Germination of Fusarium oxysporum Chlamydospores in Soils Favorable and Unfavorable to Wilt Establishment

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Accepted for publication 13 September 1971.

ABSTRACT

Chlamydospores of *Fusarium oxysporum* f. sp. *batatas, cubense*, and *lycopersici*, and three common soil saprophytic *F. oxysporum* isolates which resemble each of the three pathogenic clones culturally, were compared for their ability to germinate in soil when small concentrations of glucose and asparagine were added to the premoistened soil. A higher percentage of saprophyte chlamydospores germinated in soil known to be wilt-suppressive than did chlamydospores of the tested pathogens. In soil where wilt has been known to occur, little difference was observed in percentage germination between pathogens and saprophytes. Increasing the nutrient level increased the percentage germination in both soils and decreased the differential between pathogen and saprophyte, indicating that competition with common soil flora for small amounts of nutrients may be involved. Bacterial numbers (determined by plate counts) increased more rapidly in the wilt-suppressive soil than in the wilt-conducive soil.

Phytopathology 62:273-277.

Additional key words: nutrition of soil-borne fungi, saprophytic and pathogenic *Fusarium oxysporum* compared.

Sweet potato cuttings transplanted in the greenhouse into soils known to be either Fusarium "wilt-suppressive" or "wilt-conducive", which contained the same chlamydospore levels of *Fusarium oxysporum* f. sp. *batatas* distributed through them, differed in amount of disease development (16). To investigate reasons for wilt development which occurs in field soil of one type, but has not been known to occur in field soils of the other type, the fate of *F. oxysporum* chlamydospores incorporated into these two soils was studied in the laboratory.

Cook & Schroth (3) reported that chlamydospores of *F. solani* f. sp. *phaseoli* needed both a carbon and a nitrogen source in order to germinate in soil relatively low in nitrogen. Papavizas et al. (13) found that sources and ratios of carbon and nitrogen needed to suppress Fusarium root rot of beans varied in different soils. Soils used in the present experiments were treated with nutrients of the order that have been shown to stimulate chlamydospore germination (3, 14, 15). After 16-hr incubation, soil smears were examined microscopically for germlings.

The present work compares the behavior of certain *F. oxysporum* pathogen chlamydospores with those of saprophytes of the same species in two soils of quite similar texture, but markedly different in their ability to support Fusarium wilt pathogens.

MATERIALS AND METHODS.—The soils used were two of those previously described (16), namely "wilt-suppressive" soil (S), which is Chualar sandy loam, determined by the University of California Extension Service to contain 70.2% sand, 20.8% silt, 9.0% clay, 0.10% nitrogen (10 ppm NO_3-N), and 2.9% organic matter; and the pH measured 6.8; the slightly lighter "wilt-conducive" soil (C), which is Elkhorn sand, contained 81.2% sand, 12.0% silt, 6.8% clay, 0.11% nitrogen (12 ppm NO_3-N), and 2.1% organic matter; and the pH measured 6.0.

The *Fusarium* isolates used were the parasites, *F. oxysporum* Schlecht. formae speciales *batatas* (Wr.) Snyd. & Hans.; *cubense* (E.F.S.) Snyd. & Hans., and *lycopersici* (Sacc.) Snyd. & Hans.; and three saprophytic clones of *F. oxysporum* (Schlecht.) Snyd. & Hans. Each of the three saprophytic clones of *F. oxysporum* resembled a corresponding clone of the three parasites so closely that it was hard to distinguish them culturally, but all were isolated from the suppressive soil.

Separate lots of the two described soils were each fortified with chlamydospores of one parasitic or saprophytic clone of *F. oxysporum*. The soils were fortified with relatively high numbers of chlamydospores to make the counts easier, and to assure that probably all chlamydospores counted were those of the seeded clone rather than of the natural population of *Fusaria* occurring in the nonsterilized soil samples. In order for chlamydospores to be formed in sufficient numbers, conidia from the surfaces of several potato-dextrose agar (PDA) slants were suspended in 15 ml of water and added to premoistened soil in 150-ml beakers, then allowed to dry down for 2-4 weeks. Then the soil was mixed, remoistened, and dried for another 2-4 weeks. During this period, remaining conidia either converted to chlamydospores or died. Numbers of chlamydospores formed were of the order of 3×10^5/g of soil. Background *Fusaria* in the samples was 10^4/g, and many of these chlamydospores exist buried in organic debris (11), and therefore are not usually visible in a soil smear.

The experiments were conducted by placing 5 g of each dry soil sample on a microscope slide, moistening it to water-holding capacity, and allowing it to remain in a petri-dish moist chamber 24 hr before adding nutrient solution drop-wise to the soil. Nutrients used in these experiments consisted simply of glucose and asparagine, added until the sample contained 0.01 or 0.1% of each of these two nutrients. Similar experiments were carried out using Difco peptone or tryptone in place of asparagine and, although results were similar, they were less reproducible. Droplets of water were added to a

control. Samples were incubated for 16 hr more in petri dishes at room temperature; then the soil was stained drop-wise with either 1% acid fuchsin in lactic acid or cotton blue-lactophenol. The samples, transferred carefully to 10-ml beakers, were moistened drop-wise with distilled water until they were a heavy slurry which could be poured onto a microscope slide. These smears were covered with a large cover slip and kept in moist chambers until counted. Four replicate samples were made for each soil-fungus combination; and duplicate slides, for each sample. One hundred chlamydospores were observed on each slide for counts of percentage germination. Lengths of germ tubes were measured for *F. oxysporum* f. sp. *batatas*.

In addition to measuring chlamydospore germination, enumeration of soil bacteria and the added *F. oxysporum* propagules were made in samples before and after incubation by plate counts. For bacterial counts, plates poured 5 days previously and containing 0.5% Difco tryptone, 0.8% glucose, 0.1% Difco yeast extract, and 2.0% agar were seeded with 1 ml of soil suspension diluted 10^{-6} or 10^{-7}. The suspensions were made from the soil just at the time samples were prepared for observations of chlamydospore germination. Dilution plates were incubated at room temperature for 7 days before counting colonies.

Propagules of *F. oxysporum* were counted on peptone-pentachloronitrobenzene (PCNB) agar plates (12). Dilution suspensions were made from air-dry soil before and after the observation of the germlings in the same samples.

RESULTS.—Not only was germination of all three pathogens higher in the C than in the S soil at the 0.01% nutrient level, but it was noted that often in S soil the germ tube, once initiated, appeared to stop growing further (Table 1, Fig. 1). Such "stunted" germlings occurred also in the C soil, but less frequently. This situation is reflected in the average measurements of the *F. oxysporum* f. sp. *batatas* germ tubes. Germlings in S soil averaged considerably shorter than in C soil. Saprophyte germlings, however, grew well in both soils, and new chlamydospores were observed forming on their thalli more frequently than on thalli of the parasites. Increasing the nutrient dosage to 0.1% resulted in higher percentages of germination, longer germ tubes, and less differences between the soils in these measurements. Stunted germlings were still observed, but germ tubes up to 150 μ were found in S soil, and up to 400 μ in C soil.

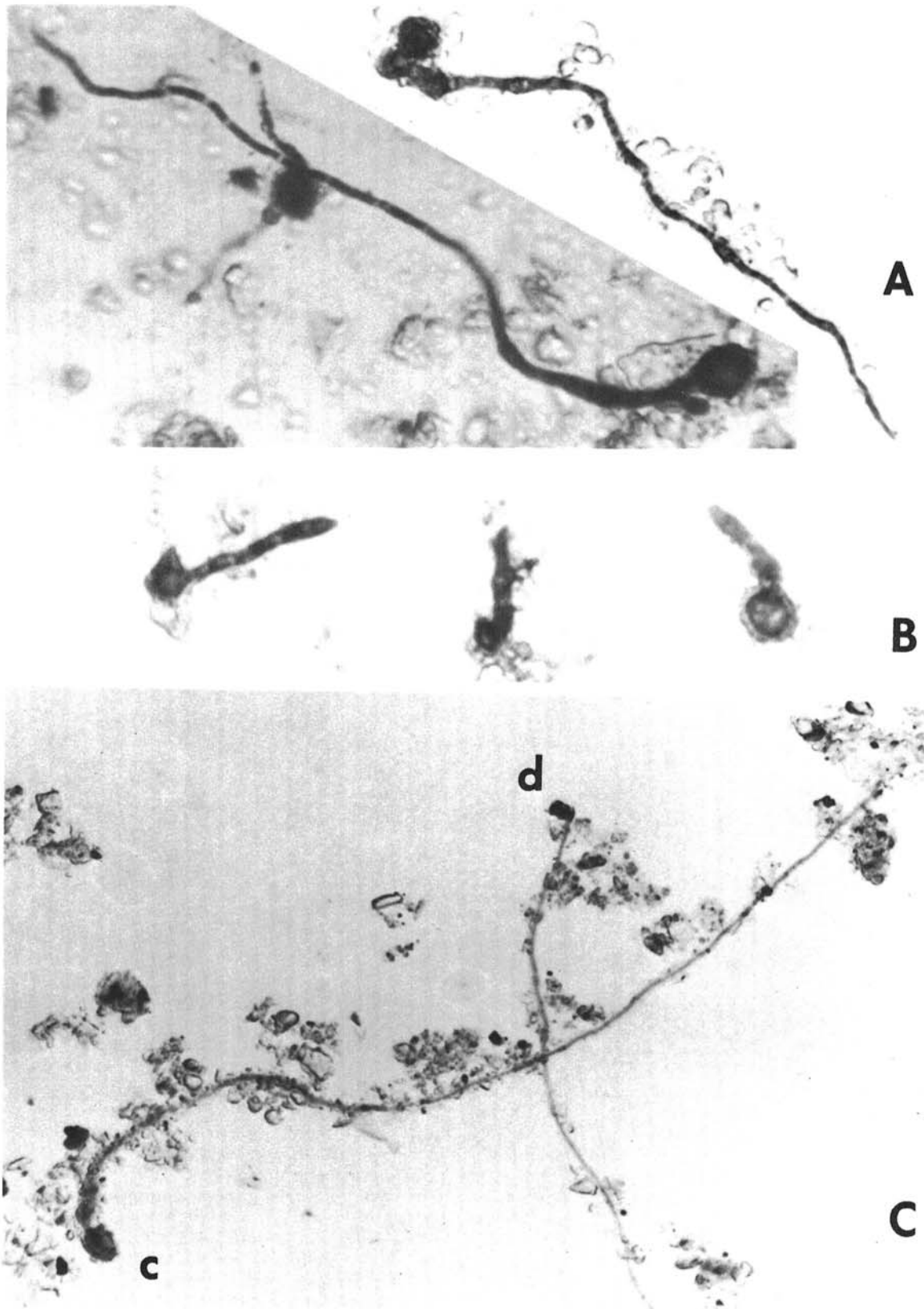
Bacterial plate counts in water checks of these two soils were remarkably similar to each other, and also, they showed little increase in numbers over the premoistened samples taken before the 16-hr incubation period. Addition of nutrients led to increases in bacterial counts in both soils, but the bacteria counted consistently increased faster in S than in C soil. When the 0.01% nutrient level was used, the numbers of bacteria that appeared on the agar plates of the 16-hr incubated S soil were 3.0 to 4.5 times that of the water checks, whereas numbers of bacteria in the C soil similarly incubated were only 1.5 to 2.0 times that of the water checks. At the 0.1% nutrient level, bacterial plate counts differed less between the two incubated soils. The numbers of bacteria counted after incubation averaged 3.5 times that of the water checks in the S soil, and 4.5 times that of the checks in the C soil.

It was noted that the S soil, after addition of nutrients, contained less variety of bacteria on the counted plates than did the similarly treated C-soil plates. A few types in particular, such as short gram-negative rods which formed smooth, opaque, milky-white colonies were responsible for most of the numerical increase. These bacteria showed little inhibition of *Fusarium* colonies on petri plates, and must be considered active as nutrient competitors in this soil rather than as antagonists. Furthermore, it appeared that at the higher nutrient level (0.1%), competition was no longer of consequence, because the bacterial flora increased substantially and the *Fusarium* chlamydospores germinated well (Table 1) in both soils.

Propagule counts of each seeded isolate of *F. oxysporum* made on air-dry samples before and after microscopic observations revealed a tendency for pathogen populations to decline after addition of a low level (0.01%) of glucose and asparagine (Table 2). There was less decline in C than in S soil. At a higher nutrient level (0.1%), the soil population of pathogens changed little. Saprophyte populations did not decrease after application of low nutrient levels in S soil, and they exhibited a marked increase after application of 0.1% nutrients to the soil. These data show ability of the isolates to form replacement chlamydospores with limited nutrients available. These replacement chlamydospores may substitute for those that commenced to germinate, but then used up the energy source necessary for continued growth or for a return into a resting state.

DISCUSSION.—Several workers have reported that germination of *Fusarium* chlamydospores is stimulated by nutrients exuded from plant roots (8, 14, 15), and may be brought about in vitro by adding

Fig. 1. Chlamydospores of *Fusarium oxysporum* (formed from conidia seeded into the soil) germinated within 16 hr in response to 0.01% glucose and 0.01% asparagine added to the soil sample. **A)** *Fusarium oxysporum* f. sp. *batatas* chlamydospores germinating in wilt-conducive (C) soil (X 900). **B)** *Fusarium oxysporum* f. sp. *batatas* chlamydospores germinating in wilt-suppressive (S) soil (X 900). Those chlamydospores that did germinate produced short germ tubes as illustrated; then the hyphae stopped growing. **C)** Saprophytic *F. oxysporum* chlamydospores in S soil produced long hyphae (X 500). Sometimes daughter chlamydospores (d) were beginning to form at 16 hr. Note the hyphae from two germlings growing crosswise; only one chlamydospore (c) appears on the photo.


A
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C

TABLE 1. Percentage of *Fusarium oxysporum* chlamydospore germination, and average germ tube length 16 hr after nutrient (glucose and asparagine) addition. Each pathogen is compared with a saprophytic *F. oxysporum* soil isolate of similar cultural appearance in wilt-suppressive (S) and wilt-conducive (C) soil

F. oxysporum culture	Soil	Water check germination (%)	0.1% Nutrient level		0.01% Nutrient level	
			Germination (%)	Germ tube length (μ)	Germination (%)	Germ tube length (μ)
f. sp. *batatas*	S	0	12	14.5	66	56
	C	1	47	53.5	82	98
Saprophyte No. 1	S	1	47	107.0	78	115
	C	2	67	105.0	85	122
f. sp. *cubense*	S	0	7		60	
	C	0	49		64	
Saprophyte No. 2	S	0	40		72	
	C	0	86		89	
f. sp. *lycopersici*	S	0	14			
	C	0	42			
Saprophyte No. 3	S	0	50			
	C	1	68			

TABLE 2. *Fusarium oxysporum* counts X 10^3 in Fusarium wilt-suppressive (S) and wilt-conducive (C) soil samples previous to and after incubation with applied nutrient solutions. Counts represent the average number of propagules of the fungi per gram of soil in four replicate samples plated on peptone-pentachloronitobenzene agar. Plating was done on air-dry samples

Soil fortified with *F. oxysporum*	Soil	No. propagules of *F. oxysporum* per g soil			
		Before nutrient addition	Water check	0.01% nutrient level	0.1% nutrient level
f. sp. *batatas*	S	390		236	70
	C	339		233	363
Saprophyte No. 1	S	470		507	850
	C	312		143	360
f. sp. *cubense*	S	277	200	46	110
	C	176	136	40	158
Saprophyte No. 2	S	250	196	144	464
	C	173	210	116	288
f. sp. *lycopersici*	S	200		50	
	C	70		32	
Saprophyte No. 3	S	90		92	
	C	57		60	

nutrients to soil samples (2, 3, 13, 14, 15). Competition for these nutrients by other soil organisms has been reported to suppress *F. oxysporum* f. sp. *cubense* (10). Moreover, competition for nutrients has been cited by Ko & Lockwood (6, 7) as the cause of soil fungistasis. Ford et al. (4, 5) found specific soil bacteria which suppress *F. solani* f. sp. *phaseoli* thallus growth and stimulate chlamydospore formation in culture.

In the present work, it was found that certain rhizosphere bacteria multiplied faster in S than in C soil, and may, at least in part, account for a depletion of nutrients before the comparatively slow-starting pathogens have grown much. Adams et al. (1), in experiments using *F. solani* f. sp. *phaseoli* chlamydospores in cellulose-amended soils, suggested that the critical period during which fungistasis stimulated by cellulose may be operative is within the first 2 hr of the germination process. If such fungistasis applies also to *F. oxysporum* pathogens, it explains the arrested growth of the stunted germlings.

Lochhead & Cook (9) found that the rhizospheres of a flax cultivar resistant to Fusarium wilt supported a different bacterial flora than did the susceptible cultivar. They suggested that the presence of numerous thiamine-requiring bacteria in the resistant cultivar rhizosphere removed this vitamin, which had a depressing effect on *F. oxysporum* f. sp. *lini* growth. If this sort of phenomenon commonly occurs in nature, conceivably soil type could also have an effect in selection for bacteria that subsequently remove a Fusarium growth-suppressing factor or a stimulatory one.

Whatever mechanisms are involved in stimulating pathogenic *Fusaria* in one soil and suppressing them in the other, the saprophytes used in these experiments germinated well in both soils at both nutrient levels, and formed a rather extensive thallus.

Often daughter chlamydospores were observed starting to develop on thalli 16 hr after the addition of nutrient.

The reasons that some soils are conducive to Fusarium wilt establishment, whereas others are suppressive, are undoubtedly complex and varied, as Stover (17) suggested. However, the ability of a *Fusarium* chlamydospore to germinate in the nutritional atmosphere of a plant rhizosphere, to form a vigorous thallus that will either infect a host plant or give rise to one or more replacement chlamydospores at low nutritional levels encountered in soil, must be important to the establishment of a pathogen in a particular soil.

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