Host Relations of *Myriogenospora atramentosa* and *Balansia epichloë* (Clavicipitaceae)

D. M. Rykard, C. W. Bacon, and E. S. Luttrell

Biology Department, Coker College, Hartsville, SC 19550; Toxicology and Biological Constituents Research Unit, USDA-ARS, Russell Agricultural Research Center, Athens, GA 30613; and Department of Plant Pathology, University of Georgia, Athens 30602.
Accepted for publication 27 March 1985.

ABSTRACT

Rykard, D. M., Bacon, C. W., and Luttrell, E. S. 1985. Host relations of *Myriogenospora atramentosa* and *Balansia epichloë* (Clavicipitaceae). Phytopathology 75:950-956.

Attempts to inoculate *Paspalum notatum* and *P. laeve* with *Myriogenospora atramentosa* and *Sporobolus poiretii* with *Balansia epichloë* through inflorescences, seed, seedlings, leaf whorls, and cut stubble failed. Both fungi were culturable biotrophs on several warm-season perennial grass species. They overwintered in dormant buds and produced systemic infections of new shoots the next year. Mycelium of *M. atramentosa* lay on the surfaces of leaf primordia in buds and growing points of *Eremochloa ophiuroides, P. laeve*, and *P. notatum*. Near the tip on the upper surface of developing leaves it thickened into stromata in which sporocarps were produced after the leaf emerged from the sheath. The fungus did not penetrate the host, and no alterations in the host cuticle could be demonstrated with electron microscopy. Mycelium of *B. epichloë* was intercellular in buds, stems, and leaves of *S. poiretii* and *Chasmanthium laxum*. In localized areas on the upper leaf surface, hyphae emerged between epidermal cells or through stomates and formed a superficial fertile stroma. When hyphae grew laterally beneath the cuticle, electron-transparent pockets appeared in the overlying cuticle, and the cuticle disintegrated.

Additional key words: Balansiae, culturable biotrophs, cuticle digestion, growth alterations, hyphal egress, toxicoses.

Diehl (8) recognized Gäumann's (10) *Epichloë-Claviceps* phylogenetic series in the Clavicipitaceae as the subfamily Clavicipitoideae with the tribes Clavicipiteae, Balansiae, and Ustilaginoideae. These subfamily and tribe names were not validly published and are of questionable utility (24). They serve, however, to point to a difference in parasitism between the Clavicipiteae and the Balansiae. The tribe Clavicipiteae contains only the genus *Claviceps* whose species cause the local infections of grass ovaries resulting in ergot. The tribe Balansiae contains the genera *Epichloë*, *Balansia*, *Balansiopsis*, and *Atkinsonella* whose species cause systemic infections of grasses and sedges. Infected plants are commonly sterile but otherwise show no evidence of infection until the intercellular mycelium emerges from the host in localized areas and forms superficial stromata that bear first conidia and then perithecia (5,8,25). A fifth genus, *Myriogenospora*, in which the mycelium appears to be entirely superficial, was added to the Balansiae by Luttrell and Bacon (19).

Host-fungus relationships in these essentially symptomless, systemic infections caused by species of the Balansiae with their nonhaustoriate, intercellular mycelium and strongly histotrophic sporulation are of obvious theoretical interest (29) but have received little attention because they have been considered of no appreciable economic importance. The only familiar disease is the choke disease of various forage and wild grasses caused by *Epichloë typhina* (Pers.) Tul. which forms its stroma around the sheath of the flag leaf and prevents development of the inflorescence (5). Choke is of minor importance even in forage grasses grown for seed (25). Interest has been excited recently, however, by the association of fungi in the Balansiae with various cattle toxicoses ranging from debility to nervous and gangrenous disorders resembling ergotism and referred to by names such as summer syndrome, fescue foot, ryegrass staggers, and Bermudagrass tremors (1). Results of studies beginning in 1975 (2,3,22) have demonstrated the ability of *Balansia* spp., including *B. epichloë* (Weese) Diehl infecting various wild grasses in pastures with histories of toxicity problems to produce various ergot alkaloids. In 1977, the presence of an endophytic fungus causing a symptomless infection of tall fescue grass (*Festuca arundinacea* Schreb.) (21,27) was correlated with toxicity of the grass (4). In 1981, the presence of a similar endophytic fungus in *Lolium perenne* L. (26) was correlated with ryegrass staggers (9,28). In an early study of *E. typhina* on various grasses Sampson (25) demonstrated systemic infection and survival of the fungus in the dormant buds of perennial grasses. Infected plants of *Festuca rubra* L. rarely produced either the sporodochial conidial state (*Sphacelia typhina* Sacc.) or the stromatic perithecial state. The infection commonly remained symptomless, and the fungus was transmitted through seed produced in apparently normal inflorescences (25). The endophyte causing a similar infection of tall fescue, which remains consistently symptomless, also has been referred to *E. typhina* (4,21). On the basis of growth in culture, in which sporodochia are not produced, the conidial state of *E. typhina* has unjustifiably been removed from *Sphacelia* and renamed *Acremonium typhina* Morgan-Jones & Gams (20). Primarily because of its larger conidia (21), the endophyte from fescue was considered a distinct species, *A. coenophiala* Morgan-Jones & Gams (20). The endophyte from ryegrass (26) has been described, with reservations, as a third species, *A. loliae* Latch, Christensen & Samuels (17). Available evidence from growth within the host (4,12,14,25), morphology of the conidial states in culture (17,20), and serology (13) suggests that *A. typhina, A. coenophiala*, and *A. loliae* are closely related (17). This relationship would be better expressed by placing all three of these species in *Sphacelia*, the generally accepted genus for conidial states of *Claviceps* and *Epichloë* (24). The combinations in *Acremonium, A. coenophiala*, and *A. loliae*, should be used only with the understanding that they do not imply a separation of these fungi from *E. typhina* at a level higher than that of species.

To extend observations on host relationships in the Balansiae, which have been mostly limited to *E. typhina* (5,14,25), a comparative study was made of *B. epichloë*, which was taken as a typical representative of the Balansiae, and *Myriogenospora atramentosa* (Berk. & Curt. apud Berk.) Diehl, which is the most discordant element of the group. Although these observations confirmed previous reports that the mycelium in *M. atramentosa* is entirely superficial, a point by point comparison showed a remarkable degree of similarity between *M. atramentosa* and *B. epichloë*.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

In rare symptomless infections with *E. typhina* (25) and with the fescue and ryegrass endophytes (16,27,28), the fungus is transmitted vertically through the seed. Although the terminal inflorescences of *Danthonia spicata* (L.) Beauv. infected with *Atkinsonella hypoxylon* (Pk.) Tul. are aborted, cleistogamous seed produced in the lower leaf sheaths carry the fungus. In infections of perennial grasses by all species of the Balansiae (7,8,19,25) the fungus may be transmitted through vegetative propagation of the host. The question of the source of the original infection, however, seems absurdly difficult to answer. Western and Cavett (32) obtained infection of *Dactylis glomerata* L. only by applying spores to freshly cut stubble. In a series of inoculations of grass florets with spores of various species, including *B. epichloë* and *M. atramentosa*, Diehl (7,8) had only one success. He (8) obtained infections on three of 20 plants of *Cenchrus echinatus* L. grown from seed produced by plants inoculated with *B. obtecta* Diehl. Consequently, a major effort was made in the present study to obtain infection of healthy plants with *B. epichloë* and *M. atramentosa*. All inoculation experiments failed. These experiments are reported briefly only to record the inoculation methods tried.

MATERIALS AND METHODS

Infected grasses were collected during surveys of pastures with toxicity problems and transplanted in Athens (Clarke County), GA, to outside field plots or into plot culture in a greenhouse. Species, hosts, and original habitats were as follows: *M. atramentosa* on *Paspalum laeve* Michx., Clarke County, on *Eremochloa ophiuroides* (Munro) Hack. (centipede grass), Tift County, and on *Paspalum notatum* Flügge (Bahia grass), Laurin County, GA; *B. epichloë* on *Sporobolus poiretii* (Roem. & Schult.) Hitchc., Newton County, GA, and on *Chasmanthium laxum* (L.) Yates, College Station, TX.

Inoculations. Inoculations with *M. atramentosa* were made on healthy plants of *P. notatum* and *P. laeve* and with *B. epichloë* on *S. poiretii*. Inocula were prepared as aqueous suspensions of conidia produced in culture (23) or as suspensions of conidia or ascospores produced on infected plants. Inocula were misted onto plant parts with a chromatography sprayer, applied with a camel-hair brush, or injected into stems or placed in whorls with a hypodermic needle. Entire inflorescences or individual marked florets were misted or brushed with spore suspensions at anthesis and at various times thereafter. The following materials were inoculated by misting or applying inocula with a hypodermic needle: freshly cut stubble, cut and uncut leaves of 2-, 4-, 6-, and 8-wk-old seedlings, and clones of mature plants. Following inoculations, plants from the greenhouse were placed in a dew chamber at 25 C for 24, 48, or 72 hr or left on the greenhouse bench. Three-month-old uninfected plants were removed from the greenhouse and placed in the laboratory and left unwatered for 3 days to induce stress. The plants were cut back to within 7.5 cm of the soil line, and drops of spore suspension were placed on the cut stubble until they were no longer absorbed by the plant. Plants were placed in a dew chamber at 25 C from 72 to 96 hr.

The following additional inoculations were made with *M. atramentosa*. Leaves of tillers on 3-mo-old uninfected plants from the greenhouse were cut back to within 5.0 cm of the soil line. Pots were placed in a desiccator jar and a spore suspension was placed on the cut surfaces of the plants. A partial vacuum of -1.1 kg/cm^2 (-15 psi) was placed on the plants for three 5-min periods. Each time the vacuum was broken, fresh inoculum was placed on the cut surfaces. Pots were removed from the desiccator and placed in plastic bags under the greenhouse bench for 48 hr. Similar cut tillers with the soil removed were submerged in a beaker of spore suspension in a desiccator jar and similarly subjected to partial vacuum. The plants were potted and placed in plastic bags under a high-energy fluorescent light for 4–5 days. Seeds of *Paspalum notatum* were germinated for 2 wk under a high-energy fluorescent light in a moist chamber on filter paper. Tips of leaves were cut back and seedlings were placed in a beaker containing a spore suspension in a desiccator jar. A partial vacuum of -1.1 kg/cm^2 (-15 psi) was placed on the seedlings for two 5-min intervals. Seedlings were returned to the moist chamber for 48–72 hr, then potted and placed in the greenhouse. The experiment was repeated with seeds and uncut seedlings. After inoculation, seeds were germinated for 3 wk on filter paper in a moist chamber under the fluorescent light, then planted in paper cups and placed in the greenhouse. Additional seeds were germinated in conidial or ascospore suspensions in petri dishes under fluorescent light, transplanted to pots, and placed in the greenhouse. Coleoptiles, intact or punctured with a needle, of seeds germinated on filter paper were misted with a spore suspension every day from 1 to 5 days. Seedlings were placed back in the moist chamber for 48 hr then planted in pots and placed in the greenhouse.

To assess natural infection and seed transmission in *M. atramentosa* and *B. epichloë*, seeds were collected from inflorescences occasionally produced by infected plants and from healthy plants growing in close proximity to diseased plants. The seeds were germinated under fluorescent light, transplanted to individual pots, and placed in the greenhouse for observation. Plants were observed for infection (stromata or systemic hyphae) for a 3-yr period.

Microscopy. Whole mounts of leaf tissue with hyphae of *M. atramentosa* were cleared in lactophenol and stained with 0.5% cotton blue in lactophenol, destained on the slide overnight in lactophenol, and examined with phase-contrast optics. Leaf tissue infected with *B. epichloë* was embedded in Tissue Tek O.C.T. compound (Ames Division, Miles Laboratory, Elkhart, IN), sectioned at 15 mm on a Tissu-freez specimen freezing stage (Bailey Instruments, Saddle Brooke, NJ) attached to an American Optical Corporation freezing microtome, stained with an aniline blue solution (aniline blue, 0.1 g; lactic acid, 50 ml; and distilled water, 100 ml), mounted in water, and examined under an oil immersion lens at ×1,000.

For electron microscopy 1-mm squares of host leaf tissue were excised in a drop of glutaraldehyde fixative on a sheet of dental wax, fixed in 2% glutaraldehyde in 0.05 M phosphate buffer (with 1 mm $CaCl_2$ added) for 1.5 hr, postfixed in 2% OsO_4 for 2 hr, left overnight in 0.5% aqueous uranyl acetate, dehydrated in acetone, embedded in Spurr's hard medium, and sectioned on a DuPont Sorval MT-2-B ultramicrotome. Sections were stained on the grids with lead citrate for 2 min, and examined under a Zeiss Em-10-A microscope.

RESULTS

Disease cycles and infection. All hosts on which *M. atramentosa* and *B. epichloë* were studied were warm season perennials that died back to the crowns during the winter. The fungi developed on new leaves during the spring from mycelium overwintering in dormant buds in the crowns. Conidomata were produced in superficial linear to elliptical black stromata on the upper surface of the leaf early during leaf emergence and before the leaf was fully expanded. Production of conidia on new leaves continued throughout the growing season. Ascomata developed within the same stromata following the conidia. Inflorescences were aborted or suppressed and in infections by *M. atramentosa* were bound up in a stroma, but occasionally a few viable seeds were produced. Seeds collected from infected inflorescences and from healthy inflorescences of plants in proximity to diseased plants never produced infected plants during the 3-yr observation period. All inoculation attempts were unsuccessful.

With *M. atramentosa*, natural spread of the fungus was observed at one location from infected transplants of *Paspalum notatum* to neighboring plants of *P. laeve, Panicum hians* Ell., and *Panicum* sp. No other source of inoculum was evident. Recovery from infections was observed also. Occasional shoots from infected plants showed no evidence of infection. These shoots were much more vigorous than infected shoots. The contrast was most striking in bunch grasses in which the clumps were distorted by the more vigorous recovered tillers on one side. This created a problem in maintaining sources of infected plants. Unless the more

competitive recovered shoots were weeded out, they obscured and suppressed the infected shoots.

Histology of infections with *M. atramentosa* on *Paspalum laeve* and *Eremochloa ophiuroides*. Sparse superficial mycelium that was found on the upper surfaces of leaf primordia (Fig. 1) in active buds during the growing season was also found in dormant buds on infected plants during the winter. Mycelium was found on the upper surface inside the delicate young leaf when it was still folded and before it emerged from the protection of the sheathing leaf bases. As the leaf elongated from the base, patches of mycelium near the tip of the leaf became thicker (Figs. 2 and 3) and developed into a stroma with conidia present before the leaf expanded. The stroma was then isolated in the upper third of the leaf with no connection to the mycelium in the apical meristem from which it originated. The fungus stroma prevented the leaf blade from expanding and the isolated stromata on mature leaves were enclosed in chambers bounded below and laterally by unexpanded leaf blade tissue and sealed across the exposed surface by a cortex of dark, thick-walled cells. Whole mounts of young leaves from the earliest that could be dissected from the apical meristem to those just prior to emergence from the basal sheath and with the stroma developing on the surface showed mycelium on the upper surface of the leaf (Figs. 1–3). Sections of older stages with the stroma thickened showed the mycelium restricted to the surface of the leaf (Figs. 5–7). There was no evidence of penetration or modification of the cuticle. No mycelium was ever observed within tissues of the leaf.

Histology of infections with *B. epichloë* on *Sporobolus poiretii* and *Chasmanthium laxum*. The mycelium of *B. epichloë* was systemic and intercellular as observed with light microscopy (Fig. 4) and transmission electron microscopy (Fig. 8). Fungal hyphae ramified between cells of stem and leaf tissues, including pith (Fig. 4) and mesophyll (Fig. 8). Hyphae emerged between host epidermal cells (Fig. 9) and between guards cells of stomates (Fig. 10). The stroma in which sporocarps developed in *B. epichloë* was entirely superficial on the upper surface of the leaf above the cuticle; however, it was connected with the internal mycelium at numerous points by hyphae passing between epidermal cells and through stomates (Figs. 9 and 10). Some hyphae grew laterally over the surface beneath the cuticle (Figs. 11 and 12). The raised cuticle over the subcuticular hyphae was progressively destroyed by enlarging electron-transparent pockets originating on the inner surface in contact with the hyphae (Fig. 11). These pockets enlarged until only a thin outer layer of cuticle remained intact (Fig. 11) and then until only remnants of the cuticle remained (Fig. 12).

DISCUSSION

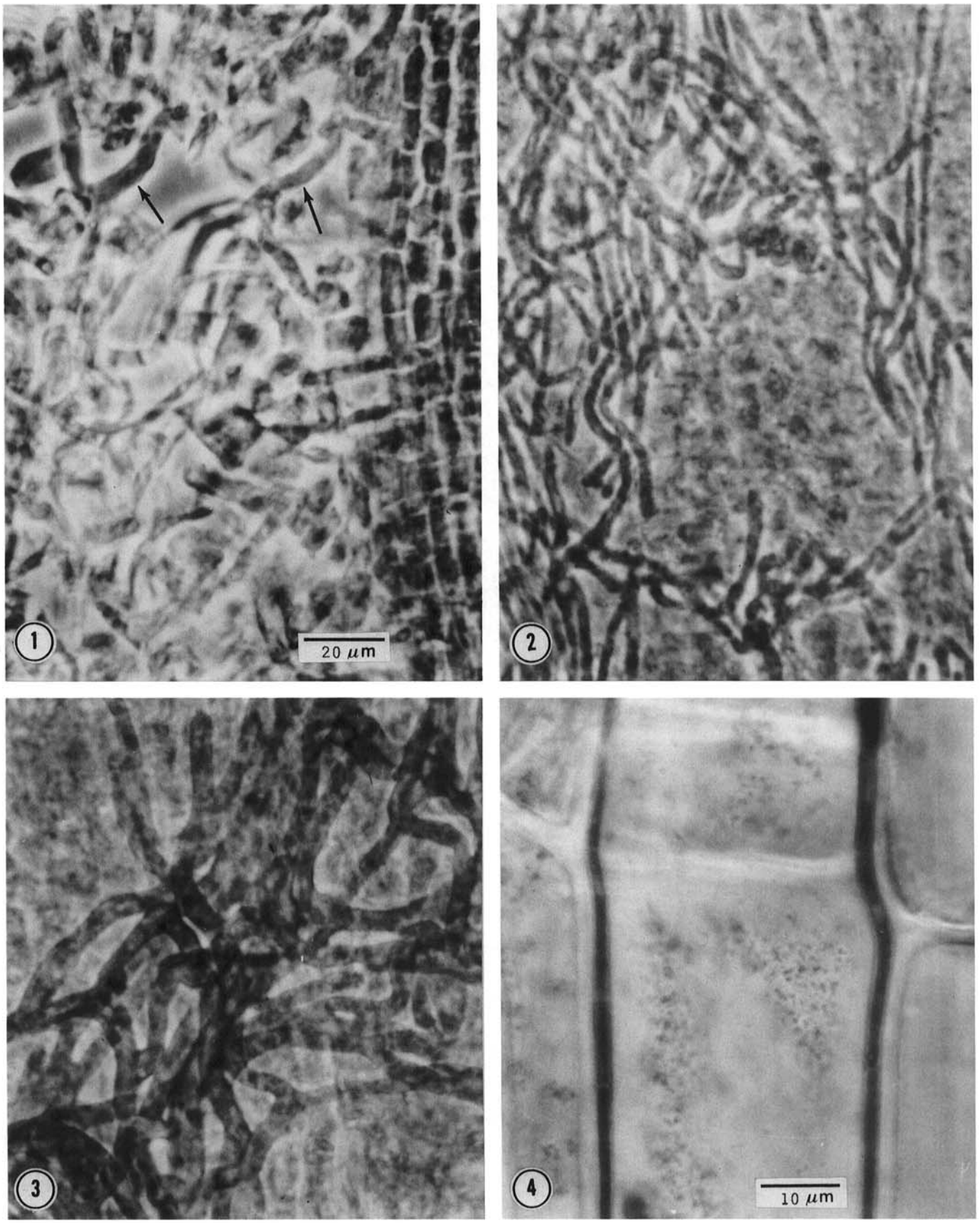
The host ranges reported for *M. atramentosa* (species of *Andropogon, Arthrolophis, Axonopus, Cymbopogon, Panicum, Paspalum, Saccharum* [31], and *Eremochloa*) and for *B. epichloë* (species of *Andropogon, Calamagrostis, Chloris, Ctenium, Eragrostis, Gymnopogon, Panicum, Sporobolus, Thrasya, Triodia* [8], and *Oryzopsis* [31]) are surprisingly broad for such highly compatible biotrophic parasites (18). Observation of apparent natural spread of *M. atramentosa* from introduced transplants of infected *Paspalum notatum* to neighboring plants of three species of weed grasses suggests a low degree of host specificity. Definitive evidence on the question of host specificity and on other questions such as delimitation of species in the Balansiae, identity of the endophytes of fescue and ryegrass (17,20,21,25,27,28), and the relationship of host-parasite interactions to alkaloid production and toxicities of infected plants could be obtained only through development of a reliable procedure for producing infection experimentally. A method has been developed for producing conidia for inoculum in culture (23) and infected perennial hosts furnished conidial and ascoporic inoculum for years. The possible routes of infection seem obvious. Vertical transmission through seed infected from the plant on which they are produced has been demonstrated for fescue and ryegrass plants with symptomless infections with the fescue (27) and ryegrass (16,28) endophytes. In normal infections of various grasses in which the fungus produces symptoms and sporocarps on the host almost no viable seed are produced (8,26). However, Clay and Jones (6) reported that in *Danthonia spicata* infected with *Atkinsonella hypoxylon* even though the terminal, wind-pollinated inflorescences were aborted and engulfed by the stroma of the fungus, self-pollinated cleistogamous spikelets in the axils of the lower leaf sheaths produced viable seed. From 25 cleistogamous seed produced by infected plants, four of six progeny plants brought to maturity showed the usual signs of infection. The timing of fungus sporulation, especially in members of the Balansiae that produce sporocarps only on aborted inflorescences of infected hosts, suggests invasion of developing seed by germ tubes from spores deposited on the stigmas at anthesis as in loose smut of wheat and barley (8). Seedling infection from seedborne spores as in covered smut of wheat and barley is possible, but spores produced by the Balansiae have none of the characteristics of resting spores. Infection of meristematic tissue of stems from spores introduced into leaf whorls of seedlings or older plants or between bud scales would be a probable route for *M. atramentosa*, and a possible one for other Balansiae. Finally, cut stubble resulting from mowing or grazing might serve as an invasion route to the basal meristem. Investigations of all of these possibilities gave negative results.

Little success with artificial inoculation has been reported previously. Diehl's (7,8) attempts at inoculation of florets, including inoculations with *B. epichloë* on *Ctenium* sp. and with *M. atramentosa* on *Adropogon scoparius* Michx., *Paspalum dilatatum* Poir., and *Saccharum* sp., were unsuccessful. However, Diehl (8) reported infection on three of 20 plants of *Cenchrus echinatus* L. grown from seed produced by plants inoculated by brushing conidia of *B. obtecta* onto the stigmas of blooming florets. No infection resulted when the florets were sprayed with a suspension of conidia in water. Western and Cavett (32) were unable to obtain infection of *Dactylis glomerata* L. with *Epichloë typhina* by inoculating florets. They succeeded in demonstrating invasion through freshly cut stubble inoculated with ascospores and conidia by growth of hyphae down the pith.

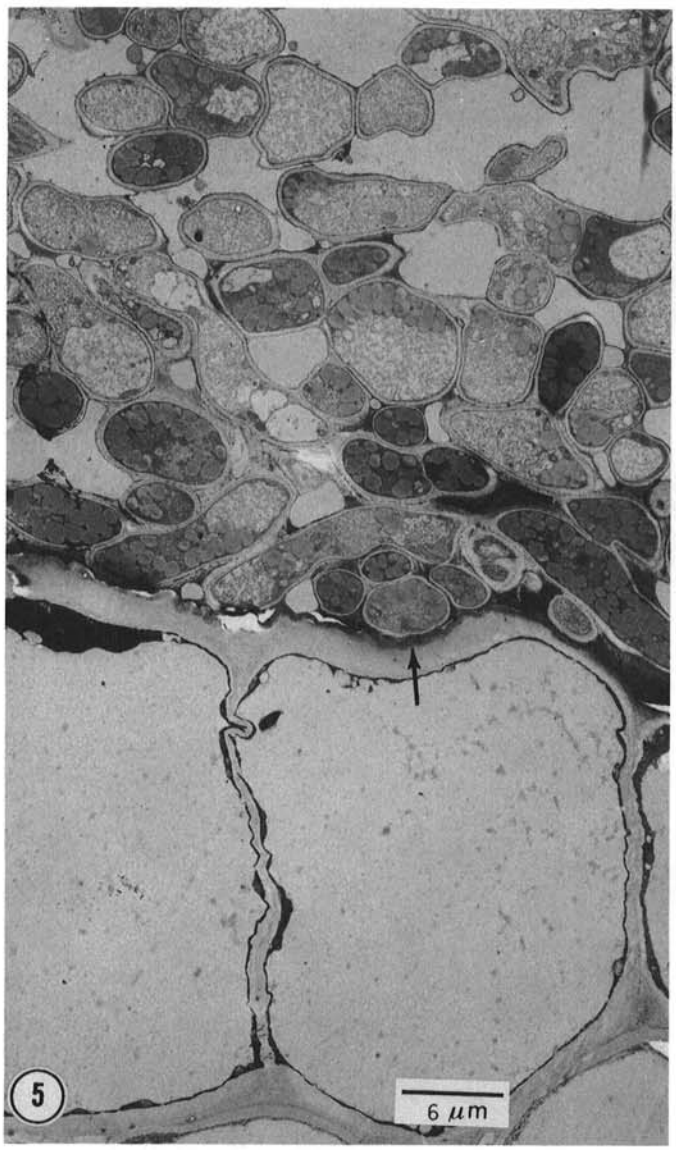
B. epichloë is typical of the majority of the Balansiae. All of these species are culturable biotrophs (18), produce systemic infections, and may cause generalized symptoms such as dwarfing and alterations in floral development. These fungi become conspicuous only at sporulation, which occurs in localized areas of the host that are characteristic of the fungus species. In all of these characters, species of the Balansiae are remarkably similar to the smuts that cause systemic infections. They differ from the smuts in that the mycelium is uniformly intercellular and host cells are neither penetrated nor destroyed even at sporulation, since the mycelium emerges between the epidermal cells or through stomates and the stromata and sporocarps develop on the surface of the host.

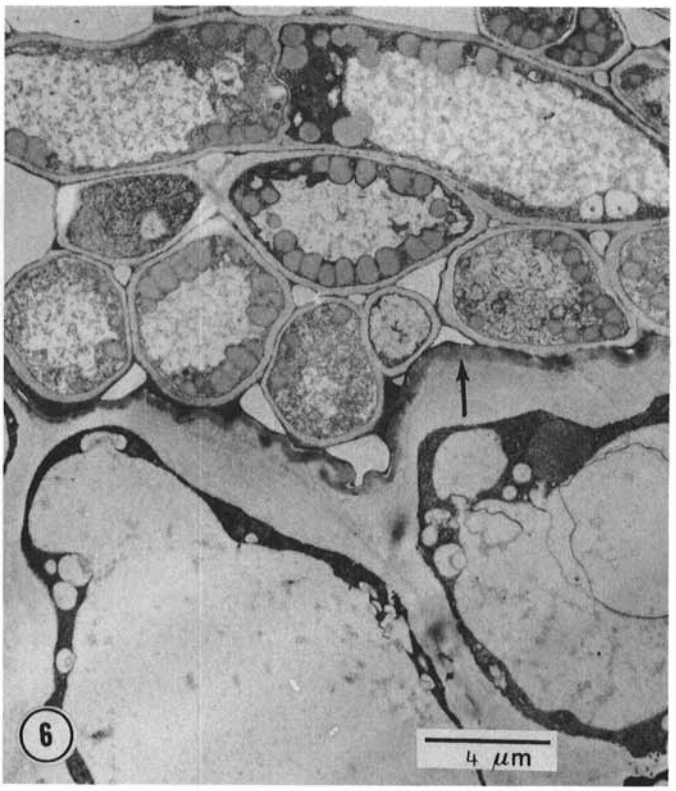
In species such as *Balansia pilulaeformis* (Berk. & Curt.) Diehl apud J. H. Miller (23) and *Atkinsonella hypoxylon* (8,23) the inflorescences are bound up in mycelium that develops into a stroma, and mechanical binding offers a simple apparent explanation for abortion of the inflorescence (6). *B. epichloë* forms stromata only on the leaves. The generalized symptoms of suppression of inflorescences, therefore, must have some explanation other than mechanical binding. An effect of infection on the hormonal system of the plant may be postulated. Hormonal imbalance may cause suppression of inflorescences in all cases, and mechanical binding may be a secondary effect.

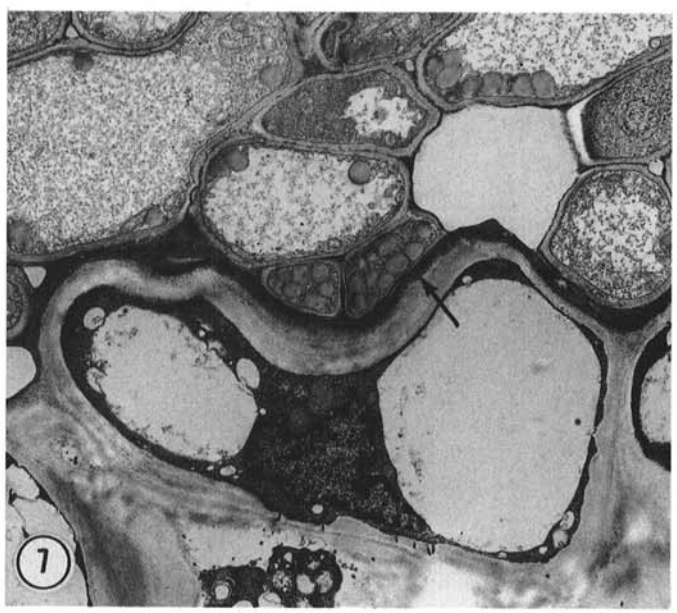
Although *M. atramentosa* differs from *B. epichloë* and all other typical Balansiae in that the mycelium, as reported previously (19,29), is entirely superficial, a comparison of these two fungi shows a remarkable degree of similarity. *M. atramentosa* may be classed as a biotroph (18) because in nature it has been found only in contact with living host tissue and has been demonstrated to be highly efficient in absorption of nutrients through the host cuticle (29). Oddly, in its nutritional requirements it seems to be the most fastidious of the Balansiae. It was not cultured until special media were devised (23). Without penetrating the host it produces what is most conveniently termed a systemic infection, although narrow

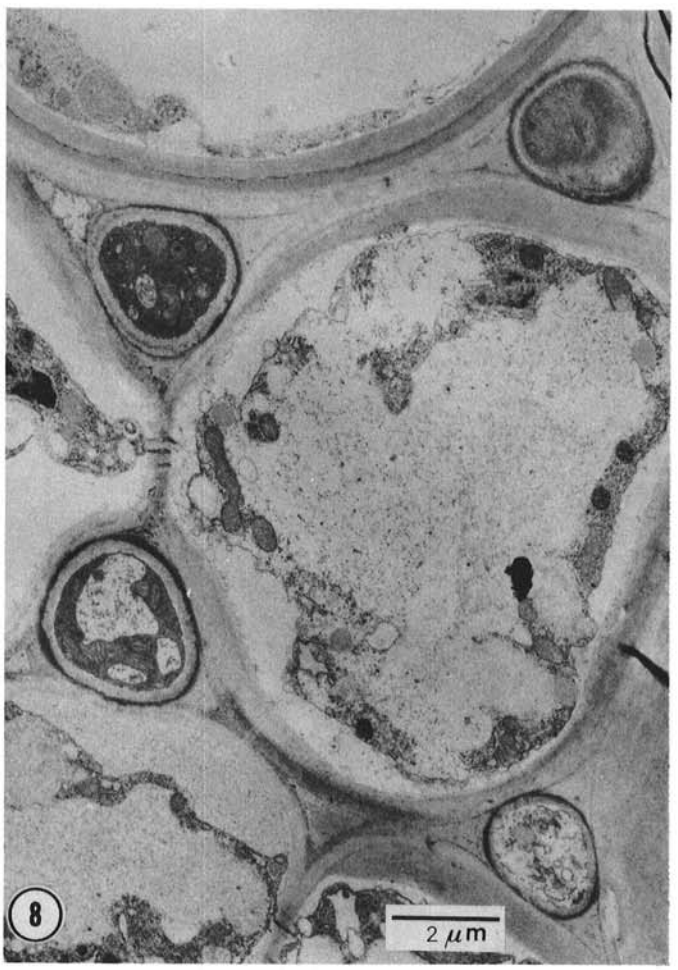


Figs. 1–4. Light micrographs of mycelium. **1–3,** *Myriogenospora atramentosa* on upper surface in whole mounts of leaves of *Paspalum laeve*, to scale in Fig. 1. **1,** On leaf primordium in lateral bud (arrows); **2,** on second leaf with differentiated blade; **3,** thicker hyphae at base of stroma on third differentiated leaf. **4,** *Balansia epichloë*. Two deeply stained hyphae running vertically in intercellular spaces at junctures of host cells in pith squash of *Sporobolus poiretii*.

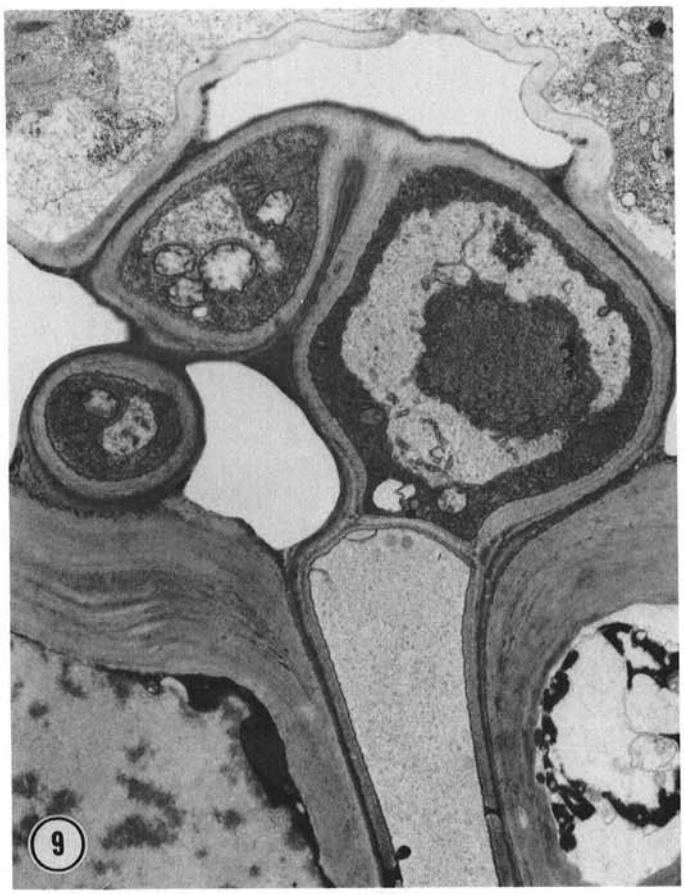


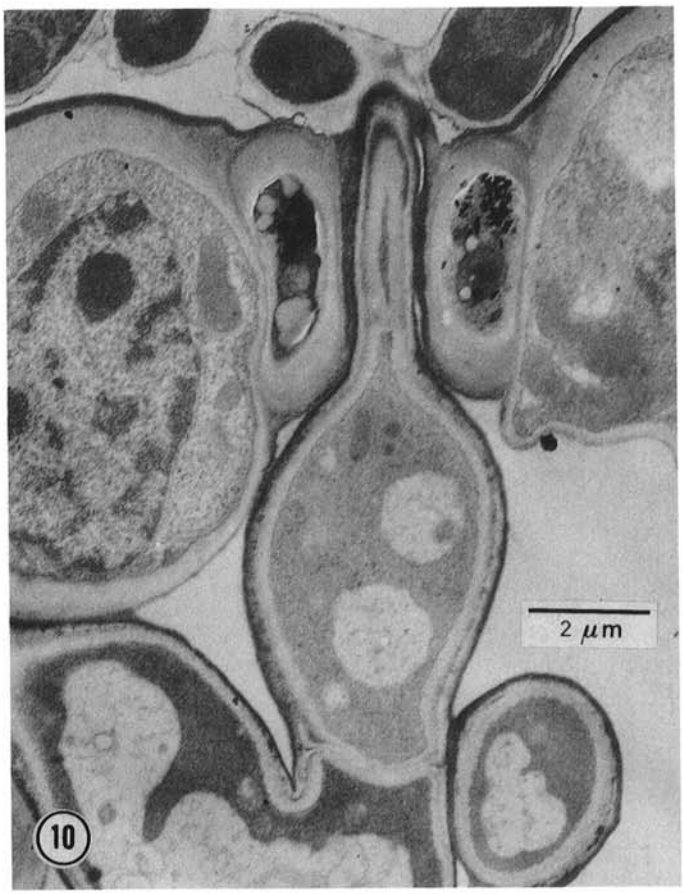


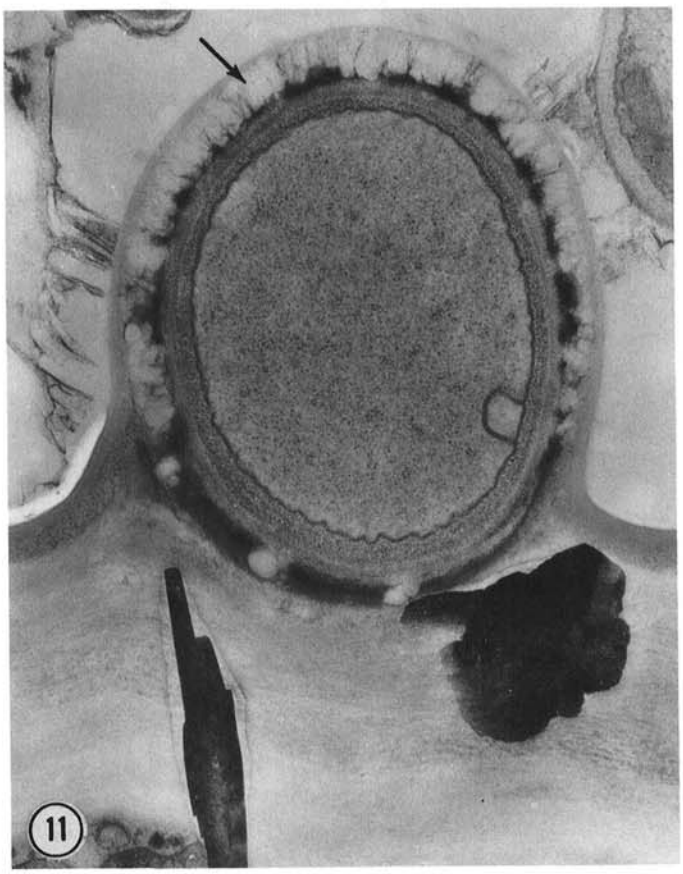


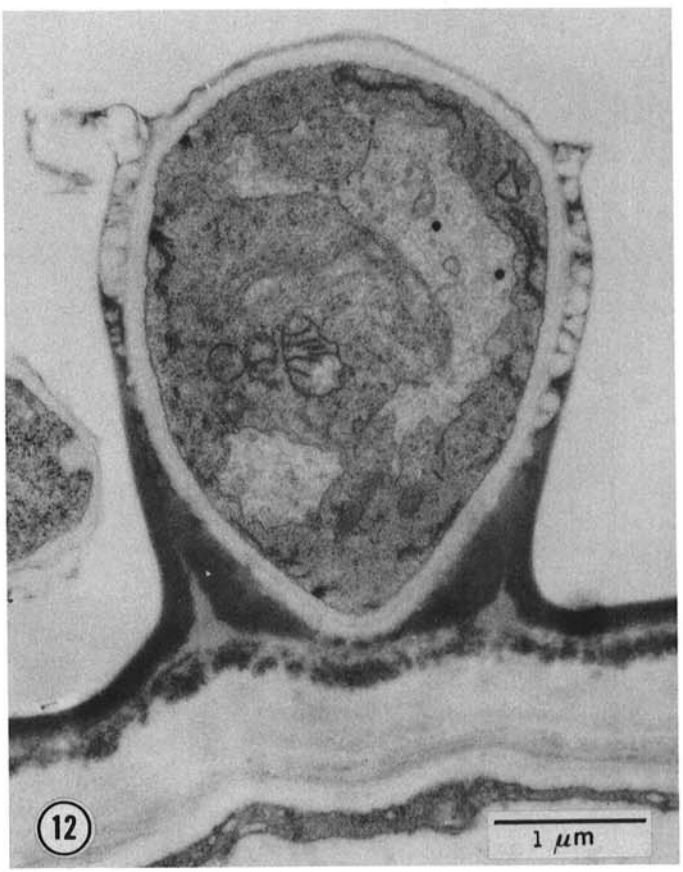


Figs. 5–8. Transmission electron micrographs. **5–7,** Stroma of *Myriogenospora atramentosa* above apparently unaltered cuticle (arrows) on upper surface of leaves of *Eremochloa ophiuroides*. **7,** To scale in Fig. 6. **8,** Cross sections of four hyphae of *Balansia epichloë* in intercellular spaces around mesophyll cell of leaf of *Chasmanthium laxum*.









Figs. 9–12. Transmission electron micrographs of *Balansia epichloë*. **9,** Egress between epidermal cells in upper surface of leaf of *Sporobolus poiretii*, to scale in Fig. 10. **10,** Egress through stomate in upper epidermis of *Chasmanthium laxum*. **11,** Electron-translucent pockets (arrow) in raised leaf cuticle of *S. poiretii* above subcuticular hypha, to scale in Fig. 12. **12,** Cuticle disintegrated and ruptured over top of subcuticular hypha on leaf of *C. laxum*.

definitions of both "systemic" and "infection" may be strained by this application. Its presence on the host induces such localized growth responses as the folds in the leaves that enclose its stromata and modifications of shapes of underlying epidermal cells as well as the generalized symptoms of dwarfing, reduction in vigor, and suppression of inflorescences. The fungus is present in the buds, although only on the surface of the leaf primordia, and stromata and sporocarps generally appear on every leaf and inflorescence as it develops. The similarity extends to the localization of the stroma in both *M. atramentosa* and *B. epichloë* on the upper surface of the host leaf.

At the time when the developing stroma of *M. atramentosa* requires a major source of food for production of spores it is separated from host tissues by the cuticle. Although cuticles in general are leaky enough to support a varied flora of ectocommensalistic fungi (11,30), it might be expected that a parasite such as *M. atramentosa*, which is capable of inducing growth alterations in host tissues and is efficient in absorption of nutrients (29), would prove capable of inducing alterations in the cuticle to increase the flow of nutrients. Negative evidence from microscopy (19,29) may be questioned, but it is sufficient to indicate that conspicuous alterations in structure of the cuticle such as those produced by *B. epichloë* are lacking in leaves infected with *M. atramentosa*.

In infections with *B. epichloë* the progressive disintegration of the cuticle over the subcuticular hyphae suggests digestion of the cuticle by fungus enzymes. Cutinase has been found in seven species of pathogenic fungi and has been demonstrated to function in penetration in two species (15). There is no indication, however, that digestion of the cuticle is essential to the nutrition or egress of *B. epichloë*, since the stroma is connected with the systemic internal mycelium at innumerable points by hyphae passing through stomates as well as between epidermal cells.

LITERATURE CITED

1. Bacon, C. W., Lyons, P. C., Porter, J. K., and Robbins, J. D. 1985. Ergot toxicity from endophyte-infected grasses: A review. Agron. J. (In press).
2. Bacon, C. W., Porter, J. K., and Robbins, J. D. 1975. Toxicity and occurrence of *Balansia* on grasses from toxic fescue pastures. Appl. Microbiol. 29:553-556.
3. Bacon, C. W., Porter, J. K., and Robbins, J. D. 1979. Laboratory production of ergot alkaloids by species of *Balansia*. J. Gen. Microbiol. 113:119-126.
4. Bacon, C. W., Porter, J. K., Robbins, J. D., and Luttrell, E. S. 1977. *Epichloë typhina* from toxic tall fescue grasses. Appl. Environ. Microbiol. 34:576-581.
5. Butler, E. J., and Jones, S. G. 1949. Plant Pathology. Macmillan, London. 979 pp.
6. Clay, K., and Jones, J. P. 1984. Transmission of *Atkinsonella hypoxylon* (Clavicipitaceae) by cleistogamous seed of *Danthonia spicata* (Gramineae). Can. J. Bot. 62:2893-2895.
7. Diehl, W. W. 1933. The *Myriogenospora* disease of grasses. Phytopathology 25:677-681.
8. Diehl, W. W. 1950. *Balansia* and Balansiae in America. U.S. Dep. Agric. Monogr. 4. 82 pp.
9. Fletcher, L. R., and Harvey, I. C. 1981. An association of *Lolium* endophyte with ryegrass staggers. N.Z. Vet. J. 29:185-186.
10. Gäumann, E. 1949. Die Pilze. Birkhauser, Basel, Switzerland. 382 pp.
11. Godfrey, B. S. 1976. Leachates from aerial plants and their relation to plant surface microbial populations. Pages 433-439 in: Microbiology of Aerial Plant Surfaces. C. H. Dickinson and T. F. Preece, eds. Academic Press, New York.
12. Hinton, D. M., and Bacon, C. M. 1985. Distribution and ultrastructure of the endophyte of toxic tall fescue. Can. J. Bot. 63:36-42.
13. Johnson, M. C., Siegel, M. R., and Schmidt, B. A. 1985. Serological reactivities of endophytic fungi from tall fescue and perennial ryegrass and of *Epichloë typhina*. Plant Dis. 69:200-202.
14. Kirby, E. J. M. 1961. Host-parasite relations in the choke disease of grasses. Trans. Br. Mycol. Soc. 44:493-503.
15. Kolattukudy, P. E., and Köller, W. 1983. Fungal penetration of the first line defensive barriers of plants. Pages 79-100 in: Biochemical Plant Pathology. J. A. Callow, ed. John Wiley & Sons, New York.
16. Latch, G. C. M., and Christensen, M. J. 1982. Ryegrass endophyte, incidence and control. N.Z. J. Agric. Res. 25:443-448.
17. Latch, G. C. M., Christensen, M. J., and Samuels, G. J. 1984. Five endophytes of *Lolium* and *Festuca* in New Zealand. Mycotaxon 22:535-550.
18. Luttrell, E. S. 1983. Fungi. Pages 258-266 in: Challenging Problems in Plant Health. Diamond Jubilee Volume. T. Kommedahl and P. Williams, eds. American Phytopathological Society, St. Paul, MN. 538 pp.
19. Luttrell, E. S., and Bacon, C. W. 1977. Classification of *Myriogenospora* in the Clavicipitaceae. Can. J. Bot. 55:2090-2097.
20. Morgan-Jones, G., and Gams, W. 1982. Notes on Hyphomycetes and the anamorph of *Epichloë typhina*, new taxa in one of two new sections of *Acremonium*. Mycotaxon. 15:311-318.
21. Neill, J. C. 1941. The endophytes of *Lolium* and *Festuca*. N.Z. J. Sci. Technol. 23A:185-193.
22. Porter, J. K., Bacon, C. W., Robbins, J. D., Himmelsbach, D. S., and Higman, H. C. 1977. Indole alkaloids from *Balansia epichloë* (Weese). J. Agric. Food Chem. 25:88-93.
23. Rykard, D. M., Luttrell, E. S., and Bacon, C. W. 1982. Development of the conidial state of *Myriogenospora atramentosa*. Mycologia 74:648-654.
24. Rykard, D. M., Luttrell, E. S., and Bacon, C. W. 1984. Conidiogenesis and conidiomata in the Clavicipitaceae. Mycologia 76:1095-1103.
25. Sampson, K. 1933. The systemic infection of grasses by *Epichloë typhina* (Pers.) Tul. Trans. Br. Mycol. Soc. 18:30-47.
26. Sampson, K. 1937. Further observations on the systemic infection of *Lolium*. Trans. Br. Mycol. Soc. 21:84-97.
27. Siegel, M. R., Johnson, M. C., Varney, D. R., Nesmith, W. C., Buckner, R. C., Bush, L. P., Burrus, P. B., II, Jones, T. A., and Boling, J. A. 1984. A fungal endophyte in tall fescue: Incidence and dissemination. Phytopathology 74:932-937.
28. Siegel, M. R., Latch, G. C. M., and Johnson, M. C. 1985. *Acremonium* fungal endophytes of tall fescue and perennial ryegrass: Significance and control. Plant Dis. 69:179-183.
29. Smith, K. T., Bacon, C. W., and Luttrell, E. S. 1985. Reciprocal translocation of carbohydrates between host and fungus in bahia grass infected with *Myriogenospora atramentosa*. Phytopathology 75:407-411.
30. Tukey, H. B. 1970. The leaking of substances from plants. Annu. Rev. Plant Physiol. 21:305-324.
31. U.S. Dep. Agric., Agric. Res. Serv. 1970. Index of plant diseases in the United States. Agric. Handb. 165. U.S. Government Printing Office, Washington, DC. 530 pp.
32. Western, J. H., and Cavett, J. J. 1959. The choke disease of cocksfoot (*Dactylis glomerata*) caused by *Epichloë typhina* (Fr.) Tul. Trans. Br. Mycol. Soc. 42:298-307.