Scanning Electron Microscopy of *Fusarium moniliforme* Within Asymptomatic Corn Kernels and Kernels Associated with Equine Leukoencephalomalacia

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ABSTRACT

Bacon, C. W., Bennett, R. M., Hinton, D. M., and Voss, K. A. 1992. Scanning electron microscopy of *Fusarium moniliforme* within asymptomatic corn kernels and kernels associated with equine leukoencephalomalacia. Plant Dis. 76:144-148.

Fusarium moniliforme, a pathogen of corn, produces a variety of mycotoxins. Scanning electron microscopy was used to determine the association of this fungus with asymptomatic kernels of corn and kernels associated with a specific animal toxicity, equine leukoencephalomalacia. The location of *F. moniliforme* in all asymptomatic kernels examined was always the pedicel or tip cap end of kernels. These observations suggest that, although it is unknown, the point of entry into asymptomatic kernels by the fungus is probably the same. The fungus was found within the embryo and endosperm in kernels associated with animal toxicity. In some toxic kernels, the fungus had undergone extensive growth and sporulation, producing microconidia that were similar to a known isolate of this species cultured on agar media.

Additional keywords: *Gibberella fujikuroi*, toxic corn, *Zea mays*

Fusarium moniliforme J. Sheld. (*Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura) has been implicated as being one of the causal factors of seedling blight, ear rot, and stalk rot of corn (*Zea mays* L.) (14,17,20,26,31,32). Most of these implications are derived from the fact that *F. moniliforme* is the most prevalent fungus isolated from corn kernels and plant parts (9,18). In addition to its involvement in corn diseases, the occurrence of the fungus on corn also is important because it is known to produce several mycotoxins which include the fumonisins (10), fusarin C (2), and moniliformin (5). Furthermore, corn naturally and experimentally infected with cultures of *F. moniliforme* is carcinogenic (11,19,22), immunosuppressive (4), and hepatotoxic and nephrotoxic to animals (21,33). Cracked kernels and plant parts obviously infected with fungi are toxic, as are asymptomatic kernels. There is no indication when corn becomes toxic or if there are any associated differences between toxic and nontoxic corn. Therefore, the nature of the association of *F. moniliforme* with corn is important because any control must take into account the mode of entry, the dissemination, and the survival of this fungus within corn kernels.

Although *F. moniliforme* is one of the earliest known examples of a mycotoxic and phytotoxic species (21), various aspects of its relationship to corn are confusing or unknown. *F. moniliforme* is an endophyte because hyphae are recovered from surface-sterilized stems, leaves, cobs, and roots (9,20,32). Additionally, we know that this fungus can cause a surface-borne infection of kernels (17,26, 31). Further, because a heat treatment is required to eliminate the fungus (6), we must conclude that it is also located interiorly. Considerable controversy exists concerning its pathway of infection into kernels. The endophytic habit sug-

Accepted for publication 2 August 1991.

gests that the fungus arrives at its internal location in kernels from growth through the stalk, into the cob, rachilla and pedicel (9), or by entering the ruptured scar on the pericarp produced by the emerging coleorhiza after kernel germination (20). Others feel that it enters the kernel by growth along the silks, through tip ends of ears, over the surface of kernels into bracts and pedicels, into the vascular cylinder of the cob, and finally into kernels (17,26). In the latter case, insects may play a role in the ear infection route (7). If the fungus enters the kernels via wounds on the kernel pericarp (17), hyphae should not be uniformly localized but randomly distributed within the kernel.

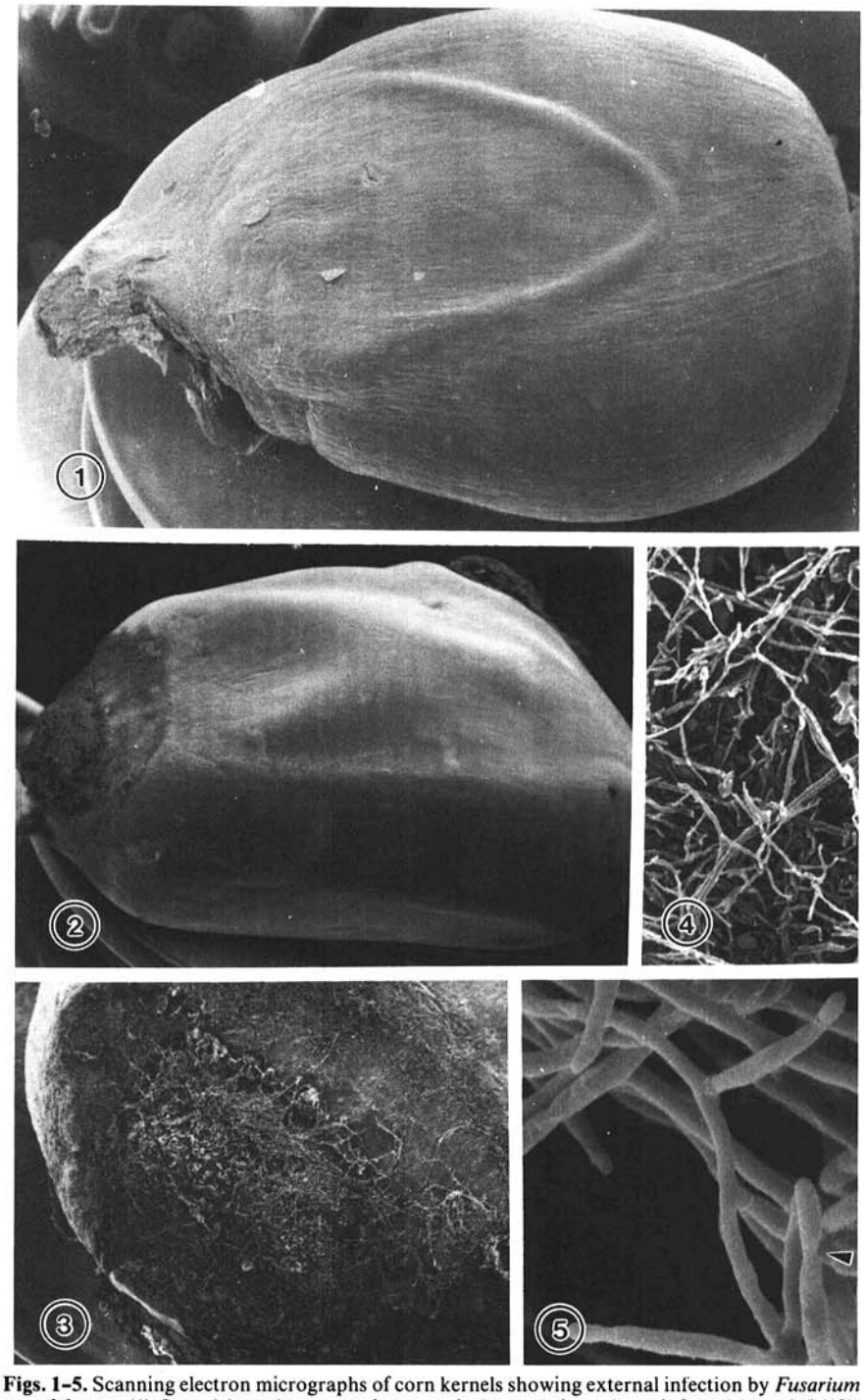
Although several histological studies have been reported for various aspects of this fungus on corn (17,24,26,32), they neither provide evidence for the uniform location of the fungus within tissue of the vascular cylinder at the hilar tip end, nor, if contrary, indicate a random distribution within an infected kernel. We report using scanning electron microscopy on the systemic location of *F. moniliforme* within dormant kernels of corn and compared this with kernels known to be infected by this fungus and associated with a mycotoxicosis specific to *F. moniliforme*, equine leukoencephalomalacia.

MATERIALS AND METHODS

Corn samples. The asymptomatic kernels of corn, sweet and field, used in this study were those established as naturally systemically infected with *F. moniliforme* from field surveys from southern Georgia and the Midwest. The toxic corn kernels used were those associated with equine leukoencephalomalacia and established earlier as infected with *F. moniliforme* after plating on an agar medium (33). These kernels were whole and showed no obvious external signs of infection, although they were from samples that were nephrotoxic and hepatotoxic to rats (33). Control kernels included nontreated seed grade Trucker's Favorite field corn, which was less than 10% infected with *F. moniliforme*. The mean germination percentages of asymptomatic (sweet and field) and toxic corn kernels were 90 and 20%, respectively.

To observe the egress of the fungus, kernels were surface-sterilized with 5.25% sodium hypochlorite for 5 min and rinsed with sterile distilled water for 1 min. The treated kernels were placed on Difco potato-dextrose agar (PDA) or wet filter paper and incubated for 3–4 days at room temperature. After the incubation period, kernels developed coleoptiles while the fungus was emerging from the germination scar. To determine the distribution of the fungus within the kernel, corn was surface-sterilized as described earlier, soaked in water at 45 C for 15 min, and cross or longitudinal sections were made. In several instances, the opposite half of a kernel used for microscopy was plated on PDA to confirm the identity or presence of *F. moniliforme*. A known culture of *F. moniliforme* was inoculated on autoclaved corn kernels (33) and its ultrastructure compared with fungi obtained from naturally infected kernels.

Scanning electron microscopy. The results reported represent more than 20 observations, each randomly selected from five toxic samples and four samples of sound corn. Samples were fixed in a 2% glutaraldehyde-cacodylate buffer (0.1 M) for 1 hr, rinsed in buffer, and postfixed with a 1% osmium tetroxide-cacodylate buffer for 2 hr. These samples were dehydrated through an ethyl alcohol series, critical-point dried, and coated with gold-palladium on a Hummer X sputter coater (Anatech Ltd, Alexandra, VA). Some samples were fixed in vapors from a 1% osmium tetroxide-cacodylate buffer for 24 hr, air-dried, and coated with gold-



Figs. 1–5. Scanning electron micrographs of corn kernels showing external infection by *Fusarium moniliforme*. **(1)** Sound kernel representing a typical nontoxic and noninfected kernel (×12); **(2)** kernel showing fungus over a damaged area just distal to the tip cap end (×10); **(3)** the tip cap end in Figure 2 showing external hyphae (×19); and **(4)** and **(5)** further enlargements of the same area of Figure 3, showing the hyphae in Figure 4 (×367) and a conidium at the tip of a conidiophore (arrow) in Figure 5 (×2,743).

palladium. All samples were observed with a Philips 505T scanning electron microscope at 15 kV.

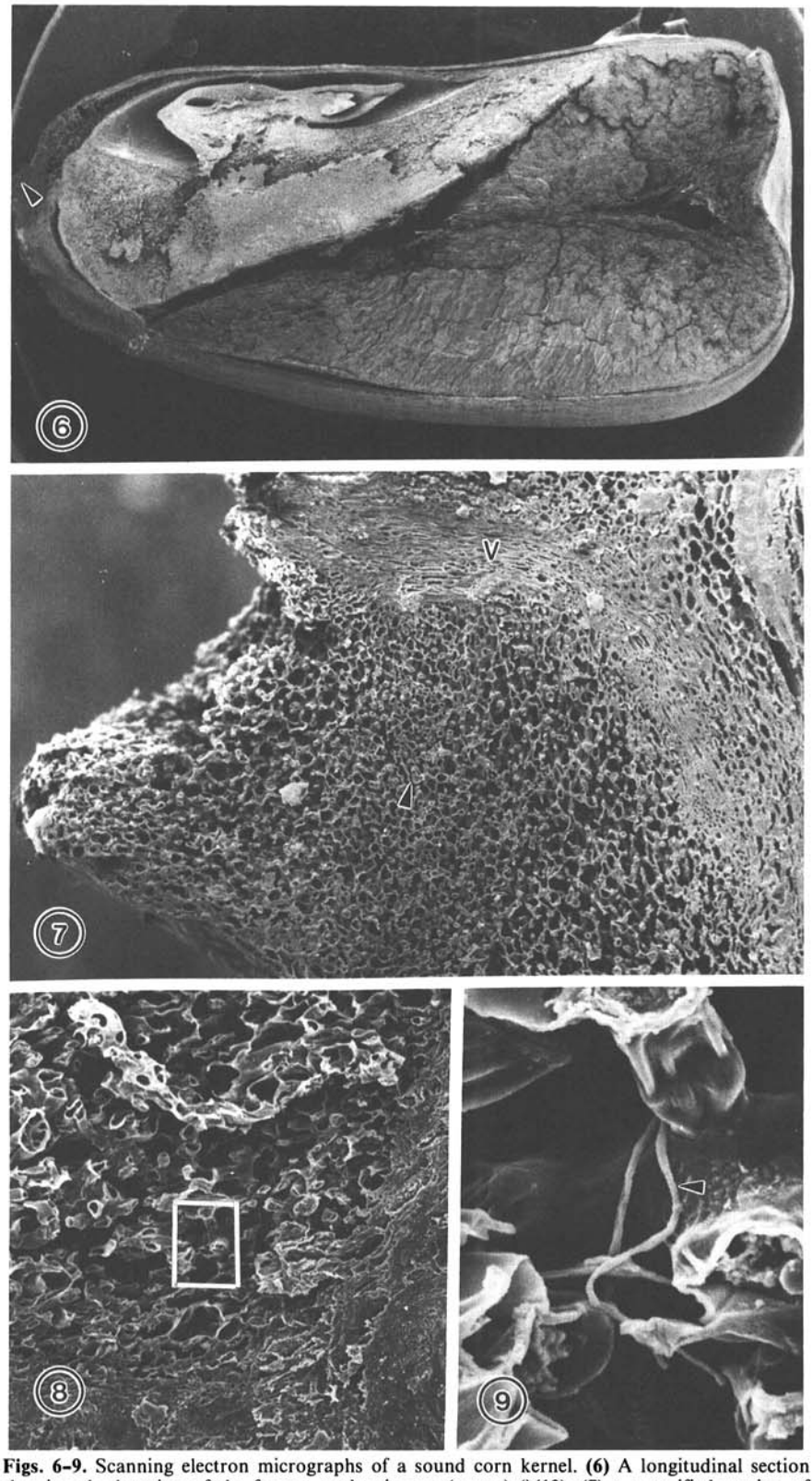
RESULTS

A survey of fungi from field corn used in this study indicated that although kernels were not visibly damaged (Fig. 1), an average of 80% of surface-sterilized kernels were internally infected with fungi. This represented a survey of 50 different samples. Of these, 90% were identified as *F. moniliforme*. The other fungi consisted of *F. subglutinans* (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun & Marasas, *F. graminearum* Schwabe, *F. culmorum* (Wm. G. Sm.) Sacc., *Aspergillus flavus* Link:Fr., and two or more *Penicillium* species. In addition to identifying these fungi on media, the hyphae of the latter two were easily distinguished on the basis of their smaller size when viewed under the scanning microscope and their tendency to be associated mainly with the pericarp. Only those corn samples that had an infection of 90% and more of *F. moniliforme* were used in the scanning microscopic study. Ninety-nine percent of the samples of corn kernels associated with leukoencephalomalacia in horses were infected with *F. moniliforme*.

Some kernels had abrasions or cracked areas in the pericarp, particularly at the tip cap and areas immediately distal to it, and such areas contained fungi (Figs. 2–5). Fungi isolated and identified from kernels with damaged pericarp consisted primarily of *F. moniliforme*, although other fungi as indicated above also were isolated.

Scanning micrographs of a longitudinal section of matured asymptomatic corn kernels showed that the endosperm, inner pericarp, and embryo were entirely free of fungal hyphae. In these kernels, evidence of the fungus was seen as a sparse welt of hyphae that always was located in the upper region of the tip cap (pedicel) (Figs. 6–9). There was no evidence of spores or conidiophores at this location. Although it was difficult to determine by scanning microscopy, the hyphae appeared to be intercellular and were not associated with any vascular tissue area found within the pedicel (Fig. 7). In a few kernels, hyphae were associated with the hilar layer of the kernel and occasionally the cavity between the tip cap but did not invade the endosperm or embryo. Fungi were not seen within or around the scar left by the silk attachment nor in endosperm immediately below this scar. When surface-sterilized toxic samples of corn infected with *F. moniliforme* were allowed to germinate, internal hyphae emerged through the rupture produced by the radicle (*data not presented*). The results are similar when surface-sterilized corn is germinated on sterilized filter paper.

In corn associated with leukoencephalomalacia, the distribution of the fungus was entirely different and extensive. Hyphae were observed externally (Figs. 3 and 10) and randomly distributed within the embryo (Fig. 11), the endosperm, and pericarp (Figs. 12 and 13). Scanning micrographs of *F. moniliforme* cultured on PDA illustrated the distinguishing characteristic of microconidia formed in chains on monophialides, although the presence of false heads also was observed (Figs. 14–18). When halves of a kernel



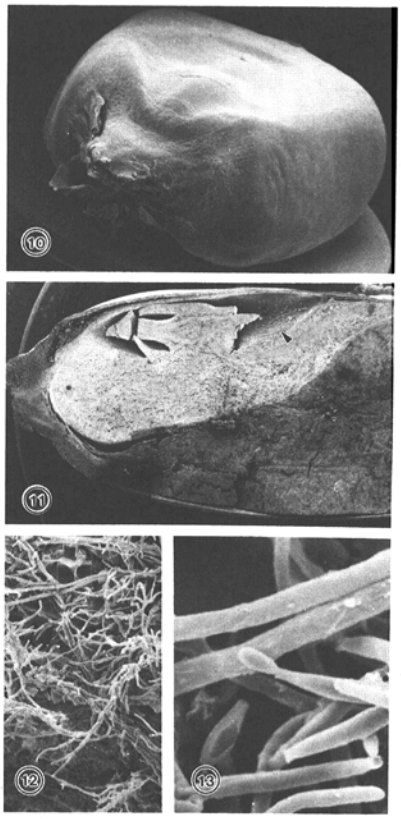
Figs. 6–9. Scanning electron micrographs of a sound corn kernel. **(6)** A longitudinal section showing the location of the fungus at the tip cap (arrow) (×13); **(7)** a magnified version of Figure 6 showing the location of the fungus (arrow) below the vascular tissues (V) (×107); **(8)** higher magnification of Figure 7 showing location of fungus in boxed area (×207); and **(9)** hypha of *Fusarium moniliforme* (arrow) within this area (×783).

were sectioned and allowed to incubate for 24 hr, similar chains of microconidia also were observed (Fig. 16), indicating that the fungus observed at the ultrastructural level was *F. moniliforme*. In some sections of corn associated with leukoencephalomalacia, there was evidence of internal fungal growth and sporulation (Fig. 17). Occasionally, within corn kernels and on the agar medium, collarettes were observed on some phialides (Figs. 17 and 18). Conidiation within dried kernels was found only in samples that caused leukoencephalomalacia, although not all kernels within this type of sample showed internal conidiation. These kernels were also characterized by external growth of hyphae, primarily at the tip cap end (Fig. 3).

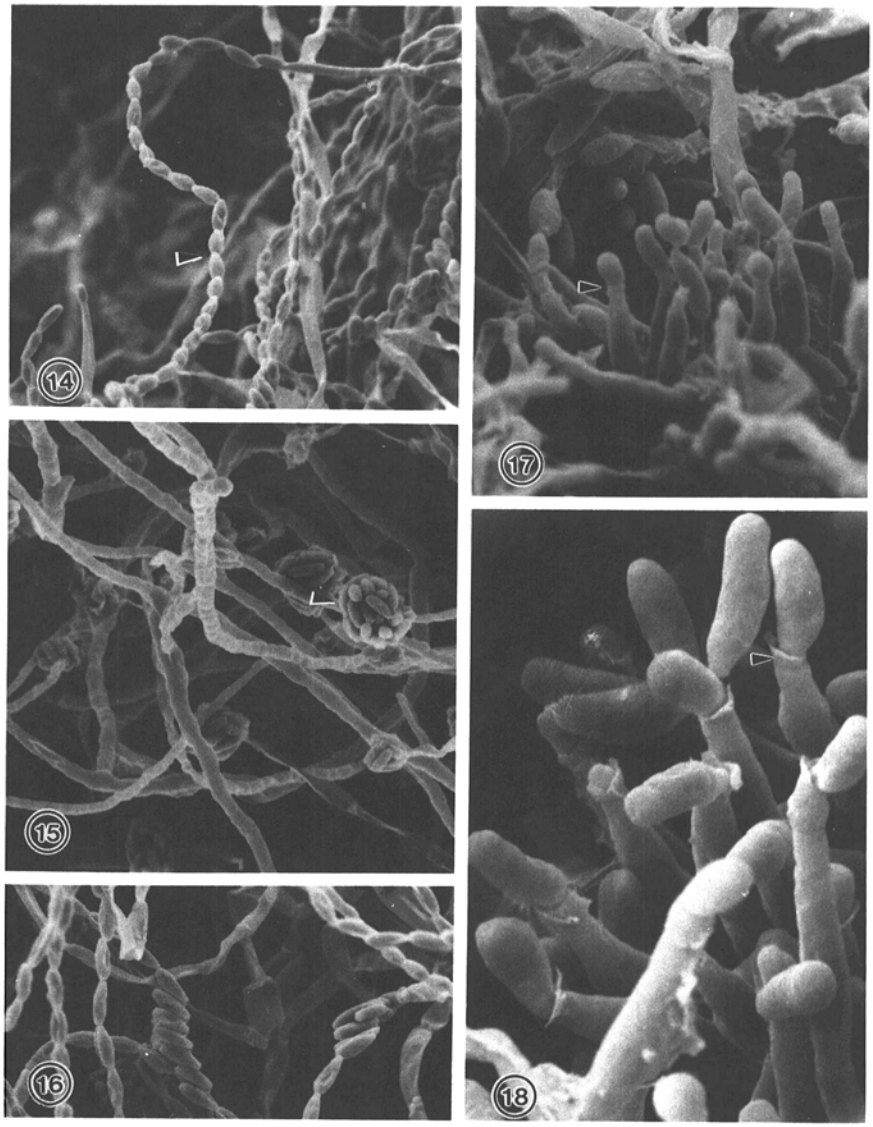
DISCUSSION

F. moniliforme was isolated from sound field grown corn where it was associated with other fungal species. In sound kernels, this fungus was associated with tissue of the upper pedicel and not the embryo or endosperm. This fungus is not known to produce overwintering structures (i.e., chlamydospores) but has been reported to reside in corn debris in soil as thickened hyphae (9,24). The hyphae observed in kernels were localized and sparse and there was no evidence of a thickening of the walls indicative of a survival structure as was described for hyphae of this fungus in corn residue in soil (24). The hyphae in this location is viable as evidenced by its growth through the ruptured scar produced by the emerging radicle. Thus, the pedicel of kernels serves as an additional and important protective and survival structure for the fungus from which it might infect germinating kernels. The finding of *F. moniliforme* in this location substantiates earlier reports on the isolation frequency of this fungus from the pedicel part of the kernel (34).

If, indeed, the fungus enters the kernel via the vascular bundles (17,26), hyphae should be associated with bundle tissue. We found no evidence that fungi were associated with vascular bundle tissue within the tip cap. Furthermore, we found no evidence of this fungus invading vascular bundles of germinating seedlings within a 2-wk period. Pennypacker (25) considers *F. moniliforme* to belong to the cortical rot group, one of two disease categories produced by species of *Fusarium*. This group does not colonize the vascular system (25) and initially are further characterized as being intercellular. However, because *F. moniliforme* can produce a series of pectic and cellulolytic enzymes (C. W. Bacon, *unpublished data*), it has the potential of becoming intracellular and producing disease symptoms. Corn plants infected with *F. moniliforme* are characterized as being asymptomatic and symptomatic (14). The symptomatic condition prob-



Figs. 10–13. Scanning electron micrographs of a kernel associated with equine leukoencephalomalacia. **(10)** A kernel showing the presence of exterior hyphae, primarily at the tip cap (×12); **(11)** a longitudinal section through a similar toxic kernel showing the fungus within the embryo axis (arrow) (×26); **(12)** an enlargement of Figure 11 showing the fungus (×329); and **(13)** a higher magnification showing that the fungus is sporulating (×2,838).



Figs. 14–18. A comparison of *Fusarium moniliforme* grown on PDA and corn. **(14)** Growth of the fungus on culture medium showing chains of microconidia (arrow) (×1,360); **(15)** an older culture showing microconidia in false heads (arrow) (×1,591); **(16)** the growth of the fungus on a sectioned corn kernel incubated for 2 days (×1,545); **(17)** the appearance of the fungus sporulating inside a corn kernel associated with leukoencephalomalacia (×2,720)—note the presence of collarette (arrow); and **(18)** *F. moniliforme* cultured on a corn kernel also showing a collarette on a conidiophore (arrow) (×26,220).

ably is the result of a complex interaction of virulence of an isolate (20), corn variety (14,23,30), and environment (28).

The observation of conidiation in kernels associated with leukoencephalomalacia indicates that these kernels were exposed to conditions, probably moisture, necessary for fungal growth. This corn was further characterized as having a low percent germination. The minimum moisture content of corn kernels for vegetative growth of this fungus is 18.4% (16), although this is temperature- and air-dependent (29). It is not known if these conditions also favor sporulation and mycotoxin synthesis, but oxygen limitation may not be important for growth as this fungus can tolerate anaerobic conditions (29).

Because this is the first report of scanning electron microscopy of this fungus, it was necessary to compare naturally infected toxic kernels with sterilized kernels inoculated with a known *F. moniliforme* isolate. Conidiogenous cells observed on naturally infected and laboratory inoculated corn kernels and media indicated that the phialides of *F. moniliforme* were simple and consisted of both prominent and inconspicuous collarettes. Simple phialides are characteristic of the section Liseola to which *F. moniliforme* belongs (3). The occurrence of collarettes also has been reported in one other *Fusarium* species, *F. semitectum* Berk. & Ravenel, although it is not clear if these structures have any value as a character in delimiting species within this genus. Of the conidia observed in situ, only microconidia were found, although macroconidia also are produced by this fungus.

Corn kernels high in moisture content are very good substrates for the production of two mycotoxins by *F. moniliforme*, fusarin C and fumonisin B_1 (1,8,10). Because the kernels used were dry, a premoisture period must have occurred during storage or harvest. These samples were further characterized as having fungi associated with the embryo and endosperm and low percent germination. The extensive hyphae in kernels associated with toxicity reflects growth of *F. moniliforme*. According to Pennypacker (25), once fungi of the cortical rot group colonize the host cortex, they may become intracellular but rarely sporulate within the plant. Our data indicate that in corn kernels, *F. moniliforme* invades the embryo and sporulates internally, probably when there is high moisture, resulting in mycotoxin production. These data suggest that mycotoxins are probably not produced by the sparse welt of hyphae but rather during pre- or post-harvest storage.

F. moniliforme within sound kernels is sequestered at the tip cap, apparently cannot penetrate the pericarp, and probably becomes latent or dormant during kernel development. This suggests that the placental chalazal region and other maternal tissues might be important in the resistance to this fungus. Several studies indicate that resistance to *F. moniliforme* is maternally inherited (13,15, 27) and that several factors in maternal tissue have a strong influence on the nature of resistance to *F. moniliforme* (12). At maturity, corn kernels consist of the pedicel or tip cap and associated materials of the cob (i.e., glumes). Because *F. moniliforme* is associated with tip caps of asymptomatic kernels, it would appear that it arrived at this location from growth occurring on or within the cob. Further, its universal location within the pedicel of asymptomatic kernels indicates that it arrived there from one infection point.

ACKNOWLEDGMENTS

We thank Paul E. Nelson, The Pennsylvania State University, University Park, PA, for confirming the identity of isolates and supplying additional cultures of *Fusarium moniliforme*. We also thank Norman McGlohon, University of Georgia, Athens, for supplying the corn samples used in this study.

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