A Boll Rot of Cotton Caused by Phytophthora parasitica

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ABSTRACT

A Phytophthora boll rot of cotton (*Gossypium hirsutum*) has been found widely distributed in Louisiana. Six of 12 isolates from rotted bolls collected from different cotton-producing areas were compared on a variety of media and test plants. The six isolates were each highly pathogenic and produced similar symptoms upon contact with healthy bolls. Morphological characters, growth on various media, growth at stated temperatures and hydrogen ion concentrations revealed no obvious differences among the isolates. All evidence indicated the six isolates to be the same species, *P. parasitica* = *P. nicotianae* var. *parasitica*.

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Many species of fungi have been associated with rots of cotton bolls (12). Even though the losses caused by these organisms have been substantial over a period of years, details relating to the biology, pathology, and epidemiology of most of these species are meager. Annual disease surveys of the Louisiana cotton crop have been made regularly to estimate losses from cotton boll rots and to identify the causal agents (7). In September 1967, diseased bolls of a different character from those usually observed were found in an area on the Northeast Louisiana Branch Station near St. Joseph. Although the diseased fruit resembled those attacked by *Rhizoctonia solani* (*Pellicularia filamentosa* = *Thanatephorus cucumeris*), isolation-reinoculation studies showed the causal agent to be a species of *Phytophthora* (8).

In 1968, 19 principal cotton-growing parishes were surveyed to determine the distribution of the disease. What appeared to be the same fungus was isolated from rotted cotton bolls collected from 12 of the 19 parishes. To date, this fungus has been recovered from diseased cotton bolls each year since its discovery in 1967.

Phytophthora parasitica (4), *P. palmivora* (6), and *P. cactorum* (2) have been reported attacking cotton. *P. parasitica* has also been identified as the species found in Florida (5), Mississippi (1), and Puerto Rico (10, 11). The purpose of this study was to record the symptoms caused by the fungus on commercial cotton bolls from the humid cotton belt and to compare and identify the isolates of the fungus collected from the several major Louisiana cotton-producing parishes to determine whether one or more *Phytophthora* species were involved.

MATERIALS AND METHODS.—The fungus was isolated by plating pieces of discolored tissue, removed from rotted bolls, on plain agar. Of the 12 cultures, six were arbitrarily selected for this study. The code letter and parishes from which they were collected were as follows:

Bossier-A, East Carroll-B, Lafayette-C, Natchitoches-D, Rapides-E, and Tensas-F. Isolates were maintained by alternate transfers from oatmeal agar to healthy, surface-sterilized, greenhouse-grown cotton bolls and reisolation.

Pathogenicity studies were made by placing very small pieces of agar or bits of mycelia and sporangial scrapings of each isolate from infected bolls on a boll surface beneath the bracts of 20 healthy green cotton bolls ('Deltapine 16') of several ages. Plants with inoculated bolls were then placed in a mist-type moist chamber. Noninoculated checks consisted of plants with healthy bolls placed in the same chamber with inoculated ones.

The morphology of the fungus was studied by microscopic observations of cultures grown on water agar at 30 C for 48 hr. Disk transfers of the six isolates growing on oatmeal agar for 72 hr were seeded on water agar plates.

The isolates were grown on various culture media to determine the one best suited to each isolate. The four media used were malt, nutrient, Leonian's, and bean pod agar, all of which were prepared according to the Difco Manual (3).

Other media used were oatmeal, lima bean, cotton boll, and cottonseed agar. All the media were autoclaved at 10.6 kg-force/cm^2(15 lb/in^2) 121 C for 15 min after 15 g of agar was added to each filtrate.

To determine the optimum temperature for growth, isolates were seeded by placing a 6-mm mycelial disk in the centers of oatmeal agar plates at 5, 10, 15, 20, 25, 30, 35, and 40 C. Colony diameters were measured after 72 hr.

The effects of hydrogen ion concentration between pH 2 and 13 on the growth of the isolates was determined by the method of Riker & Riker (9). Five plates of oatmeal agar for each pH level, were seeded with 6-mm mycelial disks. Diameters of colonies were measured at 24, 48, and 72 hr. To investigate the host range of the fungus, it was introduced in the indicated part of several plant species after the part had been surface sterilized with 5% Clorox (0.25% sodium hypochlorite) for 15 min. Bits of mycelium were then placed in a small wound made in the surface of the fruit or vegetable. Following inoculation, the fruits and vegetables were placed in sterile glass moisture chambers and incubated at 30 C for 72 hr.

Phytophthora boll rot of cotton occurred most commonly after 2 or 3 days of rain, during which the plants remained continuously wet. Complete destruction of the boll usually followed in 2 or 3 days. Saprophytic fungi, usually species of *Fusarium,* soon masked the original symptoms. For this reason, we suspect that the true identity of the pathogen in many of our boll rots has been overlooked.

RESULTS AND DISCUSSION.—The infected tissue of a diseased boll was blueblack to black in color (Fig. 1). The entire surface became black, usually within 48 hr, then the surface of the boll became spongy and gradually developed a soft, watery rot. Under wet conditions the fungus grew on the surface of the boll as well as in the boll tissues. Abundant sporangial and mycelial development on the boll surface resulted in a white mealy appearance. Both would and nonwound inoculations, under conditions of high humidity, caused bolls to become completely rotted in 2 or 3 days; this was particularly true of bolls approaching maturity.

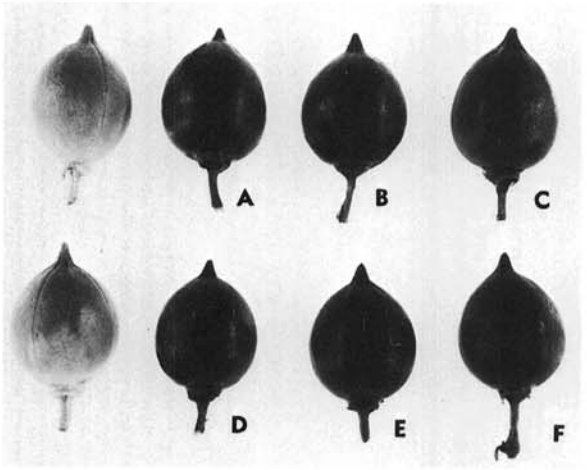

Fig. 1. Symptom expression of isolates A-F of *Phytophthora parasitica* inoculated without injury on healthy greenhouse-grown cotton bolls ('Deltapine 16'). From left to right, top: control **A, B, C**; bottom: control **D, E, F.**

There appeared to be some variation in pathogenicity of the isolates, based on total numbers of bolls diseased after inoculation. However, intermediate-aged bolls (15 to 25 days after anthesis), were more resistant to infection than were 30-day-old bolls. Furthermore, a very high humidity was required for infection.

Pathogenicity of isolates.—Under these conditions, and using 20-day-old Deltapine 16 fruit, isolates B and C were the most pathogenic and caused 80-85% infection. Isolates A, D, and E were slightly less pathogenic, causing 35% infection.

Morphology of causal organisms.—No obvious morphological differences were noted among the six isolates. Hyphae in young mycelia were branched and nonseptate, becoming septate with age in some cases. Sporangia and chlamydospores developed on boll surfaces.

Although the sporangia of the various isolates were somewhat variable in size, all were light yellow in color, each with a definite apical papilla (Fig. 2A). They germinated either directly, by the emergence of a germ tube, or indirectly, by the division of the contents into a variable number of zoospores which appeared to be fully developed within the sporangia (Fig. 2B, D). Liberation of zoospores occurred either directly or by means of a vesicle. In a majority of cases, zoospores were liberated promptly when the papilla broke open at the tip (Fig. 2B, C). The opening appeared to be smaller in diameter than the zoospores (Fig. 2C). When a vesicle was involved, zoospores differentiated in the sporangium and passed into the vesicle as mature zoospores (Fig. 2D). Liberation occurred by the bursting of the vesicular wall. A further detailed study of this process is indicated, since two methods of zoospore liberation were involved. After liberation, zoospores swam for a few minutes, became rounded, and later germinated by means of a germ tube.

Abundant chlamydospores occurred in both oatmeal and water agar. They were spherical, thin- to thick-walled, brown in color, somewhat variable in size, and

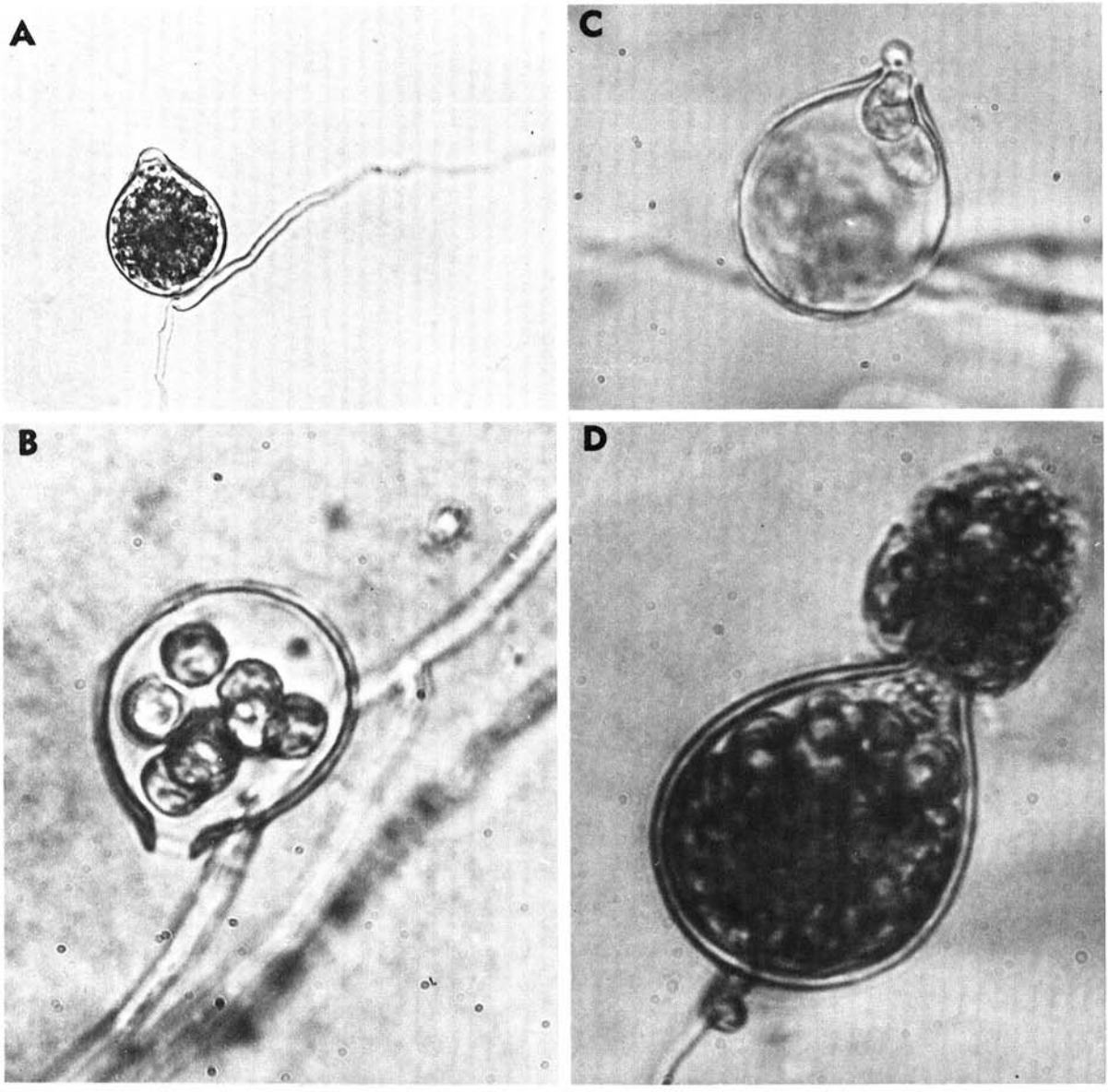

Fig. 2. Sporangia produced by isolates of *Phytophthora* from cotton. **A)** A typical sporangium. **B)** Fully developed zoospores within a sporangium wherein no vesicle was involved. **C)** Direct liberation of zoospores from a sporangium. In Fig. 2-B the last zoospore is passing through the sporangial opening. **D)** Emergence of zoospores through a vesicle from a sporangium.

were produced on short hyphae. Germination occurred directly by emergence of a germ tube (Fig. 3A).

The occurrence of the sexual stage was observed only once in a single culture (Fig. 3B). Oospores with an amphigynous antheridium attached, occurred on a 4-month-old culture of isolate C growing on oatmeal agar. Dr. Erwin mated a single culture from Louisiana (P576, University of California, Riverside) with each of five other known A1 mating types of *P. parasitica* and one isolate of *P. palmivora* type "C" on plates of V-8 juice agar. Oospores were formed abundantly where the two colonies met, indicating P576 to be an A2 mating type.

Growth on various culture media.—All six isolates grew best on oatmeal agar. The next best growth occurred on lima bean and soybean agar. Poor growth occurred on cottonseed agar, cotton boll agar, nutrient agar, Leonian's agar, potato-dextrose agar, and water agar. No growth was observed on bean pod, or malt agar. Unless otherwise noted, growth studies were conducted on oatmeal agar at 30 C.

Temperature.—With the exception of some slight growth rate variations, no significant differences among the six isolates were observed. All six isolates had a temperature range of growth from 15 to 35 C. The isolates grew best at 30 C, which is near the optimum of the host, followed by a sharp decline in growth rate at temperatures above 30 C. Although the isolates grew slowly at low and high temperatures, visible growth was detected at 15 C

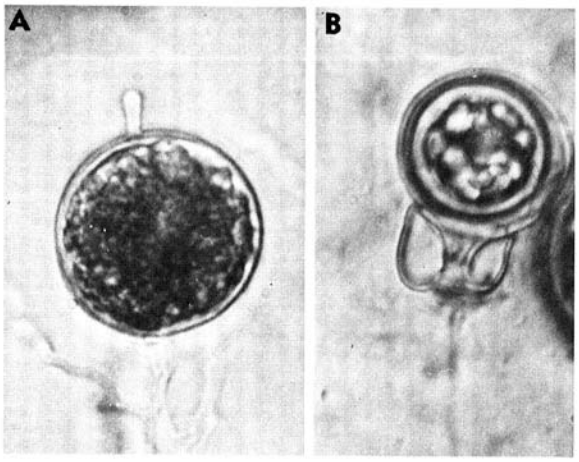

Fig. 3. A) A typical chlamydospore produced by *Phytophthora parasitica* growth on water agar. **B)** Oospore with amphigynous antheridium produced in a 4-month-old culture of isolate C of *Phytophthora parasitica* from cotton bolls.

after 24 hr and measurable growth was detected at 35 C after 24 hr.

Hydrogen ion.—The six isolates grew on oatmeal agar at a pH range from 3 to 12. Maximum growth was recorded at pH 5, growth rates at pH 6 to pH 10 were less than those of pH 5, but were not low enough to prevent additional studies at these pH values, if these were required.

Host range.—Fruits and vegetables of 12 plant species were used in the host range study. Wound inoculation tests showed that all six isolates caused a brown, soft rot on fruits of apple, bean, cucumber, eggplant, pepper, and tomato. All isolates caused a slight decay and discoloration on the exterior tissue of carrot root and orange fruit. No infection or change in color or texture occurred on Irish potato tubers or sweet potato roots. None of the isolates caused visible disease symptoms on either cotton or soybean seedlings grown in infested soil.

Results presented in this study were used for the identification of the *Phytophthora* sp. isolated from Louisiana cotton fields. With Tucker's system (11) used as the criterion, the fungus was identified as *Phytophthora parasitica* Dast. Using Waterhouse's key (13), it would be identified as *P. nicotianae* var. *parasitica* (Dast.) Waterhouse. All isolates studied were of the same species.

LITERATURE CITED

1. BAGGA, H. S. 1968. Fungi associated with cotton boll rot and their frequency. Plant Dis. Reptr. 52:582-584.
2. DRANDALL, B. S., L. ABREGO, & B. PATINO. 1951. A check list of diseases of economic plants of El Salvador, Central America. Plant Dis. Reptr. 24:545-554.
3. DIFCO LABORATORIES, INC. 1953. Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. Ninth Ed. Difco Laboratories, Detroit, Michigan.
4. HOPKINS, J. C. 1925. Notes on the soft rot of cotton bolls in the West Indies caused by Phytophthora. Ann. Bot. 39: 267-280.
5. NOWELL, W. 1923. p. 274-275 *In* Diseases of crop plants in the Lesser Antilles. The West India Committee, 14 Trinity Square, London.
6. PATIL-KULKARNI, B. G., & B. ASWATHALAK. 1962. Phytophthora boll rot of cotton (Gossypium hirsutum L.) in Mysore. Sci. Cult. 28: 233-234 (Rev. Appl. Mycol. Abstr. 42:24. 1963).
7. PINCKARD, J. A., & S. J. P. CHILTON. 1966. The economic importance and classification of cotton boll rots in Louisiana. La. Acad. Sci. 29:12-22.
8. PINCKARD, J. A., & G. F. GUIDROZ. 1968. A parasitic Phytophthora boll rot of cotton found in Louisiana. Plant Dis. Reptr. 52:780-781.
9. RIKER, A. J., & R. S. RIKER. 1936. Introduction to research on plant diseases. The J. S. Swift Co. 117 p.
10. TUCKER, C. M. 1927. Report of the plant pathologist. P.R. Agric. Exp. Stn. Rep. p. 24-40. (Rev. Appl. Mycol. Abstr. 6:599-602. 1927).
11. TUCKER, C. M. 1931. Taxonomy of the genus Phytophthora de Bary. Missouri Agr. Exp. Stn. Res. Bul. 153: 208 p.
12. UNITED STATES DEPARTMENT OF AGRICULTURE. 1960. Index of Plant Diseases in the United States. Agriculture Handbook No. 165. U.S. Government Printing Office, Washington, D.C.
13. WATERHOUSE, G. M. 1963. Key to the species of Phytophthora de Bary. Commonwealth Mycol. Inst., Mycol. Paper 92. 22 p.