (*4*). Each epibranchial organ resembles a coiled snail shell and has the overall shape of a hemisphere with its convex surface facing medially. The right epibranchial organ forms a left-handed, or clockwise, spiral, and the left organ, a right-handed spiral.

The entire coiled tube is enclosed in a cartilaginous supporting capsule (Fig. 1). Peripherally, the lumen of the tube forms a trough or canal that opens posteriorly into the opercular cavity. This trough is bordered at either edge by a row of modified gill rakers that serve as a sieve or screen separating the peripheral trough from the remainder of the lumen of the tube. Centrally the lumen of the tube forms a small groove that is confluent with the buccal cavity near the esophageal opening. Between the peripheral trough and the central groove the lumen is walled on either side by striated muscles and lined by an epithelium containing many mucous glands and numerous taste buds (*4*).

In teleostean fishes, as in all vertebrate groups, taste buds are innervated by sensory components of the facial (VII), glossopharyngeal (IX), and vagal (X) cranial nerves. The gustatory fibers terminate centrally in the special visceral sensory region of the medulla, which in most teleosts consists of paired longitudinal columns of cells and neuropil that bulge somewhat into the fourth ventricle. In a few groups—notably

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