Suitability and Efficacy of Ground Corncobs as a Carrier of *Fusarium solani* Spores

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ABSTRACT

Batson, W. E., and Trevathan, L. E. 1988. Suitability and efficacy of ground corncobs as a carrier of *Fusarium solani* spores. Plant Disease 72:222-225.

Ground corncobs were tested as a carrier of spores of *Fusarium solani* for application in pathology investigations. Particles were infested with suspensions of *F. solani* spores ranging in concentration from 0 to 2.0×10^5 spores per milliliter. The total number of particles developing colonies (CFU) was determined for infested material stored for various periods by incubating on potato-dextrose agar for 4 days. In material stored at 4 C, CFU numbers declined through 16 wk. More than one spore per square centimeter of particle surface was required to obtain a CFU efficiency ≥90%. Scanning electron micrographs confirmed that spores were located in protected areas on the particles and that spores stored on corncob particles over time were similar in appearance to freshly harvested spores. *F. solani* caused significant reductions in emergence of cotton and increased root discoloration of surviving seedlings when spores carried on ground corncob material were used to infest soil at temperatures of 16, 20, and 24 C in root zone chambers. The fungus was recovered from 81% of root systems of surviving seedlings at 16 C and from 100% at 20 and 24 C.

Investigations of the etiology of soilborne diseases require the utilization of soil infestation techniques. We have had varying degrees of success in our use of the methods available for studying soilborne pathogens of cotton and forage crops. Thus, we developed a technique to permit uniform distribution of inoculum, to allow prediction of the viable propagule concentration over time, and to provide a method of infestation that could be standardized and repeated.

Commercially available ground corncobs, milled and screened to size specifications, are absorbent, free-flowing, nonhygroscopic, and degradable and have a pH of 4.9 in bulk. These characteristics are useful in the application of insecticides and herbicides that are highly toxic and/or active at very low rates. Likewise, most pathogens are active at very low rates of inoculum concentration and are capable of causing disease when applied under proper conditions. Despite their relative efficiency, high inoculum densities are often necessary to obtain desired infection rates for a given plant pathogen.

The main objective of this study was to evaluate ground corncobs as a carrier for spores of *Fusarium solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. in pathology studies. Suitability was based on efficiency of recovery of *F. solani* from corncob particles and pathogenicity of the fungus to cotton seedlings.

MATERIALS AND METHODS

F. solani, culture FSO-12 isolated from diseased seedling cotton roots from Stoneville, MS, was grown in shake

Published with the approval of the director of the Mississippi Agricultural and Forestry Experiment Station as Paper No. 6315

Accepted for publication 5 October 1987 (submitted for electronic processing).

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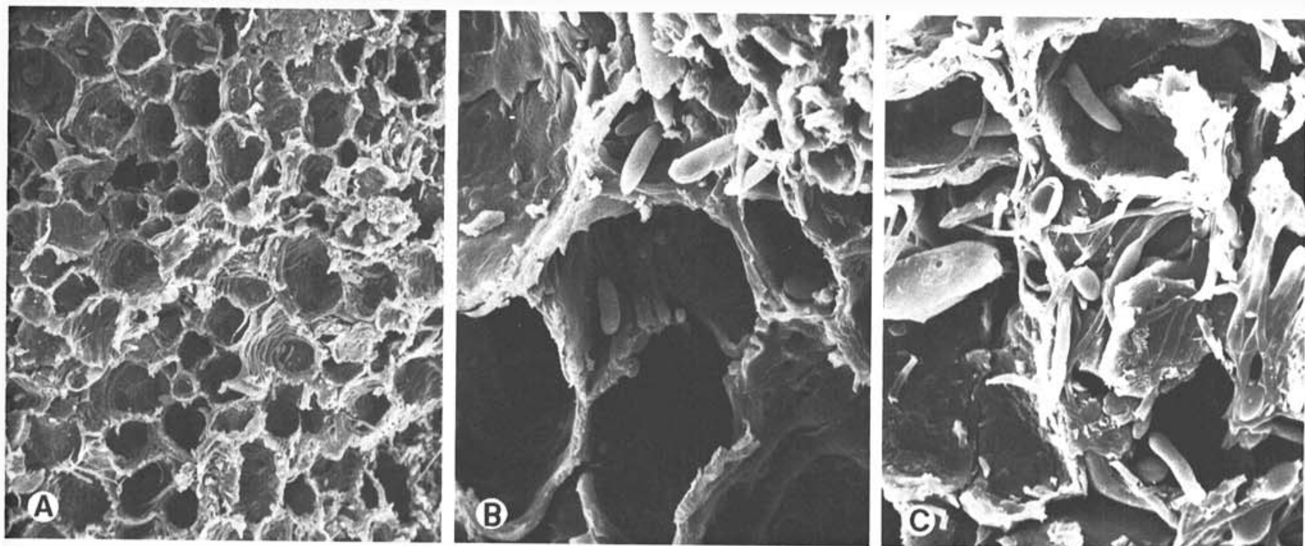

Fig. 1. Surface of ground corncob particle showing: **(A)** distribution of sites for collection of spores of *Fusarium solani*, **(B)** condition and location of spores in collection sites after application, and **(C)** condition of spores after 32 wk of storage.

culture in Czapek-Dox broth. After 21 days, cultures were filtered through a Büchner funnel, and macroconidia and microconidia trapped on filter paper were removed by washing with distilled water. The concentration of the resultant spore suspension was determined with a hemacytometer. Ground corncobs (Grit-O'Cobs, size 1014, The Andersons Cob Division, P.O. Box 119, Maumee, OH 43537) were autoclaved in paper bags for 20 min at 1.05 kg/cm^2, then spread in a steam-sterilized transfer room and allowed to cool. After cooling, 150 g of particles were infested in bulk in a seed treater by spraying with 50 ml of a spore suspension containing 0, 0.05, 0.1, 0.5, 1.0, 1.5, or 2.0 $\times$ 10^5 spores per milliliter. Infested particles were then spread on butcher paper in a steam-sterilized room and allowed to air-dry for 12 hr.

Three experiments were conducted from July through September 1983. For each experiment, three separate 1-g subsamples of particles infested with each spore concentration were incubated on potato-dextrose agar (PDA). Each subsample was divided among eight to 10 plates (52 particles per plate). The remaining infested material from the third experiment was stored in a plastic bag at 4 C, and subsamples were removed at 4-wk intervals for assay. The total number of particles with developing colonies was determined with a colony counter for each assay after 4 days of incubation.

For electron microscopy, subsamples of dry corncob particles (infested with 2.0 $\times$ 10^5 spores per milliliter) were submerged immediately after spore application or 32 wk thereafter in 2% osmium tetroxide in 0.05 M sodium cacodylate buffer for 2 hr. Nonfloating particles were gently rinsed twice in buffer, then dehydrated with alcohol. The dehydration series consisted of two

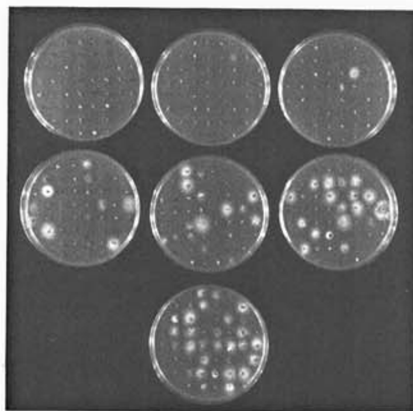

Fig. 2. Colonies of *Fusarium solani* from ground corncobs infested in bulk (150 g) with a 50-ml spore suspension of 0, 0.05, 0.1, 0.5, 1.0, 1.5, or 2.0 $\times$ 10^5 spores per milliliter, top left to bottom center, respectively.

10-min periods each in freshly prepared solutions of 30, 50, 70, and 95% ethyl alcohol. To ensure complete dehydration, samples were then passed through four changes of absolute ethyl alcohol for 1 hr. Samples were critical-point dried, mounted on stubs, coated with gold, and examined in a Hitachi HHS-2R scanning electron microscope operated at 20 kV. The samples observed 32 wk after spore application were stored at reduced temperature as described above.

Ground corncob material was treated with 3.0 $\times$ 10^6 spores of *F. solani* per gram as previously described. One hundred fifty grams of the infested material was mixed uniformly with 9,500 cm^3 of fumigated soil. Then, 350 cm^3 of this inoculum was layered onto 950 cm^3 of fumigated soil in a 14-cm square plastic pot and leveled. Five nontreated cottonseeds (*Gossypium hirsutum* L. 'Stoneville 213') were placed on the soil surface and covered with an additional 2 cm of inoculum. Seed planted in soil similarly prepared with sterilized corncob material served as a control. Pots were watered from below, and five replications of each treatment were placed in root zone chambers where soil temperatures could be maintained at 16, 20, or 24 C. Ambient temperature ranged from 24 to 30 C. Light was provided at 6,990 lx for 12 hr of each 24-hr period. This experiment was repeated.

Cotton seedling emergence was recorded 21 days after planting, and percentage emergence of seed planted was calculated. Roots of surviving seedlings were washed free of soil and indexed relative to discoloration, where 0 = no discoloration, 1 = yellowish roots, 2 = brownish roots, 3 = blackened roots, and 4 = nub root. Roots of surviving seedlings were surface-sterilized in 0.5% NaOCl, and root tissue pieces were placed on PDA. Fungal colonies growing from root tissue were identified.

RESULTS

The porous nature of the surface of milled corncob particles provides numerous sites for retention of *F. solani* spores (Fig. 1A). Spores adhered to the surface and to cell walls within pores and crevices of the particles and appeared to be well preserved (Fig. 1B). These spores were ungerminated, and the particle surface was almost devoid of mycelium. By contrast, spores germinated and produced abundant mycelial growth on stored particles (Fig. 1C).

Developing colonies of *F. solani* were evident on PDA 2 days after plating (Fig. 2), but counts were made 4 days after plating to avoid overlapping of colonies and because few colonies were initiated after this time. The number of particles developing colonies (CFU) increased from 0 to 93% with increasing spore concentration. At the time of infestation, a concentration of 0.5 $\times$ 10^5 spores per milliliter applied to 150 g of ground

corncobs in the ratio of 1:3 v/w yielded a CFU efficiency ⩾50% (Fig. 3). Regression equations for the lowest to the highest spore concentrations were: $ln\ Y = 8.59 - 0.40X$; $ln\ Y = 15.64 - 0.72X$; $ln\ Y = 50.98 - 0.54X - 0.08X^2$; $ln\ Y = 67.62 + 0.09X - 0.15X^2$; $ln\ Y = 77.91 + 1.04X - 0.21X^2$; and $ln\ Y = 87.20 + 2.40X - 0.27X^2$. The resultant R^2 values were 0.91, 0.81, 0.88, 0.90, 0.93, and 0.86, respectively.

Of the spore concentrations tested, the maximum particle efficiency (MPE) attained was 93% after application of 2.0×10^5 spores per milliliter. Particle efficiency declined slightly in storage through 16 wk for all spore concentrations tested. However, efficiencies ⩾50% were maintained through 12 wk when particles were infested with 1.0×10^5 spores per milliliter and 16 wk when infested with 1.5 and 2.0×10^5 spores per milliliter. After 20 wk of storage, particle efficiency for all treatments had fallen below 20% (Fig. 3).

Emergence of cotton at 16, 20, and 24 C from fumigated soil infested with *F. solani*/ground-corncob inoculum was 32, 66, and 98%, respectively (Table 1). Emergence at 16 and 20 C was significantly lower than that in soil infested only with ground corncobs. At all three temperatures, roots of surviving seedlings grown in *F. solani*/ground-corncob infested soil were more discolored than those from soil infested only with ground corncobs. *F. solani* was readily recovered from root systems of surviving seedlings in soil infested with ground-corncob inoculum.

DISCUSSION

The use of carriers for plant pathogen propagules is important in quantifying amounts of inoculum delivered, especially for pathogens that produce small propagules and/or where low concentrations of inoculum are required (3). Previous studies have reported that some granular materials are suitable for growth and delivery of certain fungi (1,4). The limited success reported from other studies, however, seems to depend on the fungal species involved and the desired end result (2). Walker (4) feels that the development of different carriers in delivery of fungal inoculum for biological weed control is an area of particular research need.

The very large surface area of milled corncob particles and the availability of protected sites within that surface make these granules suitable carriers for spores of fungi such as *F. solani*. The CFU efficiency ⩾50% for this fungus is a function of spore concentration. A CFU efficiency ⩾50% could probably be attained at lower initial inoculum concentrations with further refinements in the method of application and enhanced spore viability. With such improvements, the MPE could also be accomplished with fewer than 2.0×10^5 spores per milliliter.

Slight declines in particle efficiency over time are considered to reflect death of spores of *F. solani* under storage conditions of this study. Drying of ground corncobs under sterile conditions after spore application prevented subsequent contamination and seemed to minimize moisture accumulation in storage. Over long periods of time, however, some condensation formed in the sealed plastic bags used for storage. This may account for the overall decline in CFUs through 20 wk, which apparently was the result of spore germination (Fig. 1C). Despite CFU decline in storage, the similarity of slopes for five inoculum concentrations lends confidence to the applicability of the method. Even under the storage conditions described above, it is possible to predict CFU efficiency for ground corncobs infested with a specific inoculum concentration and stored up through 20 wk.

Storage efficiency, reduced emergence, increased root discoloration, and recovery of *F. solani* from cotton root systems established the utility of the *F. solani*/ground-corncob system in pathology studies. Further studies are needed to determine if this medium can be used for uniform distribution of quantifiable inoculum of other soilborne pathogens. Such utility would be advantageous in providing for efficient infestation of field plots in investigations of disease etiology and biological control of weeds, insects, and diseases.

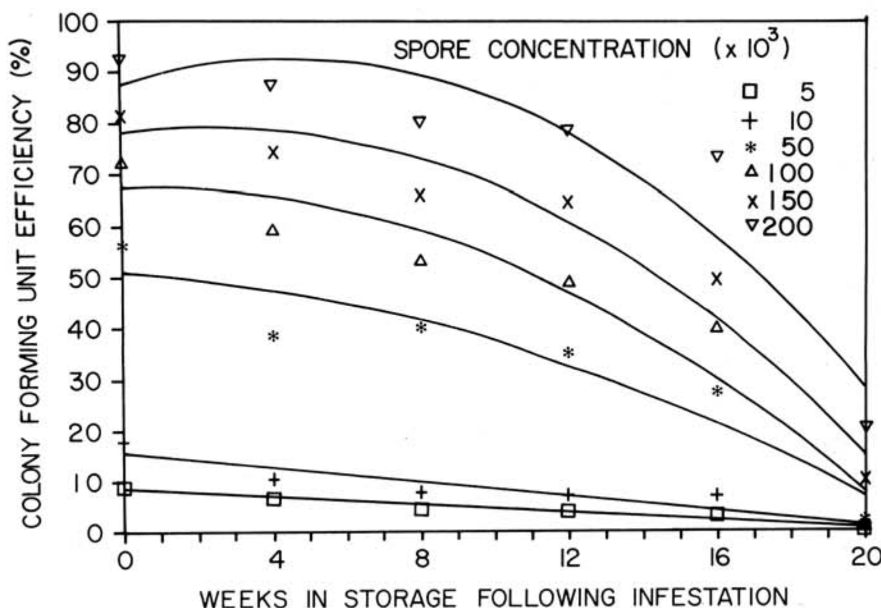

Fig. 3. Percentage of ground corncob particles with colonies of *Fusarium solani* over time in relation to initial inoculum concentration.

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Table 1. Emergence and root discoloration[w] of cotton and rate of *Fusarium solani* recovery from seedlings grown in *F. solani*/ground-corncob infested soil[x] at three soil temperatures

	Temperature								
	16 C			20 C			24 C		
Inoculum	Emergence (%)	Root grade[y]	Recovery (%)	Emergence (%)	Root grade	Recovery (%)	Emergence (%)	Root grade	Recovery (%)
F. solani/ground corncobs	32 b[z]	2.9 a	81	66 b	2.5 a	100	98 a	2.1 a	100
Ground corncobs	70 a	1.2 b	0	83 a	0.8 b	13	94 a	0.6 b	10

[w] Emergence and root grade values are means of two experiments, each with five replications. Variances of the two experiments were tested for homogeneity before being pooled.

[x] One hundred fifty grams of ground corncobs (3×10^6 spores per gram) per 9,500 cm^3 of fumigated soil.

[y] 0 = No discoloration, 1 = yellowish roots, 2 = brownish roots, 3 = blackened roots, and 4 = nub root.

[z] Means within columns not sharing a letter in common differ significantly ($P = 0.05$) according to Student Newman-Keul's multiple range test.

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