# Passage of Boll Rot Fungi Through Alimentary Canal of Cotton Boll Weevil

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**ABSTRACT**

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Conidia of *Alternaria, Fusarium, Colletotrichum*, and *Curvularia* spp. causing boll rot of cotton (*Gossypium hirsutum*) remained viable after passage through the alimentary canal of the cotton boll weevil, *Anthonomus grandis*. The duration of discharge of conidia in the feces varied with the fungus. Most of the conidia of all the fungi were expelled within the first 6 hr after a 24-hr exposure to a sporulating culture.

Additional key word: epidemiology

Inoculum of many fungi causing boll rot of cotton (*Gossypium hirsutum* L.) originates in the soil and increases on naturally shed corollas, flower buds, and bolls (3). The inoculum is moved primarily by air currents (4) to developing bolls where boll rot is initiated. However, air currents are not responsible for all inoculum movement. Insects have also been associated with transfer of inoculum of boll rot fungi. Bagga and Laster (1) reported that the adult boll weevil (*Anthonomus grandis* Boh.) transfers inoculum of *Alternaria tenuis* Auct. or *Fusarium moniliform* Sheldon from pure cultures to healthy bolls. However, the mode of inoculum transfer was not determined.

The weevil has also been associated with transfer of inoculum of fungi causing other cotton diseases. Inoculum of *F. oxysporum* Schlect. f. sp. *vasinfectum* (Atk.) Snyd. & Hans. caused Fusarium wilt after passage through the alimentary canal of boll weevil larvae (5).

The present study was undertaken to determine whether adult boll weevils ingest inoculum of boll rot fungi (four species previously isolated from weevils) and pass the inoculum in a viable condition to healthy bolls.

## MATERIALS AND METHODS

Laboratory-reared boll weevils were fed surface-sterilized flower buds and young bolls (less than 1 wk old) from greenhouse-grown plants for 24 hr to eliminate from their system antibiotics found in the laboratory diet. Plant material was surface-disinfested in a 0.05% solution of sodium hypochlorite for 1.5 min and rinsed at least three times with sterile distilled water. The plant material was transferred to a sterile glass container into which the boll weevils were also placed. After 24 hr, the boll weevils were removed from the container and placed on 2- to 4-wk-old sporulating cultures of *Colletotrichum* sp., *Alternaria* sp., *Curvularia* sp., or *Fusarium* sp. for 24 hr. Fungal isolates were obtained from boll weevils collected in the summer of 1979. Cultures were maintained on potato-dextrose agar, and sporulation was confirmed before weevils were placed on the cultures.

After feeding on cultures for 24 hr, the weevils and cultures were refrigerated at 4 C until weevil movement stopped. The quiescent boll weevils were dipped in a 0.13% solution of sodium hypochlorite for 45 sec and in 95% ethyl alcohol for 5 sec, with both liquids kept at 4 C. The surface-sterilized insects were transferred to a sterile glass container lined with sterile paper towels to remove excess liquid.

Two surface-disinfested squares were placed in each sterile, filter-paper-lined petri dish as a food source for the insects. Twenty-five surface-disinfested insects were placed in each dish. After 2 hr, weevils were aseptically transferred to a second collection dish. Pellets were collected at 2-hr intervals for 10 hr. After 10 hr, collections were made every 10 hr until conidia were no longer detected in the pellets. Feeding and transferring were repeated until the test was terminated. The test was stopped two collection periods after the first collection in which conidia could no longer be found in the pellets by examination with a microscope.

Twelve pellets were chosen at random from each collection and placed on potato-dextrose agar plates to determine the viability of conidia within the pellets. Growth and sporulation of cultures were checked after incubation for 1 wk at room temperature.

The number of conidia per pellet was determined by the method outlined by Tuite (6). Eight pellets were chosen randomly from each collection, and each pellet was placed in a test tube with 1 ml of distilled water. Each pellet was crushed with a sterile glass rod and agitated until a uniform suspension was obtained. Conidia in six microscopic fields per pellet were counted, and an average number of conidia per field was calculated. The control consisted of boll weevils allowed to feed on surface-sterilized plant material for 48 hr. After 48 hr, the weevils were surface-sterilized as previously described and transferred to petri dishes. Fecal pellets were analyzed for the presence of conidia. Pellets were stored at 4 C in the dishes when the counts could not be done immediately. Two replicates were done for each fungus.

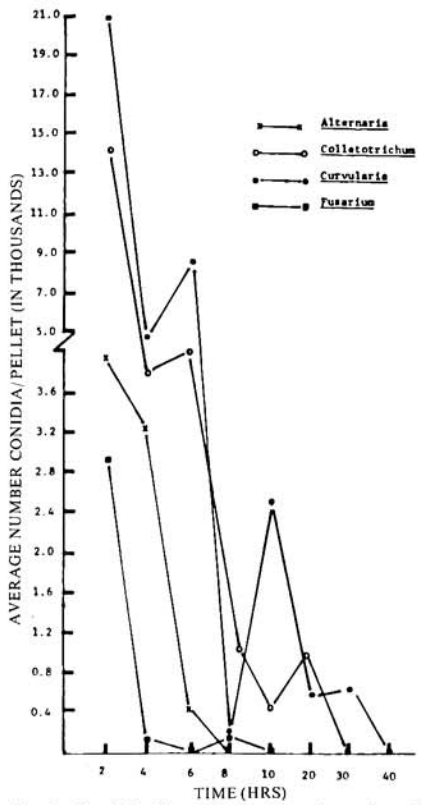


**Fig. 1.** Conidia from four selected species of boll-rotting fungi found in fecal pellets from boll weevils that had fed on sporulating cultures for 24 hr. Collection of the pellets continued two more collection periods after conidia could not be found in the pellets by examination with a microscope.

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Fungi in fecal pellets were tested for boll-rotting ability in the following manner. Bolls 30–40 days from anthesis were excised from cotton plants grown in the greenhouse and surface-disinfested in a 0.13% solution of sodium hypochlorite for 5 min. Bracts were removed from the bolls before disinfestation to reduce contamination. Bolls were rinsed in sterile distilled water and placed in sterile jars containing 2 ml of sterile distilled water. Each boll was punctured once with a sterile needle. A fecal pellet containing one of the fungi was placed on the wound. Bolls were incubated in the dark for 7 days at 27 C and observed for symptoms.

## RESULTS AND DISCUSSION

Conidia of *Curvularia* sp. were excreted by the boll weevil for the longest period of time (Fig. 1)—40 hr—of any of the fungi. A sharp decrease in the number of conidia excreted in the feces occurred between 6 and 8 hr after initial exposure to the culture. At 6 hr after exposure, each pellet contained approximately 9,000 conidia and at 8 hr, approximately 200 conidia. At 10 hr, the number of conidia per pellet increased to 2,700. At the end of the test, about 38,000 conidia had been excreted by each weevil.

Conidia of *Alternaria* sp. were excreted for a shorter time than the other fungi. At 8 hr, conidia could no longer be found in the pellet suspensions. About 7,500 conidia per weevil had been excreted after 8 hr.

*Fusarium* sp. conidia were observed in pellets up to 10 hr after the weevils fed on a culture. However, conidia were found only in trace amounts in the pellets after 2 hr. At 10 hr after the weevils had fed, approximately 3,300 conidia had been excreted.

*Colletotrichum* sp. conidia were found in fecal pellets up to 30 hr after initiation of the test. About 2,500 conidia were excreted 30 hr after the beginning of the test.

Conidia of the four species remained viable after passage through the alimentary canal of the weevils (Fig. 1). All of the fungi produced viable cultures (Fig. 1) from pellets collected at the time of the last observation of conidia in the suspensions.

No attempt was made to determine whether viable mycelial fragments passed through the alimentary canal. Some of the cultures produced from contaminated pellets could have originated from mycelial fragments. However, no mycelial fragments were observed in fecal samples analyzed for conidia. Bolls inoculated with fecal pellets containing inoculum of the fungi developed boll-rotting symptoms within 5–7 days.

Boll weevils are exposed to inoculum of boll-rotting fungi throughout their life cycle and become contaminated internally and externally. The passage of inoculum of boll-rotting fungi through the alimentary canal of the weevil probably contributes to the spread of the inoculum to boll tissue susceptible to these fungi. Fecal pellets of overwintered adult weevils contain inoculum of several boll-rotting fungi (M. L. Schroeder, *unpublished M.S. thesis*, Louisiana State University, 1981).

The female adult weevil deposits eggs in puncture wounds in flower buds and bolls of cotton and seals the opening with contaminated feces (2). The squares flare in response to ovipositing, drop to the soil, and become contaminated with soilborne organisms. Larvae complete their development within the flower buds and emerge as adults. The adult becomes contaminated externally with fungal propagules on the litter and then moves up the plant, contaminating the healthy flower buds and bolls on which it feeds.

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