Postharvest Infection of Cottonseed by Rhizopus arrhizus, Aspergillus niger, and Aspergillus flavus

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I thank Ms. M. Seminara for technical assistance and Dr. H. Schroeder and Dr. J. Ellis for identification of fungal isolates.

Accepted for publication 23 May 1975.

ABSTRACT

Infection of cottonseed (*Gossypium hirsutum*) by *Rhizopus arrhizus, Aspergillus niger,* and *A. flavus* was studied at 35 C and 20% seed moisture. Nine days after inoculation of seeds with *Aspergillus* spp., 95 to 100% of the seeds and 85 to 95% of the embryos were infected. *A. flavus* was the fungus most often isolated from either control (noninoculated) or *R. arrhizus*-inoculated seeds or their embryos. *R. arrhizus* was isolated less often as the incubation progressed to 21 days. Fungi were isolated more often from the chalazal than the micropylar ends of embryos. Infection of embryos by fungi may not be involved in all cottonseed deterioration, because fungi were not isolated from 25% of the dead embryos of control seeds.

Phytopathology 65:1229-1232

Additional key words: seed deterioration, accelerated aging, seedling decay.

Rhizopus spp. (16) and *Aspergillus* spp. (1, 2, 6, 13, 16) are among the fungi which have been isolated from field-infected cottonseed (*Gossypium hirsutum* L.). Similarly, *Rhizopus arrhizus* Fischer, *Aspergillus niger* Van Tiegham, and *A. flavus* Lk. ex Fr. were the predominant fungi isolated from samples of laboratory-deteriorated cottonseed (7, 8). Seeds that showed abundant fungal growth on the seed coat often germinated and produced normal seedlings. This study was done to determine (a) whether these fungi invade embryos as rapidly as seed coats, and (b) whether all deteriorated embryos are invaded by fungi. Preliminary reports are published (7, 8).

MATERIALS AND METHODS.—Acid-delinted cottonseed (cultivar Deltapine 16) from Arizona were graded for removal of seed that were visibly damaged or immature, or floated in water.

Fungal isolates were obtained from seeds deteriorated at 40 C and 100% relative humidity. Conidia of *R. arrhizus, A. niger,* and *A. flavus* were rinsed from 7-day-old cultures on potato-dextrose agar with 0.5% Tween 20 and filtered through facial tissue to remove mycelial debris. They were then adjusted to a spore concentration with an absorbance of 0.02 at 600 nm. Cottonseed were disinfected with 1% sodium hypochlorite for 5 minutes, rinsed twice for 2 minutes with sterile distilled water, and shaken for 2 minutes in a conidial suspension (500 ml per 2,000 g seed). Excess liquid was drained off, and seeds were air-dried to approximately 8% moisture. Control seed were surface-disinfected and rinsed with sterile distilled water before they were dried.

Dried seed were placed in 4-liter containers (500 g per container) and adjusted to 20% moisture by addition of distilled water. The seed mass was agitated until all water was imbibed by the seeds. Sealed containers (four replications) were then incubated at 35 C, and samples were removed and air-dried at intervals up to 21 days.

For germination, cottonseed were wrapped in strips of moistened Whatman No. 3 filter paper, 10 × 46 cm, and incubated at 30 C for 48 hours. Seed that produced a radicle 3 mm or longer were called "germinated". Seed infection was determined by surface sterilizing seed as described previously and plating them on water agar. Fungal growth was noted after 5 days of incubation at 22 C. Infection of embryos excised from seed was determined in the same way. Fungi of the *A. flavus* group (14) were counted as *A. flavus* in this study; of this group, the species most often isolated were *A. flavus* and *A. tamarii* Kita. Percentages of germination and infection and seedling lengths are reported as the means of four replications of 100 seeds.

RESULTS AND DISCUSSION.—Moist seed decreased simultaneously in viability (% germination) and vigor (seedling length) during incubation at 35 C (Fig. 1). Germination of seed inoculated with *Aspergillus* spp.

declined to zero more rapidly than that of other species. Only seed inoculated with *R. arrhizus* showed an immediate (0 incubation time) decrease in vigor. This decrease was associated with rotting by *Rhizopus* during seed germination (Fig. 2-A). The percentage of cottonseed infected by *R. arrhizus* decreased from 0 to 21 days (Fig. 3-C, D), indicating that *R. arrhizus* functions as a field fungus as defined by Christensen and Kaufmann (3, 4, 5), rather than as a storage mold. Except for the high initial infection by *Rhizopus,* both control and *Rhizopus*-inoculated seed and embryos were infected similarly (Fig. 3-A to D).

In addition to the fungi used as inoculum, *Penicillium* spp., *Alternaria* spp., yeasts, and bacteria were isolated from deteriorated seeds and embryos. The extent of fungal infection of seeds and embryos during deterioration is summarized in Fig. 3. The cottonseed used in these experiments had natural infection of about 8% (Fig. 3-A). Other lots of cottonseed tested in our laboratory have had equal or greater percentages of natural infection by fungi. The difficulty of obtaining cottonseed free of fungi has been discussed by Christensen et al. (6). Laboratory-inoculated seed were first infected between the time of inoculation and drying of the seed. This infection might be due either to rapid germination and entry by the fungi, or to passive entry of conidia into seeds with the imbibitional flow of water. Simpson et al. (15) reported that water enters cottonseed mainly through porous tissues at the micropylar and chalazal ends.

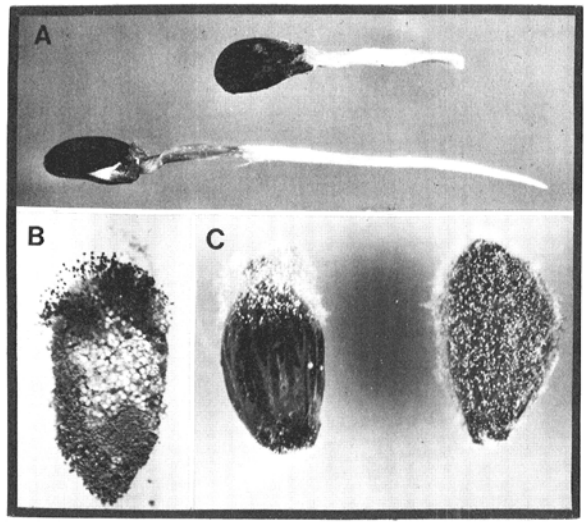

Fig. 2-(A to C). A) Seedlings attacked by *Rhizopus arrhizus,* showing (top) stunting and decay and (bottom) decay of upper radicle, hypocotyl, and cotyledons. **B)** Embryo showing multiple infection by *Aspergillus niger* (top), *A. tamarii* (center), and *A. flavus* (bottom). **C)** Seeds infected with *A. flavus,* showing mycelial growth from micropylar and chalazal ends (left) and over entire seed coat (right).

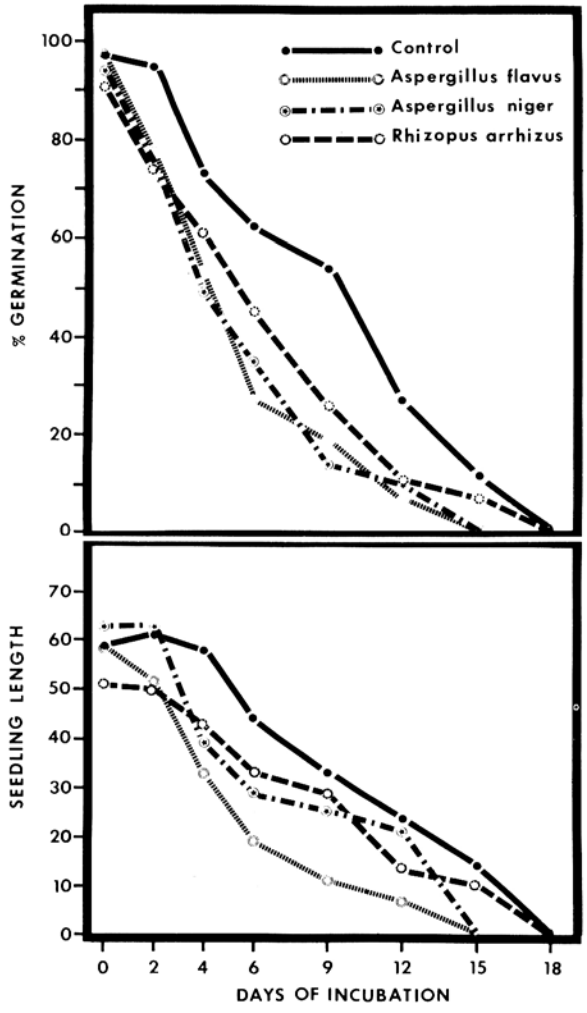

Fig. 1. Effect of inoculation with fungi and incubation at 35 C and 20% seed moisture on viability (% germination) and vigor (seedling length in mm) of cottonseed.

After 9 days of deterioration, *Aspergillus* spp. had invaded 95 to 100% of inoculated seeds and 95% of embryos (Fig. 3-E to H). Other fungal species infected less than 5% of cottonseed when competing with *Aspergillus* spp. In both *R. arrhizus*-inoculated and noninoculated seeds, *A. flavus* (and to a lesser extent *A. niger*) rapidly established dominance over other microorganisms. This presence of *Aspergillus* spp. within both nondeteriorated and noninoculated seeds of cotton differs from the findings for cereal crops (5). However, *Aspergillus* spp. are important field fungi in cotton and infect cottonseeds as boll rot organisms (1, 2, 13, 16). Simpson et al. (16) reported that multiple infections of individual freshly harvested cottonseeds with different species of fungi is unusual. In these studies, as shown in Fig. 2-B, infection was often multiple.

The presence of fungi within embryonic tissues was not required for loss of viability. *Rhizopus*-inoculated and noninoculated control seed failed to germinate after 18 days of incubation at 35 C and 20% moisture (Fig. 1). Although these seed were infected by fungi (Fig. 3-A, C), about 25% of the embryos were not infected (Fig. 3-B, D). The exact percentage of noninfected seed was probably greater, since some noninfected embryos may have been inoculated with fungi during their excision from seed coats. Mycelia usually grew abundantly between the seed coats and the nucelli, but many embryos appeared

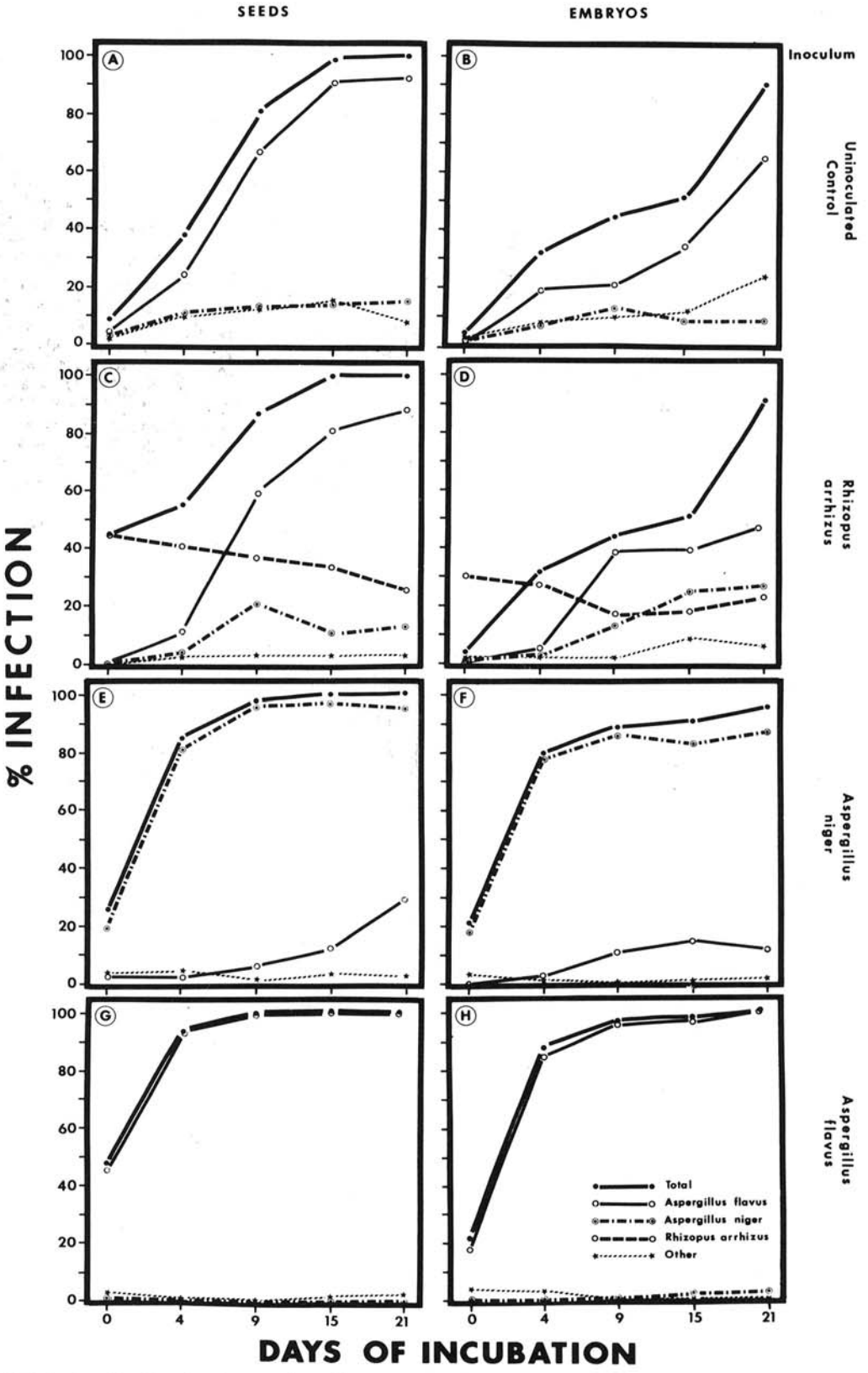

Fig. 3. Infection of seeds and embryos vs time of incubation at 35 C and 20% seed moisture. Embryos were excised from seed coats after incubation.

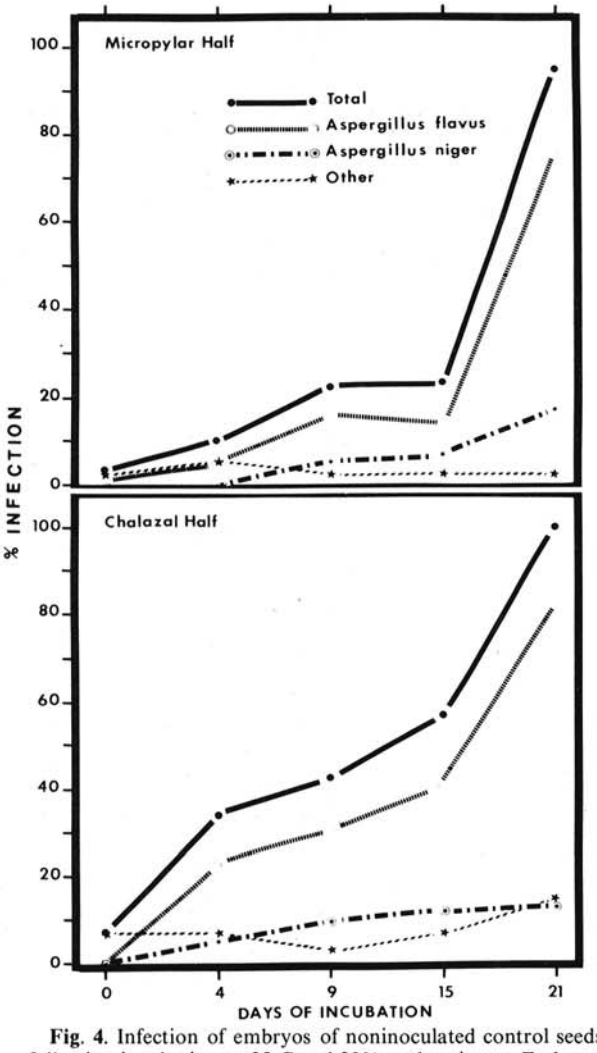

Fig. 4. Infection of embryos of noninoculated control seeds following incubation at 35 C and 20% seed moisture. Embryos were removed from seed coats and bisected into micropylar and chalazal halves following incubation.

undamaged. Similar findings were reported by others (6, 16). The nucellus might function as either a physical or a chemical barrier to invasion of the embryo by fungi.

Deterioration of some dicotyledonous seeds by *Aspergillus* spp. appears to differ mechanistically from that of the cereal grains. Christensen and Kaufmann (4) reported that direct invasion of cereal grain embryos by fungi was a primary mechanism of deterioration. My findings with cottonseed, together with similar findings by Harman and coworkers (9, 10, 11) with pea and squash seeds, and Lindsey (12) with peanut seeds, indicate that invasion of embryonic tissues by *Aspergillus* spp. does not necessarily follow invasion of seed coats, and is not essential for deterioration. Harman (9) found that germination of peas not infected with *A. ruber* is reduced by a diffusible toxin produced by the fungus. Diffusible fungal toxins and enzymes, or autolytic host enzymes may also be involved in deterioration of cottonseed by *Aspergillus* spp.

When deteriorated seed were incubated on water agar, fungal hyphae usually appeared from the chalazal and micropylar ends, but occasionally grew over the entire seed coats (Fig. 2-C). Visible fungal growth in sectioned seed was most abundant at the chalazal ends. Similar observations have been reported by others (6, 16). When excised control embryos were bisected latitudinally and plated on water agar, chalazal halves were infected more than twice as often as micropylar halves (Fig. 4). Thus, fungi appeared to invade mainly through the chalaza, which is also the main site of water imbibition (15).

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