Toxicity and Occurrence of *Balansia* on Grasses from Toxic Fescue Pastures

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Balansia epichloë (Weese) and *B. henningsiana* (Moell.) were isolated from grasses in toxic fescue pastures. *B. epichloë*, cultured in a synthetic medium, was toxic to chicken embryos. Thin-layer chromatography and ultraviolet absorption data indicated that in submerged culture the fungus produced compounds with the indole or ergoline nucleus.

Tall fescue, *Festuca arundinaceae* Schreb. (*F. elatior* var. *arundinacea* [Schreb.] Celak.), a pasture grass in many parts of the United States, occasionally becomes toxic, and cattle grazing this grass develop a condition known as "fescue foot" (6, 10). Clinical signs of this syndrome have been described (19). These signs are similar in many repects to the gangrenous-type alkaloid poisoning caused by the alkaloids from *Claviceps purpurea* as suggested by Woods et al. (18). Alkaloids from this ergot have been ruled out by other studies (6, 13), and the etiological agent remains unknown. The toxic compound is generally considered to be a vasoconstrictor (5, 13), and the sporadic and seasonal nature of the syndrome suggests that a mycotoxin might be involved. Several fungi, isolated from toxic fescue pastures, produce mycotoxins (9, 11, 20). These studies, however, did not prove that these fungi and their toxins caused the syndrome.

In recent investigations of toxic fescue pastures in Georgia two species of *Balansia* were observed. This paper reports the isolation of these systemic phytopathogens from several species of grasses in these toxic pastures and on the in vitro toxicity of one species.

Balansia epichloë (Wesse), obtained from ascospores, had not previously been cultured in the laboratory so we had to develop a medium which favored germination and growth. This medium consisted of: malt extract (Difco), 5 g; yeast extract (Difco), 20 g; glucose, 20 g; agar, 20 g; and distilled water, 1,000 ml. On this medium germination (60 to 85%) was complete after 3 days. The fungus was stored on agar slants of this medium at 4 C in capped test tubes.

Subcultures and inocula were prepared from these tubes by macerating the contents of one tube in 10 ml of sterile, distilled water. For submerged culture of the fungus a synthetic medium was used: mannitol, 30 g; sucrose, 20 g; KH_2PO_4, 5 g; NH_4NO_3, 2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; a trace element solution, 5 ml (H. J. Vogel, Microb. Genet. Bull. 13:42-43, 1956); thiamin, 0.1 μg/ml and distilled water, 1,000 ml. The inoculum (1 ml) was placed in a 2.8-liter Fernbach flask containing 250 ml of the synthetic medium, and incubated on a gyratory shaker (200 rpm, 1-cm circular orbit) for 28 days at 24 to 28 C.

For routine analysis, the medium was separated from the mycelium by centrifugation (20,000 × *g*, 15 min) and filtered through glass wool, and proteins were removed by ultrafiltration with a hollow fiber concentrator (Amicon Corp., Lexington, Mass.). The dialysate was passed through a glass chromatographic column (18 by 3 cm ID) packed with Porapak Q (80 to 100 mesh; Waters Associates Inc., Milford, Mass.). Under a pressure of 10 lb/in² the column was washed with 300 ml of distilled water and eluted with 300 ml of methanol. One portion of the methanol solution was used for chemical analysis; the other was used for toxicity studies.

The methanol solution used for toxicity studies was evaporated to dryness on a rotary evaporator (40 C), and the residue was dissolved in 3 ml of $CHCl_3$. The residue remaining after $CHCl_3$ removal was dissolved in 3 ml of distilled water. The column bypass was freeze dried, and 1 g was dissolved in 3 ml of distilled water. These fractions were bioassayed for toxicity with fertile White Leghorn eggs (17) by injecting 0.01, 0.025, and 0.05 ml on the air cell before incubation. For controls the same volumes of uninoculated medium and $CHCl_3$ ex-

FIG. 1. *B. epichloë sporulating on smut grass (S. poiretii); insert shows the black ergot-like pseudomorphic ascostromata on the adaxial leaf surfaces of the grass.*

tract of uninoculated medium were tested and found to be nontoxic (background motality ranged from 0 to 5%).

The remainder of the methanol solution was concentrated and applied to thin-layer chromatography plates of silica gel GF 254 by the method of Agurell (2). Chloroform-methanol (4:1, vol/vol) was the developing solvent. After development, the plates were examined under ultraviolet light at 254 nm, and those bands showing a dark blue absorption were marked. The portion of the plate containing the spots was sprayed with a 4-dimethylaminocinnamaldehyde reagent (12). Ultraviolet absorption spectra in ethanol were obtained on the Beckmann spectrophotometer acta CIII.

One toxic fescue pasture was observed for 1.5 years, and during July and August *B. epichloë* was noted sporulating on smut grass (*Sporobolus poiretii* [Roem. and Schult.]

Hitchc.). This weed is a serious problem in pastures in the southeastern United States (16). The fungus also was seen on fescue (*Festuca* sp.), love grass (*Eragrostis hirsuta* [Michx.] Nees), and panicum (*Panicum anceps* Michx.). Cattle were observed to graze all these grasses. This fungus was detected in the grasses by finding the black ergot-like pseudomorphic ascostromata on their adaxial leaf surfaces (Fig. 1). *B. epichloë* was noted in other toxic fescue pastures under investigation. Also in these pastures, *B. henningsiana* (Moell.) was observed growing on abaxial leaf surfaces of *Panicum anceps*, two species of *Andropogon*, and on a species of *Eragrostis*. The laboratory culture of *B. henningsiana* is being investigated.

Sporulation indicated that *B. epichloë* was the more abundant species. In agreement with Diehl (7), infected plants appeared healthy, but grasses that were heavily parasitized by *B.*

TABLE 1. *Toxicity of B. epichloë to chicken embryo*

Fraction	% Toxic		
	10[a]	25[a]	50[a]
CHCl₃	25	70	100
Water	0	0	40
Column bypass	0	30	90

[a] Dosage, microliters per egg. Each dosage is an average of two replications; each replication consists of 20 eggs.

TABLE 2. *Ultraviolet absorption of major compounds isolated from B. epichloë*

Compounds	R_f	λ max (nm)[a]
A	0.67	221, 273, 281, 289
B	0.45	221, 273, 280, 289

[a] Determined in ethyl alcohol.

epichloë did not produce inflorescences. In these plants all the leaves in a clone showed either the asexual ephelidial stage (*Ephelis* state) or the black ascostromata. These fructifications were ephemeral.

B. epichloë was toxic to the chichen embryo (Table 1). Toxicity resided in the CHCl₃, water-soluble, and column bypass fractions. Thin-layer chromatography of the methyl alcohol fraction revealed several bands that absorbed dark blue under ultraviolet light at 254 nm. When sprayed with 4-dimethylaminocinnamaldehyde reagent, several spots gave the characteristic color reaction for ergot-type alkaloids (2). Two of these major components (R_f 0.45) were scraped into glass funnels (fritted disk), and the silica gel was eluted with chloroform-methanol (1:1, vol/vol). The eluate was concentrated to dryness under a stream of nitrogen, and the residual material was taken up in ethyl alcohol. The ultraviolet absorption data of these two compounds (Table 2) were suggestive of the indole (or ergoline) nucleus characteristic of the ergot-type alkaloids (3). We are investigating the structure and toxicity of these compounds. *Balansia* is a clavicipitaceous fungus, and like *Claviceps* might be expected to produce alkaloids. Some new ergot alkaloids have been reported from unrelated genera of fungi (1).

These data suggest that in submerged culture B. epichloë can produce indole compounds in vitro. The morphology and nutritional requirements of the fungus in the synthetic culture medium will be reported elsewhere. The in vivo relationship of this fungus to the host's metabolism during various seasons of the year is unknown. Perhaps the production of toxic compounds is host or season dependent, since the variety of toxic compounds produced by a species of *Claviceps* is host dependent (1). This report is the first to indicate that B. epichloë produces toxic substances in vitro and suggests that the genus, because of its systemic nature, should be considered a potential hazard to

animals grazing on parasitized forage grasses. This endophytic systemic parasite is a better candidate for the cause of fescue foot than the saprophytic fungi studied to date. That *Balansia* may be a problem in forages has been reported from India, where cattle and goats showed signs indistinguishable from ergot poisoning of *Claviceps purpurea* after grazing on lovethron grass (*Andropogon aciculatus*) parasitized with a species of *Balansia* (14). *Claviceps* sp. was not found in that study.

The involvement of *Balansia* in the fescue foot syndrome is complicated by the general elusive nature of the fungus and fescue growth requirements. Cool temperatures favorable for the growth of fescue may suppress sporulation of the fungus. In the spring, we found only a few clones of fescue grass showing the ascostromata of the fungus. Earlier studies indicated that the fungus will not sporulate when grasses are kept at cool temperatures (lower than 20 C) but continue to grow and invade new tissue as its host grows (7). This suggests that parasitized grasses growing in their northern extremes or during cool seasons would not show signs of fungal sporulation, and thus go unnoticed. The fungi on warm-season grasses (smut grass, panicum, etc.) were heavily sporulating. Perhaps a more valid indicator for parasitism would be a test for chitin as proposed by Ride and Drysdale (15) or that suggested by Diehl (7, 8). The significance of *Balansia* has been superficially examined (4, 8). It parasitizes 10 tribes of the American *Gramineae*, many members of which are economically important forage crops (8). In more detailed investigations of this genus, we are studying its economic importance to the growth of forage grasses and to the animals grazing these parasitized grasses.

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