Basidiospore Infection of Cotton Bolls by Thanatephorus cucumeris

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ABSTRACT

Basidiospores of *Thanatephorus cucumeris* are capable of germinating on and penetrating the epidermis of cotton bolls and bracts, causing disease. Penetration took place through stomata and through the epidermis by infection pegs beneath cushions of mycelia. The latter were commonly found to have penetrated through the thin-walled accessory cells surrounding the stomata.

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Pinckard and Luke (16) reported *Pellicularia filamentosa* (Pat.) Rogers to be a primary pathogen of cotton (*Gossypium hirsutum* L.) bolls in the Louisiana-Mississippi delta. Apparently healthy bolls were easily penetrated and rotted by the fungus. Under field conditions, this type of rot follows the appearance of vast amounts of viable *Thanatephorus cucumeris* Frank (Donk) hymenial layers and basidia which Pinckard (15) observed on cotton stalks in Louisiana beginning in late July and continuing through frost. Older bolls present in the vicinity of these fungal structures, which are always under a dense foliar canopy, are susceptible to rot by *T. cucumeris*. Previous work (16) indicated that basidiospores probably initiated the boll rot from which Rhizoctonia was isolated because it is not the habit of that fungus to ascend within the cotton stalk more than a very few cm (Fig. 1A). In previous work the fungus was referred to as *Pellicularia filamentosa* (Pat.) Rogers in the fruiting stage, and to the widely used *Rhizoctonia solani* Kühn in the vegetative phase. Talbot (17) summarizes, quite well and in an acceptable form, the complexities of the nomenclature of the perfect stage associated with *R. solani*. He treats them collectively as *Thanatephorus cucumeris* (Frank) Donk. This name is used for the fungal stages mentioned in this report. The purpose of this paper was to elucidate the role of basidiospores of *T. cucumeris* in cotton boll rot.

MATERIALS AND METHODS.—*In situ inoculations.*—Cotton stalks showing profuse hymenial development (Fig. 1A) were collected from the field in the early morning during August 1967, and placed in large moist chambers. In the late afternoon a number of petri dishes were prepared with sections of the fungus hymenium attached to the underside of the covers which were allowed to discharge basidiospores on the surface of distilled water or water agar overnight at room temp. The following morning the plates were examined for spore discharge. Those plates showing numerous basidiospores (Fig. 1B) were used for boll inoculations. Within 4-6 hr basidiospore germination on check plates approached 100% (Fig. 2A). The possibility of mycelial fragments falling on the agar plates was considered, but evidence of its importance as a source of inoculum was not found. Thirty-day-old healthy bolls from greenhouse plants (cultivar 'Deltapine 16') were selected for study. Fifty bolls were dipped in a beaker containing the basidiospores in suspension. Another 50 bolls were inoculated by placing pieces of water agar with basidiospores attached between bracts and bolls growing in the greenhouse under shade. Each boll was then covered with a plastic bag and left on the plant for observation. After inception of rot, parts of the boll interiors were plated on water agar to determine the presence of *T. cucumeris*.

Inoculation of detached bolls and bracts.—Thirty-day-old bolls, with bracts, were detached from field-grown plants during early September 1971, surface-sterilized with 0.5% aqueous sodium hypochlorite for 10 min, then rinsed with sterile water. Some bolls were dipped into a spore suspension then placed in sterile,

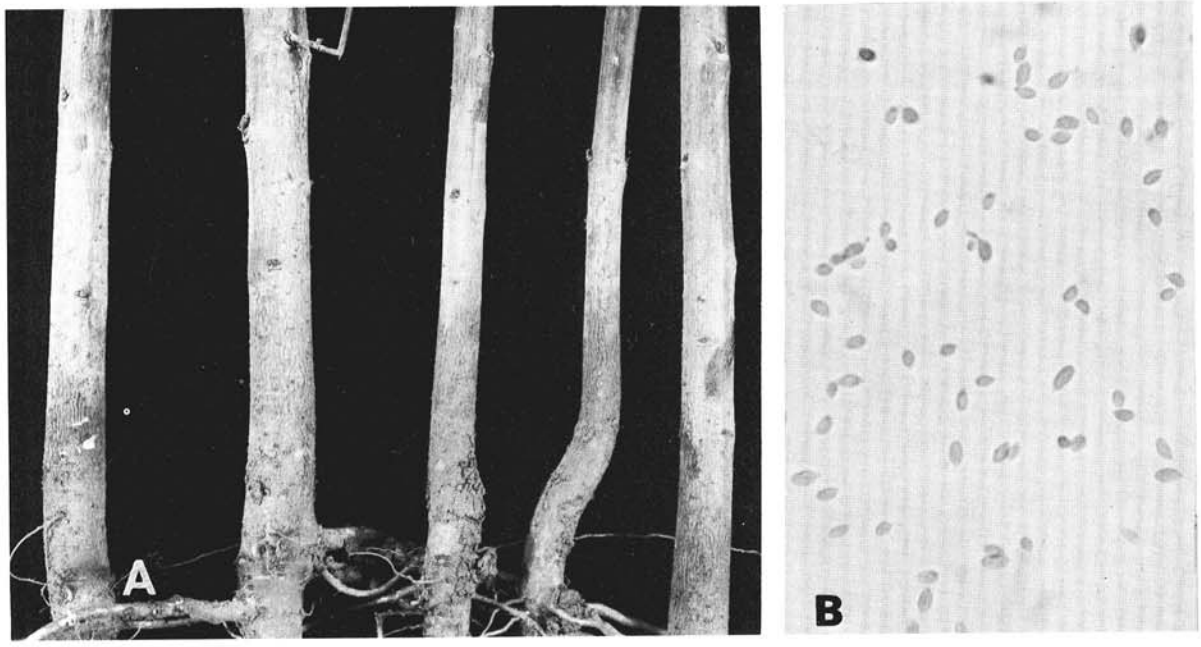

Fig. 1-A, B. A) Stalks of field cotton showing the gray hymenial fruiting structures of *Thanatephorus cucumeris* = (*Rhizoctonia solani*) = (*Pellicularia filamentosa*) (reduced). **B)** Basidiospores collected from a hymenial layer of *Thanatephorus cucumeris* soon after nocturnal release from field-grown cotton stalks (× 447).

moist chambers. These were incubated 24-48 hr, or until *T. cucumeris* boll rot symptoms appeared. Other bolls were placed in moist chambers and were inoculated by spore discharge directly from hymenia suspended above their surfaces. Cover glasses dipped in water agar were placed at random among the bolls. A microscopic examination of the cover glasses the following morning indicated areas of heavy spore discharge. After the first night of spore discharge, the cover was replaced with a fresh one if the bolls were to remain for several days. The bolls were incubated at room temp.

Preparation of epidermal strips and histological sections.—Following spore inoculation, and after a 24-48 hr incubation period, bracts were detached from bolls and fixed in a mixture of formalin, acetic acid, and alcohol (FAA). Some bolls were kept in a freezer for at least 1 hr. Epidermal strips from the frozen bolls were readily removed after they were gently thawed in warm water. The epidermal strips were stained with acid fuchsin (1 g/100 ml 70% ethanol mixed in lactophenol) and examined for spore germination and hyphal penetration. Epidermal strips with the germinating basidiospores on the surface were immersed in water and agitated to determine tthe tenacity of the germ tubes on cuticle surfaces.

Other bolls were incubated (some for 2 wk) until visible disease symptoms appeared. Part of the infected carpel area was excised and fixed in FAA. All FAA-fixed sections were carried through dehydration, infiltration, embedding, sectioning, and staining as described by Jensen (8).

RESULTS.—Under Louisiana conditions, basidiospore discharge was considered to be almost exclusively nocturnal. Spore prints indicated discharge started at approximately 10:30 PM and terminated about 3:30 AM the next morning. This agrees with our previous work and that of Flentje et al. (4). Figure 2A shows typical basidiospore germination on water agar after about 3 hr at 30 C. Germ tubes originated at one or both ends of the spore.

Bolls that began to rot 5 to 7 days after inoculation were examined microscopically for *T. cucumeris* and its presence was confirmed by isolation. *T. cucumeris* was recovered from 27 of 50 bolls inoculated with spores self-discharged from suspended hymenial growth on cotton stalks. Twenty-nine bolls inoculated with the water suspension of self-discharged spores became infected. A typical section of a boll surface with advanced growth of *T. cucumeris* mycelium is shown in Fig. 2D.

Numerous observations by the authors of basidiospore germination on water agar and boll epidermis indicated that observations should include the first 24 hr of exposure as well as later periods of development. Figure 2E shows basidiospores germinating on an epidermal strip of a 30-day-old boll incubated 24 hr at 30 C. Under proper shade and moisture conditions, this could and probably does occur in the field although with much less inoculum. Germ tube penetration into cuticle of a boll epidermal strip is demonstrated in Fig. 2F. The contention of Luke and Pinckard (9) that the bract is a factor in boll rot is substantiated by the penetration of basidiospore germ tubes through the epidermal cells and stomata of a bract as illustrated in Fig. 2-B, C.

Figures 3-A, B illustrate advanced mycelial growth of the fungus on 30-day-old bolls after 7 days. Both direct

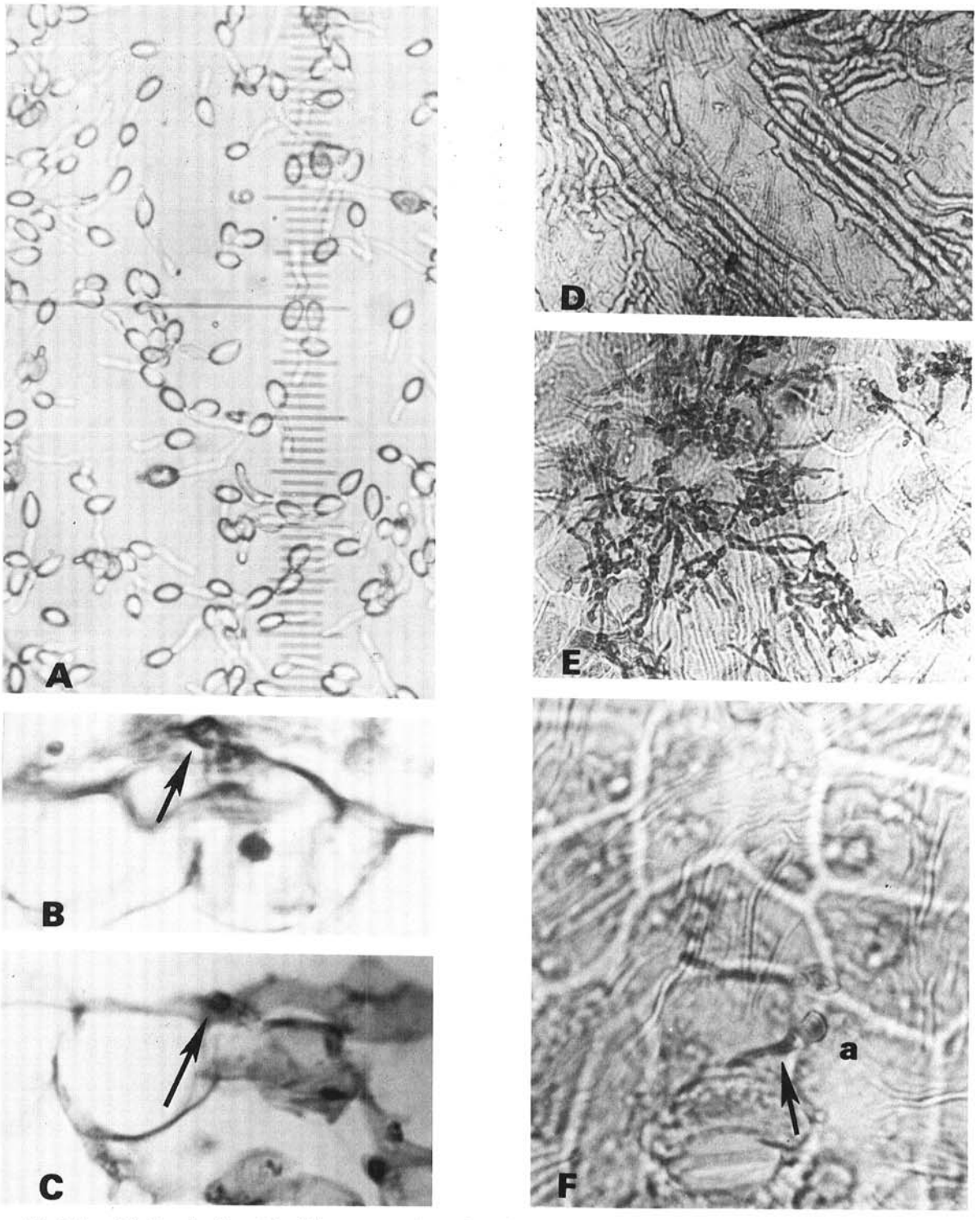

Fig. 2-(A to F). Germination of basidiospores on the surface of water agar 4 to 6 hr after nocturnal release (× 650). **B-C)** Transverse sections of bracts of a 30-day-old cotton boll showing germ tubes of basidiospores of *Thanatephorus cucumeris* entering through a stomate (8) and through an epidermal cell (9) after 48 hr at 30 C (× 860). **D)** Epidermal surface of an infected cotton boll showing typical mycelia of the vegetative stage of *Thanatephorus cucumeris* (× 210). **E)** Epidermal strip of a cotton boll showing germinating basidiospores of *Thanatephorus cucumeris* after 24 hr at 30 C. The 30-day-old boll was inoculated as in Fig. 2 (× 200). **F)** Direct penetration of germ tubes of basidiospores into the boll epidermis of cotton fruit by *Thanatephorus cucumeris*. The spore at "a" has entered an accessory cell of a stomate (× 860).

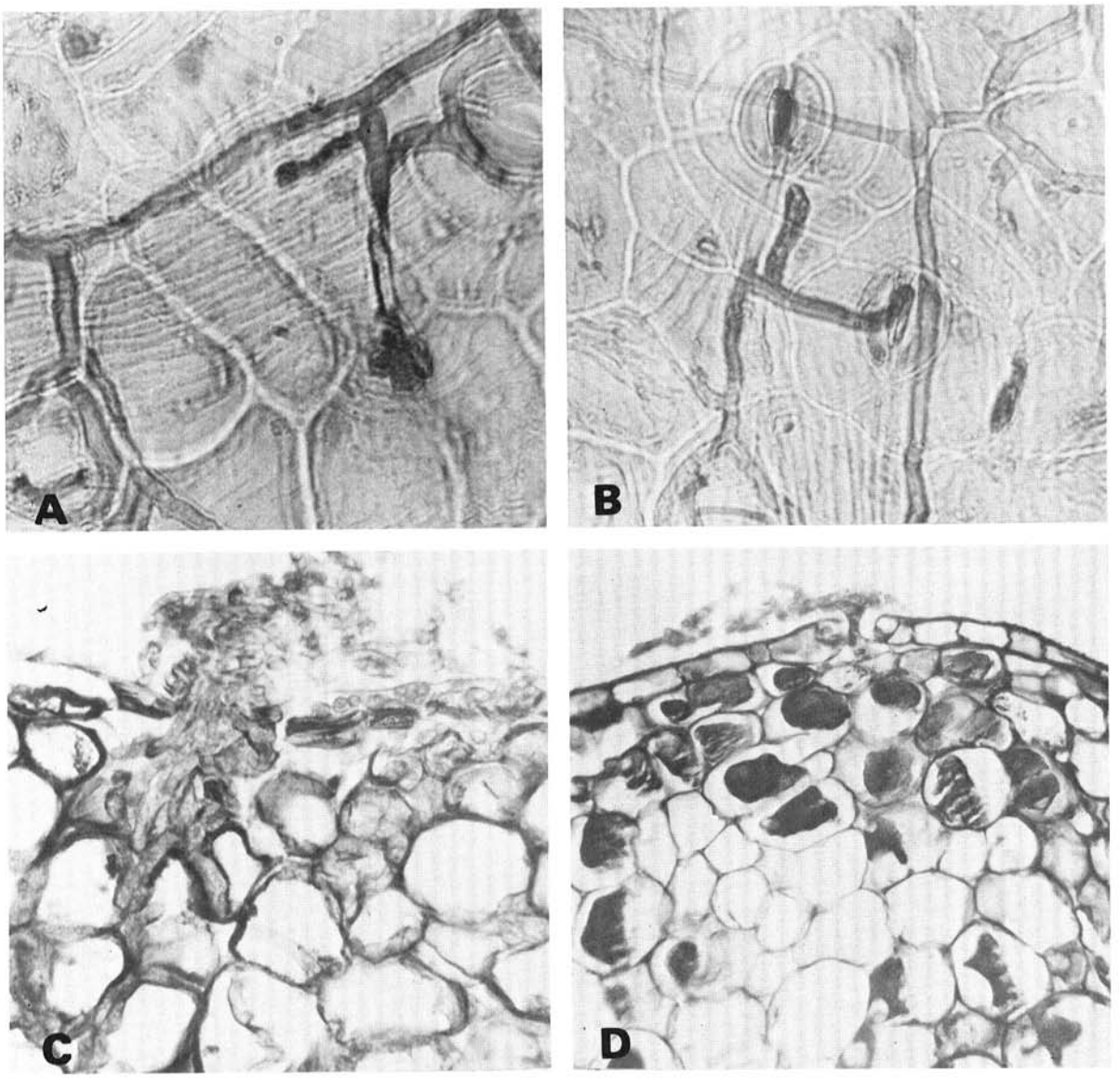

Fig. 3-(A to D). A-B). Epidermal stripes of a 30-day-old cotton boll showing hyphal growth, the vegetative stage of *Thanatephorus cucumeris*, 7 days after mycelial inoculation. **A)** Knotting of a single hyphal strand, and **B)** stomatal penetration (× 229). **C-D)** Transverse sections of the carpel wall of a 30-day-old cotton boll showing: **C)** advanced stages of penetration by mycelium of *T. cucumeris* and **D)** stomatal entry (× 229).

and stomatal penetration have been observed. Later stages of mycelial development in the carpel walls of 30-day-old bolls are illustrated in Fig. 3-B, C. Death and discoloration in advance of hyphal penetration suggested possible enzymatic and toxic action.

DISCUSSION.—The germination of *T. cucumeris* basidiospores on the surface of cotton bolls and bracts, and the consequent direct and stomatal penetration by the germ tube, or resulting hyphae, was observed. The infectious cushions described by Gonzalez and Owen (7) are similar to the cushions pictured here in Fig. 3-C, D. *T. cucumeris* is a soil-borne pathogen of cotton seedlings, bolls, leaves, and possibly the stems. Its basidiospores are commonly disseminated similarly to the aerial-borne types as described by Echandi (2).

Carpenter (1) reported infection of *Hevea* (rubber trees) and Echandi (2) observed infection of bean leaves by basidiospores of *T. cucumeris* but neither observed modes of penetration. Neal (10) reported aerial attack of cotton leaves by *T. cucumeris,* but was unable to find a source of basidiospores. Neal worked with much less dense stands of cotton than we now grow and, correspondantly, much less humidity at the soil level a key factor in the life history of *T. cucumeris*. Peeples and Bain (14) recovered *T. cucumeris* from acid-delinted cotton seed. It would be interesting to know how seed infection

occurs and how frequently it occurs. Flentje and Stretton (3) suggested that there is no reliable evidence that single basidiospores can survive and form colonies in the soil. Papavizas (11, 12) demonstrated the ability of basidiospore isolates to colonize a soil-amended cellulosic substrate. Flentje et al. (6), stressed variation in strains as a process of mutation and suggested that if single spore isolates did survive and grow they would eventually be selected toward the wild-type culture. Gonzalez and Owen (7) described basidiospore germination and direct penetration of tomato fruit by mechanical means.

The authors accept the concept (3, 5, 6) that under some or most conditions an orderly succession of mutations and recombinants as such are forces at work in establishing and maintaining the wild species in a soil. Conversely, the type or species we are dealing with on cotton in the Louisiana-Mississippi delta, and which has been identified by Parmeter (*personal correspondence*) as the "praticola" type (13), may be found on almost any cotton stem wherein the soil is adequately shaded and the temp sufficiently high (15). It is one of the most common macroscopically visible fungi in the area. The term "parasite", however, seems too confining to be used for our type of fungus; it grows on many substrates; on the stems of weeds as well as in cotton gin trash, but always in a shaded, moist location emerging to the surface when temp are above 27-28 C. Isolates collected in Louisiana, Mississippi, and Arkansas during the past several years have never failed to be pathogenic on cotton bolls and seedlings and have always resembled the praticola type. We therefore regard our type fungus as a very stable "species" and highly pathogenic on cotton fruits, leaves, stems, and seedlings under suitable environmental conditions.

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