Mycoparasitic Fungi Associated with Potential Stalk Rot Pathogens of Corn

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ABSTRACT

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Fungi were isolated from the pith of stalk-rot-affected corn plants collected from five locations in Iowa during 1979 to 1983. Known plant pathogenic fungi were isolated and identified along with potentially mycoparasitic fungi that grew in association with them. The pathogenic fungi, in descending order of their frequency of association with mycoparasites, were: *Fusarium moniliforme, Helminthosporium carbonum, Colletotrichum graminicola, Cladosporium herbarum, F. graminearum, Pyrenochaeta terrestris*, and *Diplodia maydis*. The mycoparasitic fungi, in descending order of frequency, were: *Sphaeronaemella helvellae, Trichoderma viride, Gliocladium roseum, Exobasidiellum* sp., *Gonatobotrys simplex, Trichothecium roseum, Pythium acanthicum*, and a sterile fungus. Evidence of mycoparasitism was observed as the fungi penetrated host hyphae and subsequently reduced mycelia and pigment formation in dual cultures. None of the mycoparasitic fungi were pathogenic to corn. This is the first known report of the presence of mycoparasitic fungi associated with potential stalk rot pathogens in corn. These fungi may influence the incidence and severity of the stalk rot diseases.

Additional key words: biological control, fungicolous fungi.

In the state of Iowa for the past 6 yr (1979–1984), grain corn (*Zea mays* L.) was cultivated on an annual average of 5,032,125 ha and yielded an average of 287.06 bushels per hectare (18). The price of grain corn in April, 1985, was $2.50 per bushel, which sets the annual value of this commodity for the state at $3,610,000,000. The average percentage of stalk lodge for the commercial hybrid cultivars in Iowa (between 100 to 140 cultivars tested at 18 locations in seven districts) during the past six seasons was 5.54% (26). Provided a modern combine could retrieve 80% of ears from the lodged stalks, the minimum annual financial loss to Iowa would be $40,000,000.

Corn stalk rot is caused by a complex of pathogens and is affected by differences in cultivar, culture, and environmental conditions (7,17). Research on control of stalk rot has dealt with the mode of inheritance of resistance, breeding for resistance and stiff stalk, and improvement of cultural practices (7,23). The persistence of annual losses due to stalk rot, the complexity of its etiology, and the recent trend toward biological control of diseases prompted a reexamination of fungi involved in corn stalk rot (5,7,9,13,15). A number of fungus species, known for their fungicolous or mycoparasitic activities, and hitherto undetected in corn, were encountered in association with pathogens causing stalk rot (19,21,22).

The purpose of this study was to isolate and identify suspected mycoparasitic fungi from cornstalks, to evaluate their parasitism to host fungi and to corn, to record their frequency of association with host fungi under different field conditions, and to determine their mode of parasitism and effects on fungal hosts. This paper describes six known, and one new, fungicolous fungi that are associated with potential pathogenic fungi of cornstalks.

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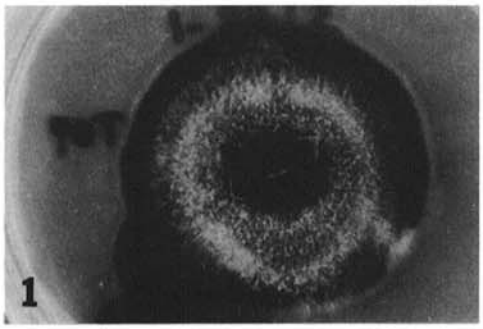
MATERIALS AND METHODS

Cornstalks from the following sources were sampled for the presence of potential stalk rot fungi within their nodal plates: pairs of healthy-appearing and stalk-lodged S_1 plants from 65 lines in BS-10 and 70 lines in BS-11 populations from 1979 harvests (10); stalk-lodged plants of commercial hybrid cultivars from the Iowa State Corn Yield Test from the 1982 harvest (26); and healthy-appearing and stalk-lodged plants of inbred lines and single-cross hybrids of converted tropical corn germplasm of the Cargill Incorporated harvest in 1982. The BS-10 and BS-11 populations and converted tropical germplasm were cultivated at a research farm near Ames, IA. The samples from the Iowa State Corn Yield Tests were collected at five other locations in the state.

The four lowest nodes above the brace roots were cut at harvest, dried at 38 C for 14 days, and stored in plastic bags. Stalks were split in half, samples (2 mm^3) were removed from the nodal plates within the pith and incubated on acidified potato-dextrose-agar (APDA, pH 3.5–4.0). Water agar, PDA plus benomyl at 400 mg/L (BPDA), or cornmeal agar plus wheat germ oil (0.5 g/L) (CMA) were used to separate and subculture the fungi. Culture plates were sealed with Parafilm, incubated at 22 C for 3 days, then placed 25 cm from germicidal lamps (2,400 AU). Exposure to germicidal light retarded floccose hyphal growth and enhanced sporulation which facilitated identification of fungi. Fungi isolated from the pith were examined under a stereomicroscope after a minimum of 14 days of incubation, and mycelium of those suspected of harboring mycoparasites was examined by bright-field and phase-contrast microscopy.

Host fungi and mycoparasites were separated by hyphal-tip isolation, by transfer to a selective medium, or by removing spores or hyphae with a finely drawn-glass needle. In addition, either *Helminthosporium carbonum* Ullstrup or *Cladosporium herbarum* (Pers.) Link ex S. F. Gray were used as trap cultures when *Fusarium* spp., especially *F. moniliforme* Sheld., were suspected of being parasitized. *H. carbonum* grows on BPDA, whereas neither *F. moniliforme* nor *Sphaeronaemella helvellae* (Karsten) Seeler tolerates benomyl. A culture block (2–3 mm in diameter) of *F. moniliforme*, suspected of being infected by *S.*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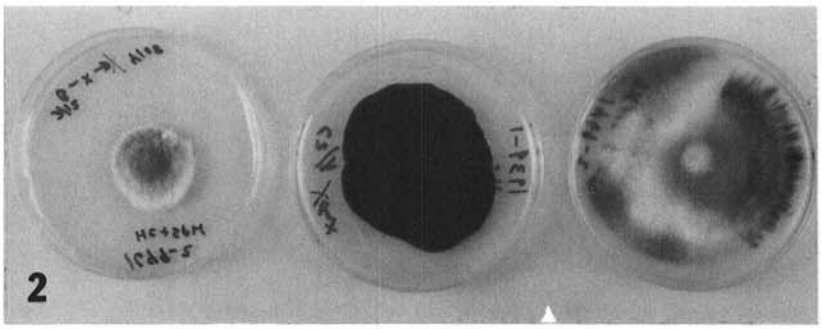
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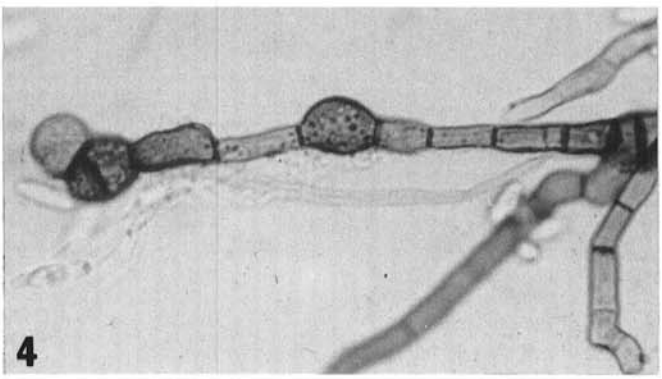
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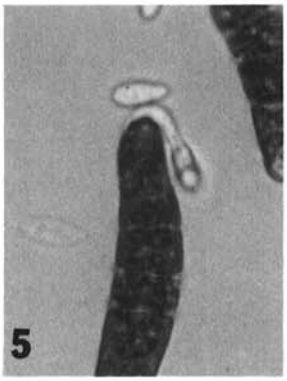
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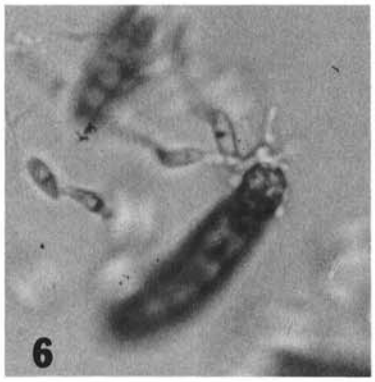
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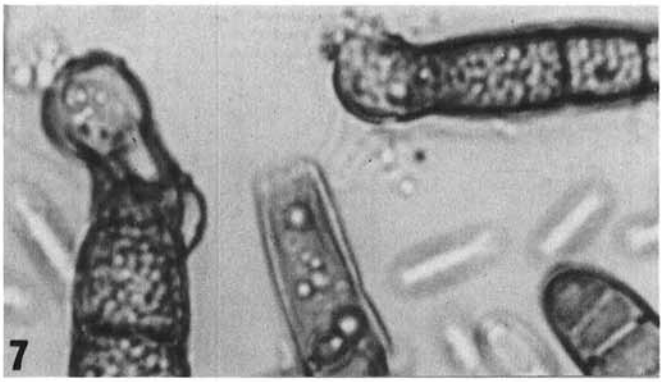
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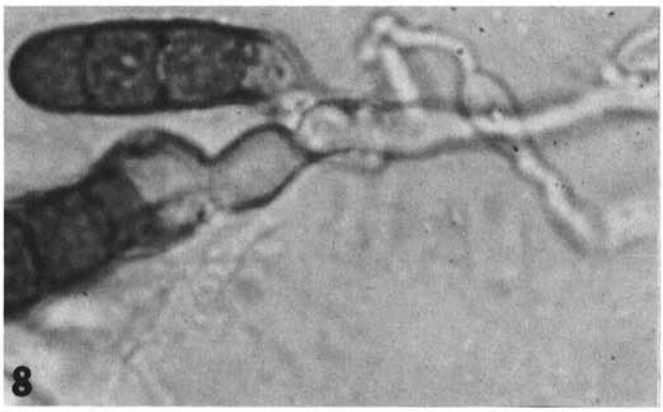
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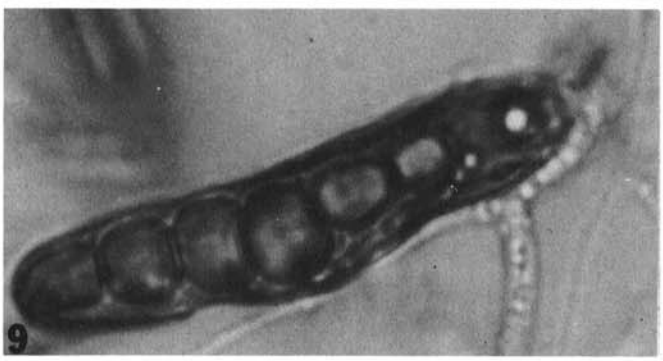
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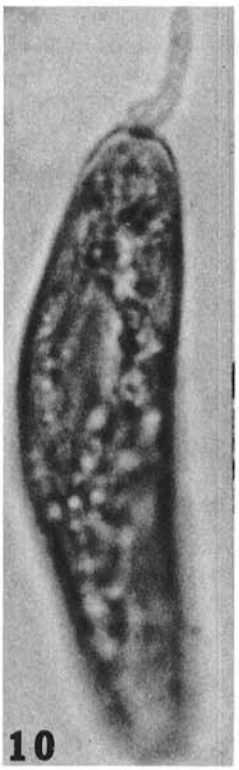
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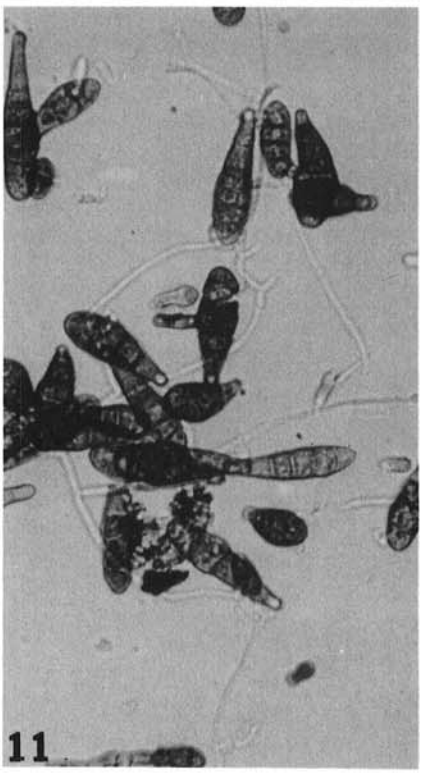
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helvellae, was placed adjacent to a culture block of *H. carbonum* on BPDA medium. *S. helvellae* parasitized the aerial hyphae and conidia of *H. carbonum* that grew on the BPDA medium.

To establish whether a test fungus was mycoparasitic, the host fungus was exposed to it by placing a mycelial mat (1–2 mm square) of the test fungus on the mycelial mat (15-mm square) of a host fungus (Fig. 1); placing the test and host fungi on opposite sides of petri plates containing PDA, APDA, or CMA; or mixing a spore suspension of the test and host fungi and placing it on one of the mentioned media. After hyphae commingled (Fig. 2), the tangled hyphae and spores of the test and host fungi were examined under a bright-field microscope for cell wall penetration (Figs. 3, 9, and 17), intracellular growth of hyphae by the parasite (Figs. 3,4,9,10,12,13,15,17–19), disruption of internal structures (Figs. 3,9,10,15,19), malformation of spores or hyphae (Figs. 4 and 7), and abnormal cell pigmentation (Figs. 2 and 8). The suspected mycoparasite was examined for formation of appressoria on germinated spores of the host fungus and for coiling around the host hyphae (Figs. 3, 5, 6, 8, 10, 13, 14, 16, and 17).

Ratings of the levels of mycoparasitic interactions were based on the responses of both the fungal host and the mycoparasite in vitro. For the host fungi, the factors considered were: rapidity of colonization of their mycelium, as measured by radial growth of the test fungus on the potential host's mycelium; amount of mycelial growth, rated visually as 1 = no growth to 10 = floccose hyphae filling the petri plate; amount of sporulation, estimated as the number of spores per unit area of the medium; stage of conidial development at time of infection, and amount of mycelial discoloration or pigment production, rated either visually as 1 = cream to 10 = dark brown or black, or measured as transmitted light measured with a photometer. For the mycoparasites, the factors considered were: incubation period before visual detection of the mycoparasite under a stereomicroscope, rapidity of smothering a host fungus, production of mycelia and asexual spores, time necessary for formation of the sexual stage; and amount of sexual reproduction. Responses were recorded at 5- to 7-day intervals up to 30 days from pairing of the host fungi and potential mycoparasites.

Leaves of inbred corn line B-73 were inoculated with spore suspensions of the seven mycoparasitic species in the greenhouse. Kernels were sown in a 8.6 × 7.4-m planting bed of silt loam soil. The experiment was a randomized complete block design with five blocks and eight plots per block. Each plot (37.5 × 50 cm) contained four plants. The distance between the plots within a block and between the blocks was 50 cm. Plants at the nine- to 11 leaf-whorl stage were inoculated by spraying 2.5 ml of a spore suspension (ranging from 5×10^5 to 5×10^6 spores per milliliter) of a given mycoparasitic fungus into the leaf whorl. One plot in each block received sterile distilled water as a control. Leaf tips in each whorl were marked and 10 days after inoculation, expanded leaves were examined for the presence of chlorotic or necrotic lesions.

Spore suspensions of *S. helvellae* (2.2×10^5 spores per milliliter), *Gliocladium roseum* (Link) Bainier (4.1×10^6 spores per milliliter), and *Pythium acanthicum* Drechs. (1.0×10^4 spores per milliliter) were injected into the internodes of plants of inbred B-73. Plants reared in the greenhouse and with physiologically mature kernels were injected either with the spore suspensions of one of the three fungi or with sterile distilled water. Four internodes, distal to the ear internode, were randomly assigned to each treatment. Each internode was inoculated in the middle with 0.1 ml of a spore suspension or with sterile distilled water. Fifteen days after inoculation, tissues from the center of the pith at the puncture point, at 5 cm above and below it, and at upper and lower nodal plates were incubated on both PDA and CMA at room temperature for 14 days before fungi growing from tissues were identified.

Seedlings of inbred B-73, at the second leaf stage of growth, were inoculated by spraying them with a suspension of mycelium of the sterile fungus, spore suspensions of the seven sporulating mycoparasitic fungi, or sterile distilled water. Seedlings were grown in a sterile soil mixture in 10-L containers placed in a controlled environment chamber (30 C day and 25 C night temperatures). There were five seedlings per container and two containers per treatment. The spore suspensions ranged between 5×10^4 to 5×10^6 spores per milliliter. Inoculated seedlings were covered with a plastic bag for 5 days, then allowed to grow for 10 more days before the second leaves of the seedlings were examined for lesions. Two seedlings were randomly selected, their leaves were surface-sterilized, and five subsamples per leaf were placed on PDA. Fungi emerging from subsamples were isolated and identified.

RESULTS

Sampling of nodal plates of cornstalks during 1979–1980 indicated that: the frequency of *H. carbonum* in S_1 progeny lines of the BS-10 and BS-11 populations was significantly greater in the stalk-lodged than in the healthy-appearing plants ($P < 0.001$), the frequency of *F. moniliforme* was similar in healthy and stalked-lodged plants of both populations, the frequency of *F. graminearum* was significantly greater ($P = 0.008$ for BS-10 and 0.052 for BS-11) in the stalk-lodged than in healthy-appearing plants, and the frequency of mycoparasitic fungi was not affected by corn populations or by the condition of the stalk at harvest. The most prevalent mycoparasite, *S. helvellae*, was primarily associated with *H. carbonum* and *F. moniliforme*.

Sampling of S_1 to S_4 progenies of the BS-10 and BS-11 corn populations during 1979 to 1982 resulted in the identification of seven mycoparasitic fungi that grew in association with the complex of fungi that are potential causes of stalk rot in corn. These mycoparasitic fungi, in descending order of their frequency, were: *S. helvellae* (16), *Trichoderma viride* Pers. ex S. F. Gray (*Hypocrea* sp. Fries) (2,3,20), *G. roseum* (*Nectria gliocladioides* Smalley & Hansen) (3,4), *Exobasidiellum* sp. Donk (1,14), *Gonatobotrys simplex* Corda (3,25), *Trichothecium roseum* Link (11), *P. acanthicum* (8,12,24), and an unidentified sterile fungus. All except *Exobasidiellum* sp. (14) previously have been reported to be mycoparasites (2,12,16).

Figs. 1–11. Macroscopic and microscopic observations of response of *Helminthosporium carbonum* to mycoparasitic fungi *Exobasidiellum* sp. and *Sphaeronaemella helvellae*. **1,** Reduced pigmentation of mycelium of *H. carbonum* in response to the mycoparasitic fungus *Exobasidiellum* sp., at 10 days after placing a 1 × 2-mm culture of the latter fungus in the center of a 15 × 15-mm culture of *H. carbonum* that was placed in the center of BPDA. **2,** Response of *H. carbonum* single-conidium isolates to dual culture with an isolate of the mycoparasitic fungus *S. helvellae*, after 10 days of incubation on APDA. Left and right petri plates contain dual cultures, and center plate contains only *H. carbonum*. In dual culture, *S. helvellae* and *H. carbonum* conidia were mixed in a 3:1 ratio, respectively. Left: *H. carbonum* mycelium was bleached, failed to grow and was smothered by *S. helvellae* mycelium which does not grow on APDA and remains restricted on *H. carbonum* mycelium. Center: *H. carbonum* with characteristic dark brown mycelium. Right: A moderately susceptible isolate of *H. carbonum*, displaying bleaching, partial overgrowth by *S. helvellae*, and infection-free periphery of a sector. **3,** Germinated conidium of *S. helvellae* forms a short germ hypha, appressorium, and an infection peg which penetrates the wall of a conidium of *Helminthosporium carbonum*. Growth of the parasitic mycelium destroys the internal structure of the conidium (×750). **4,** Malformation of conidiophore and conidium of *H. carbonum* infected by *S. helvellae*, the hyaline hypha of which is faintly discernible within cells of the conidiophore (×210). **5,** Germinated spore of *S. helvellae* attaches its germ hypha over the distal cell of a conidium of *H. carbonum* (×650). **6,** Infection and malformation of an incipient germ hypha from a conidium of *H. carbonum* by germinated spores of *S. helvellae* (×500). **7,** Malformed and nonfunctional incipient conidial germ hyphae of *H. carbonum* infected by *S. helvellae* (×750). **8,** Discoloration of germ hypha of *H. carbonum* due to infection by *S. helvellae*. The bleached infected hypha diminished in thickness and failed to grow (×650). **9,** Infection pore and intracellular deterioration of an infected mature condidium of *H. carbonum* parasitized by an invading hypha of *S. helvellae* (×750). **10,** Germinated conidium of *S. helvellae* forms an appressorium, infects a conidium of *H. carbonum* after penetration through the proximal end and fills the host with its hyphae (×750). **11,** Pincer hyphae of *S. helvellae* grip conidia of *Alternaria alternata*. Cell wall penetration occurred at pincer tips (×400).

The mycoparasitic species grew in pure culture on nutrient media. *S. helvellae, G. simplex*, and *P. acanthicum* failed to grow on APDA and BPDA. All grew well on PDA and CMA except that *S. helvellae* had weak growth on PDA. On CMA, mass ascospore cultures of *S. helvellae* developed numerous ascocarps within 5 days. Both *Exobasidiellum* sp. and *G. roseum* grew well on BPDA. This benomyl tolerance facilitated the separation of the two mycoparasitic species from *Fusarium* spp.

Sphaeronaemella helvellae. *S. helvellae* was repeatedly isolated from cornstalk nodal plates but only in association with other fungi. For example, of 1,874 nodal plates sampled in 1979, 708 were fungus-free and 1,166 supported a number of fungi which were potential causal agents of stalk rot in corn. *S. helvellae* occurred in 313 of these 1,166 nodal-plate samples. It was associated with *H. carbonum* and *F. moniliforme* in 156 samples, with *H. carbonum* and *Alternaria alternata* (Fr.) Keissler in 10, with *H. carbonum* in 110, with *Fusarium graminearum* Schw. in 14, with *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson in 11, with *C. herbarum* in two, and with *F. moniliforme* in 10.

S. helvellae was isolated more frequently in association with phytopathogenic fungi occurring in the pith nodal plate than in the epidermis of stalks of corn hybrids sampled at 153 days after planting in Wapello county in southern Iowa in 1982 (Table 1).

Homokaryons of *S. helvellae* cultured alone on CMA produced single phialides that supported relatively short chains of conidia. However, when a homokaryon of *S. helvellae* was cultured together with *H. carbonum* on either APDA or BPDA, and it depended on its host fungus for growth, it developed basitonously branched phialides with luxuriant long chains of conidia. *H. carbonum* failed to sporulate when a 3:1 mixture of conidia of *S. helvellae* and *H. carbonum*, respectively, was cultured for 10 days on CMA, while in pure culture on CMA, it produced 52.9×10 conidia per cm^2. Hyphae of *S. helvellae* wrapped around those of *H. carbonum* and then penetrated the host cell wall. The infected mycelium of *H. carbonum* lost pigmentation until it became hyaline, diminished in thickness, and ceased to grow (Fig. 8). The infected conidiophores were short, distorted, and produced malformed conidia (Fig. 4). Germ hyphae of *S. helvellae*, at points of contact with hyphae or conidia of *H. carbonum*, developed appressoria and infection pegs that penetrated the host cell walls (Figs. 3, 5, 6, 8, and 10). Germinating conidia of *H. carbonum* were attacked especially severely. The incipient germ hypha became atrophied into a spherical cell on which were attached three or more conidia of *S. helvellae* with short germ hyphae (Figs. 5 to 7). The infected immature conidia of *H. carbonum* failed to develop cross-walls and were discolored (Fig. 15). All isolates of *H. carbonum* were susceptible to parasitism by *S. helvellae*. Parasitized mycelium became bleached, sparse, and malformed, but sometimes it produced sectors that fanned out in normal growth until again overtaken by the mycoparasite (Fig. 2). *S. helvellae* parasitized *H. carbonum* rapidly at 25 C but not at 15 C.

F. moniliforme and *S. helvellae* have similarly shaped phialides and catenulate conidia. This makes it difficult to distinguish between infected and uninfected *F. moniliforme*. In the 156 pith isolates that contained *H. carbonum, F. moniliforme*, and *S. helvellae*, it was impossible to ascertain under ×140 magnification that a healthy-appearing culture of *F. moniliforme* did not harbor *S. helvellae*. The severely infected mycelia of *F. moniliforme* grew flat and did not sporulate, especially when associated with a parasitized isolate of *H. carbonum*. A number of healthy-appearing isolates of *F. moniliforme* produced dense purple pigment that diffused into the medium. The use of trap cultures of *H. carbonum* indicated that two of 10 cultures of *F. moniliforme* (sampled from densely pigmented centers) were infected by *S. helvellae*. When 12 highly pigmented isolates of *F. moniliforme* were selected and mated in all combinations, by placing a pair of isolates at opposite sides of a medium, perithecia of *S. helvellae* formed at the convergence of mycelia in two of the crosses which indicated that four cultures were infected with *S. helvellae*. Ten subcultures of *F. moniliforme* derived from the culture margins grew 1/3 faster than those derived from pigmented hyphae at the culture center. Also, hyphal tip subcultures either did not produce pigment upon aging or produced it at a reduced rate and intensity, while cultures derived from the center were densely pigmented. Inoculation of the pigment-free hyphal tip cultures of *F. moniliforme* with *S. helvellae* resulted in the formation of pigment. Uninoculated cultures remained free of pigment. Temperature affected the rate of pigment production in inoculated cultures. None occurred at 15 C, while the agar was dense purple at 25 C. The intensity of pigment in inoculated cultures varied with the isolate and ranged from lilac to purple.

The in vitro response of *Colletotrichum graminicola* (Ces.) G. W. Wils, to *S. helvellae* was similar to, but more severe than, that of *H. carbonum*. Isolates of *C. graminicola* on APDA developed grayish to brown mycelia. The walls of the hyphae were hyaline, but the cytoplasm of the mycelium was pigmented (19). The infected isolates of *C. graminicola* became colorless, failed to produce conidiomata, and grew so slowly that they were smothered by the growth of *S. helvellae*.

Trichoderma viride. Among the cornstalks sampled in 1980, *T. viride* occurred in 14 of 2,612 nodal-plate isolates in association with other fungi. All were recovered from nodal plates closest to crown and brace roots. *T. viride* did not occur in 327 pith samples isolated from greenhouse-grown cornstalks, but it did occur in 15 of 364 root samples from the same plants. The 14 field isolates were associated with six isolates of *H. carbonum*, four of *F. moniliforme*, two of *F. graminearum*, and one each of *A. alternata* and *Penicillium* sp.

T. viride was severely parasitic to a *Rhizopus* sp. that was isolated from the corn roots. It did not inhibit the mycelial growth of *Rhizopus* in vitro, but upon contact, it coiled around, penetrated, and grew within the host mycelium (Figs. 12 and 13).

Gliocladium roseum. Five isolates of *G. roseum* were found with 210 isolates of *H. carbonum* obtained from 1,166 pith samples in 1979. This fungus was highly pathogenic to *H. carbonum* and caused bleaching and cessation of growth in vitro. Its hyphae wrapped extensively around those of *H. carbonum*, penetrated the cell walls, and grew within the host hyphae (Fig. 14). It penetrated conidia of *H. carbonum* directly and prevented formation of septa in immature conidia and formation of conidia on immature conidiophores. G. roseum was highly parasitic on *A. alternata, C. acremonium*, and *F. moniliforme* but was less so on *F. graminearum* and *Penicillium oxalicum* Currie and Thom.

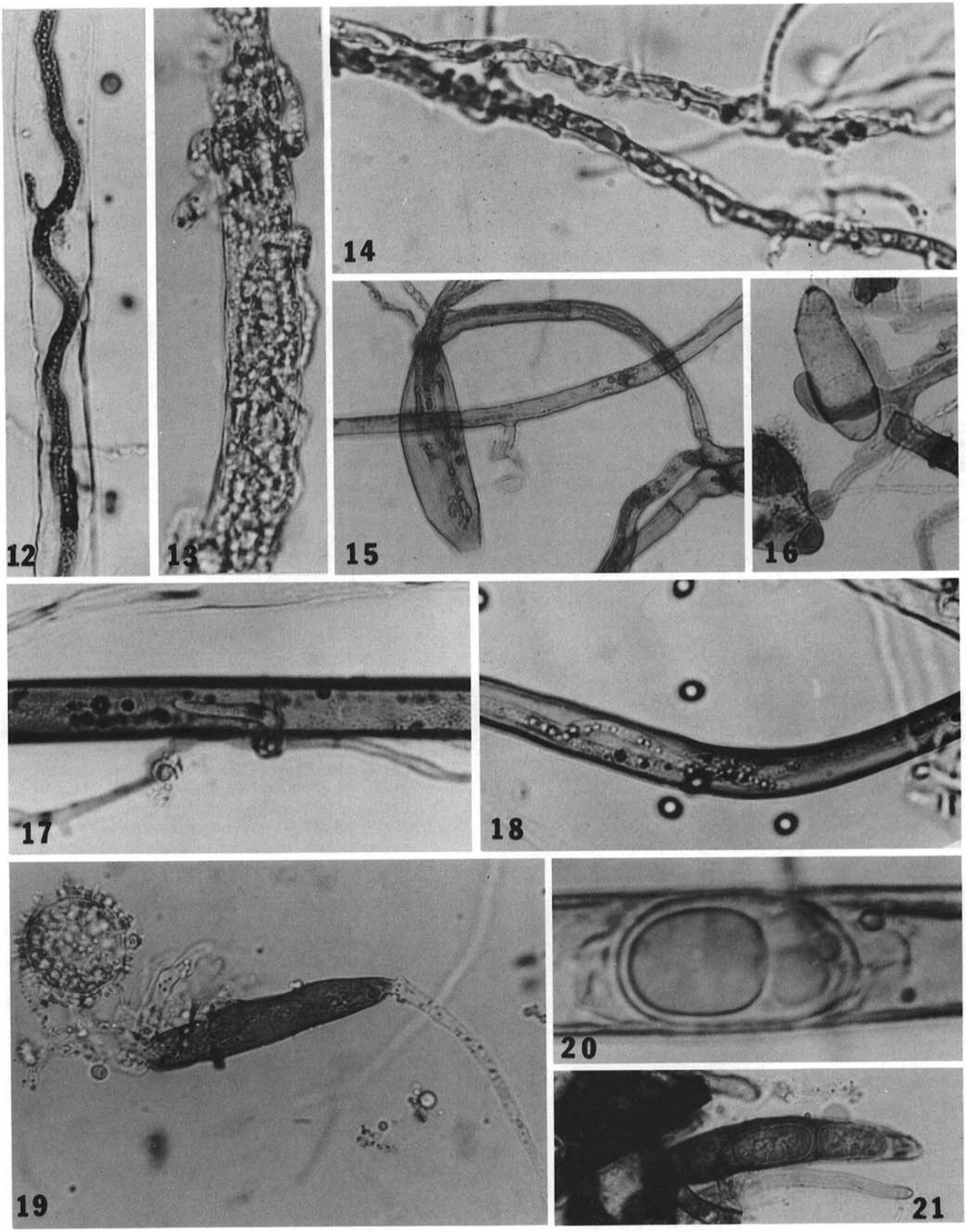
Field isolates of *G. roseum* were tolerant to benomyl, but conidial production was progressively reduced and retarded as benomyl concentration in the medium was increased from 50 to 400 mg/L. This tolerance to benomyl was used in separating *G. roseum* from isolates of *Fusarium*. Attempts to obtain the perfect stage of *N. gliocladioides*, with mature ascospore-bearing perithecia, from field isolates of *G. roseum*, were not successful.

Gonatobotrys simplex. Four of 2,612 pith samples isolated in 1980 from corn cultivated at the Hinds Research Farm (Iowa State University, Ames) had *G. simplex*, while 95 of 1,104 pith samples isolated in 1981 from corn cultivated at the Agricultural Engineering Farm (24 km from the former farm) contained *G. simplex* in association with other fungi. The four isolates from Hinds Farm were associated with two each of *F. graminearum* and

TABLE 1. Association of *Sphaeronaemella helvellae* with four phytopathogenic fungi in rind and pith tissue of hybrid corn grown in Wapello County, IA, in 1982

	Number of phytopathogen and *S. helvellae* isolates associated in tissue samples[a]	
Phytopathogen	Rind	Pith
Fusarium moniliforme	29/0[b]	57/21
F. graminearum	24/3	60/29
Colletotrichum graminicola	103/13	28/15
Helminthosporium carbonum	17/1	26/13
Total	173/17	171/78

[a] From a total of 144 samples per tissue.
[b] Phytopathogen/*S. helvellae*.



Figs. 12–21. Microscopic observations of the response of *Helminthosporium carbonum* to mycoparasitic fungi *Trichoderma viride, Gliocladium roseum, Exobasidiellum* sp., and *Pythium acanthicum*. **12,** A hypha of *T. viride* growing through the stolon of *Rhizopus* sp. (×600). **13,** Hyphae of *T. viride* coiling, penetrating, and growing within a young sporangiophore of *Rhizopus* sp. (×600). **14,** Hyphae of *G. roseum* coiling, penetrating, and growing within hyphae of *H. carbonum* (×600). **15,** Growth of hyphae of *Exobasidiellum* sp. within hyphae, conidiophore, and conidium of *H. carbonum*. No septa developed within the infected conidium (×750). **16,** Hyphae of *Exobasidiellum* sp. grip and produce appressoria upon contact with conidia of *H. carbonum* (×750). **17,** Hypha of *P. acanthicum* grips, forms an appressorium, penetrates, and grows within a sporangiophore of *Rhizopus* sp. (×600). **18.** Growth and branching of hypha of *P. acanthicum* within a sporangiophore of *Rhizopus* sp. (×600). **19,** Invasion of a conidium of *H. carbonum* by hyphae of *P. acanthicum* that have emerged from its oospore (×500). **20,** Development of parthenospore (a smooth-walled oospore) of *P. acanthicum* within a sporangiophore of *Rhizopus* sp. (×1,100). **21,** Development of parthenospores of *Pythium acanthicum* within a conidium of *H. carbonum* (×550).

F. moniliforme. The frequency of association of *G. simplex* isolates with other fungi from the Agricultural Engineering Farm was: 68% with *F. moniliforme*, 16.8% with *H. carbonum*, 6.4% with *A. alternata*, 3.2% with each of *Epicoccum nigrum* Link ex Fr. and a *Fusarium* sp., and 1.0% with each of *F. graminearum* and *Nigrospora oryzae* (Berk. & Br.) Petch.

Pythium acanthicum. *P. acanthicum* was associated only with *H. carbonum*. Of the 210 *H. carbonum* isolates obtained from 1,166 pith samples, four contained *P. acanthicum*. This fungus is a severe pathogen of *H. carbonum* and other fungi that grow in its vicinity. It was the only pathogen that produced its perfect stage within the host mycelium (Figs. 20 and 21). The infected conidia of *H. carbonum* failed to develop septa; instead, one or more oospores, without the normal echinulation, were observed occupying the conidial interior (Fig. 21). Hyphae of *P. acanthicum* penetrated the conidial cell wall of *H. carbonum*, prevented formation of septa, and grew within the conidium (Fig. 19). *P. acanthicum* produced numerous oospores on mycelia of *H. carbonum* growing on APDA, but it formed only hyphae when grown alone on APDA. Hyphae of *P. acanthicum* that contacted the stolons and sporangiophores of a *Rhizopus* sp., formed appressoria and penetrated the host wall (Figs. 17 and 18).

***Exobasidiellum* sp.** This basidiomycete did not form basidiocarps but produced bisterigmatic basidia within its floccose mycelium. *Exobasidiellum* occurred in 21 of 2,612 pith isolates collected from cornstalks in 1980. It occurred alone in 10 pith samples and was associated with *H. carbonum* in six, *N. oryzae* in four, and *F. moniliforme* in two. It was highly pathogenic to *H. carbonum* and caused bleaching of the mature spores and hyphae and smothering growth of the mycelium (Fig. 1). Hyphae of *Exobasidiellum* formed appressoria when contacting spores and mycelium of *H. carbonum*, and immature conidia failed to form septa when infected (Figs. 15 and 16).

Trichothecium roseum. Pith samples from converted tropical corn lines contained *T. roseum*. Seven of the 520 pith isolates in 1981 contained *T. roseum*, of which three were associated with *H. carbonum*, two with *Aspergillus* sp., and one each with *C. graminicola* and *Fusarium* sp. *T. roseum* was the slowest growing mycoparasitic fungus to appear among the fungi which grew on pith samples. It was similar to other fungicolous fungi in its infection of the host mycelium.

Sterile fungus. In only two of over 4,000 pith samples, this mycelial fungus was encountered parasitizing *H. carbonum*. Its hyphae coiled around those of *H. carbonum* and smothered the host mycelium rapidly. Cultures of this fungus on APDA occasionally produced sectors with a cephalate conidial form of *G. roseum*.

Phytopathogenicity. Spray application of inoculum of each of the seven mycoparasitic fungi to greenhouse-grown seedlings and mature plants of inbred corn line B-73 did not result in either foliage or pith infection. Injection of spore suspensions of *S. helvellae, G. roseum*, or *P. acanthicum* into the mature cornstalk resulted in their spread into the pith. *G. roseum* was reisolated alone from pith and nodal plates of injected plants, *P. acanthicum* was reisolated alone from the pith of one of four plants, and *S. helvellae* occurred in association with *F. moniliforme* in one of 16 isolates from the four plants.

DISCUSSION

This is the first report of the occurrence of mycoparasitic fungi in cornstalks. They were more abundant in the pith than in the rind tissues, and the species and frequency of their occurrence varied with location. These fungi were always associated with potentially phytopathogenic fungi that were also present in the pith of cornstalks. Under in vitro conditions they parasitized the potential plant pathogens.

The preliminary screening test for mycoparasitism by a fungus was the use of a differential medium such as BPDA. *H. carbonum* grew well whereas *S. helvellae* died when transfered alone to BPDA. When spores of *S. helvellae* were placed on mycelium of *H. carbonum* growing on BPDA, the white hyphae of *S. helvellae* began to grow, infect, and sporulate on the aerial hyphae of *H. carbonum*. The reisolated spores of *S. helvellae* did not grow on BPDA. The combination of the characteristics of tolerance to benomyl and susceptibility to mycoparasitic fungi in *H. carbonum* made this fungus an ideal trap culture. Cubes of a medium containing pure culture of *H. carbonum* and a fungus suspected of harboring a mycoparasite (e.g., *F. moniliforme* and *S. helvellae*) were placed adjacent to each other on BPDA. Since *F. moniliforme* failed to grow on this medium and *H. carbonum* became parasitized, the presence of *S. helvellae* could be ascertained.

The fungicolous fungi were not pathogenic to corn either at the seedling or mature plant stages. However, in the nodal plate of the pith these fungi were isolated in association with the phytopathogenic fungi. This close association suggests that a phytopthogenic host fungus could provide the means by which a fungicolous fungus enters the cornstalk.

In vitro, the mycoparasitic fungi infected their host hyphae and spores by: coiling and penetrating at the point of contact by means of infection pegs (under bright-field microscopy, the point of penetration showed as a bright puncture in the host hypha [Figs. 3, 9, and 14]); hyphal branching and formation of pincers that adhered to cells and then penetrated the host at one or both tips of the pincers (Fig. 11); and hyphal tips that formed appressoria at the point of contact with host and penetrated by means of infection pegs (Figs. 3, 5, 8, 10, and 16). The three types of infection mechanisms were observed in *S. helvellae*. Generally, coiling was observed on aerial hyphae and with minimum moisture in culture plates, whereas germ tube attachment and appressorium formation was detected when water condensed on the mycelia. After penetration, the infection peg developed into a hypha within the host cell and grew through the septa of the cell. Conidia of *H. carbonum* were often infected via their attachment points with conidiophores (Fig. 15). The thickness of the host hypha seemed to limit the number of parasitic hyphae that grew within it. For example, there was a 1:1 ratio of *H. carbonum* and S; helvellae (Fig. 4), whereas, a ratio of 1:4 or 1:5 occurred for a *Rhizopus* sp., and *T. viride* (Fig. 13). One method of ascertaining infection, especially among host fungi with hyaline mycelium, was to locate a septum of the parasitic hypha between the adjacent septa of the host hypha. Infection by *P. acanthicum* was ascertained by the presence of parthenospores (8) within the host hypha and conidia (Figs. 10 and 21).

The majority of the fungicolous fungi that grew on the pith samples were weakly to moderately parasitic to their host fungi. The fact that a field isolate of a weakly mycoparasitic fungus produces appressoria and coils around, penetrates, and grows within its host hypahae indicates that it has the genetic potential for becoming a severe parasite of its hosts. Of the seven fungicolous species, only *S. helvellae* lends itself readily to the study of the genetics of mycovirulence. The *Hypocrea* stage of *T. viride* and the *Nectria* stage of *G. roseum* are difficult to grow routinely. *P. acanthicum* has homothallic mycelium and its oospore and zoospore are diploid (6,8). Similarly, the *Exobasidiellum* sp. has two instead of four basidiospores and the nuclear make-up of its spores is considered to be mainly dikaryotic (1). The remaining species are imperfects.

The frequent association of *S. helvellae* with *F. moniliforme* in the pith isolates, and the difficulty in distinguishing between a mixture of the two species and pure isolates of *F. moniliforme*, suggest that the actual occurrence of *S. helvellae* may be more frequent than the results indicate. When 12 highly pigmented cultures of *F. moniliforme* were crossed, four were found to contain *S. helvellae* as indicated by perithecium formation. Pairing of the two fungi further indicated that pigment formation in *F. moniliforme* was due to the presence of *S. helvellae* rather than senescence of mycelium. *F. moniliforme* was the only host species that produced pigment when cultured with *S. helvellae*.

Helminthosporium carbonum is the most common host of *S. helvellae*. Up to 85% of the isolates of *S. helvellae* from corn pith were associated with *H. carbonum*. The majority of *S. helvellae* field isolates were either weakly or moderately pathogenic, but some field isolates and homokaryotic cultures were severely pathogenic to *H. carbonum*. *H. carbonum* serves as an excellent

host for the study of mycoparasitism due to its dark and robust hyphae, large conidia, and ease of handling on culture media.

Gonatobotrys simplex was often associated with *Fusarium* spp. isolated from cornstalks. In vitro, it did not smother any of its hosts but grew intermixed with their mycelium. No discoloration of host mycelium was observed in paired growth of *H. carbonum* or *F. graminearum* with *G. simplex*. This fungus is reported to be biotrophic and dependent on the presence of its fungal hosts (2,25). However, its saprophytic growth on CMA plus wheat germ oil is similar to that of other mycoparasites.

T. roseum was one of the least frequent and the slowest growing mycoparasites found with corn pith isolates. However, this fungus is reported to be highly mycoparasitic (11). In the pith samples, it was mainly associated with *Fusarium* spp. *P. acanthicum* exhibited severe mycopathogenic activity with potential pathogenic fungi of cornstalks. The choice of APDA as the initial medium for pith sample culture could have been a factor in the low frequency of isolation of this fungus.

The pith of the cornstalk may provide a protected environment for the growth of mycoparasitic fungi and may isolate them from competition and antagonism by saprophytes. This might account for the large number of fungicolous species that were encountered in the pith.

The presence of fungicolous fungi in the pith tissues and their close association with the potential phytopathogens suggests that it might be possible to utilize these fungi or their improved progenies in the biocontrol of stalk rot pathogens in the field.

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