Populations of Fusarium oxysporum f. sp. cepae in Organic Soils in New York

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ABSTRACT

A selective agar medium was developed for quantitative isolation of *Fusarium oxysporum* f. sp. cepae from organic soils in New York. Martin's rose bengal medium was supplemented with 2 ppm chlorotetracycline HCl (Aureomycin), 1 ppm thiram, 100 ppm sodium-p-(dimethylamino) benzenediazosulfonate (Dexon), and 100 ppm pentachloronitrobenzene (PCNB).

Plate counts indicated that *F. oxysporum* f. sp. *cepae* is unevenly distributed in New York organic soils cropped to onion, and is isolated from as deep

Additional key words: Allium cepa.

as 30.5 to 45.7 cm. Average populations ranged from 300 to 6,500 propagules/g oven-dry soil. Soils with a long history of *Fusarium* basal rot of onion caused by this fungus had higher populations of the fungus than soils with histories of low levels or no known occurrence of the disease. However, several organic soils with high populations of *F. oxysporum* f. sp. *cepae* did not have histories of a high incidence of *Fusarium* basal rot. Only the sporodochial form of the fungus was isolated from naturally infested soils. Phytopathology 61:1042-1048.

Fusarium basal rot of onion (Allium cepa L.) caused by Fusarium oxysporum Schlecht. emend. Snyd. & Hans. f. sp. cepae has long occurred at low levels on most onion farms in New York. However, during the late 1950's and early 1960's, the disease was severe on several farms when susceptible varieties were grown. Following commercial acceptance in the mid-1960's of certain varieties noted to be much less susceptible to the disease (6), basal rot has occurred at low levels on all onion farms in the state. The reason why severe outbreaks of Fusarium basal rot occurred regularly on specific farms in western New York and not on others was never resolved. A consideration of this phenomenon led to the study reported in this paper.

Ninety-seven per cent of the isolates of *F. oxy-sporum* f. sp. *cepae* obtained from naturally infected onion tissues were of the sporodochial type (2). In culture, the fungus mutated rather rapidly, and three morphological variants (ropy, pionnotal, and mycelial) were observed. No reversion to the parental wild type was observed in culture. The sporodochial type was the most virulent; ropy and pionnotal were intermediate; and the mycelial type was the least virulent in pathogenicity to onion as measured by the onion slice method (1, 2). These data suggested that the fungus exists in the soil primarily as the sporodochial type.

Large numbers of methods and media have been used for the assay of Fusarium populations in soils. Papavizas (11) compared 18 media under the same conditions, and concluded that peptone-pentachloronitrobenzene and V-8 juice-dextrose yeast extract agar media were the most satisfactory with the dilution plate count method. A number of these media and methods were tested for their suitability for the quantitative isolation of F. oxysporum f. sp. cepae from New York organic soils, and were found unsatisfactory in experiments preliminary to the present study.

The objectives of our experiments were to develop and utilize a selective medium for the quantitative isolation of *F. oxysporum* f. sp. *cepae* from organic

soils with and without histories of basal rot, and to ascertain the morphological variants present in these soils

MATERIALS AND METHODS.—Stock cultures of the fungus were maintained on potato-dextrose agar (PDA) at 22-24 C under 2,000 ft-c fluorescent light (Life line Sylvania warm-white tube) for 12 hr/day. Cultures were transferred frequently using the single spore technique. Inoculum was obtained from 3- to 4-week-old cultures by filtering conidial suspensions through four layers of sterile cheesecloth to remove mycelial fragments. Propagule counts were made with a Spencer Bright-Line hemacytometer.

Selective medium.—The antimicrobial activity of several fungicides against F. oxysporum f. sp. cepae and background soil microflora of several natural organic soils of New York was determined. Fungicides tested were benomyl; Botran (2,6-Dichloro-4-nitroanaline); captan; Daconil 2787 (chlorothalonil); Dexon[sodium-p-(dimethylamino) benzenediazosulfonate]; Dithane M-22 Sp. (manganese ethylenebisdithiocarbamate with ZnSO₄); ferbam; PCNB (pentachloronitrobenzene); polyram (a mixture of 83.9% of ammoniates of [ethylenebis(dithiocarbamate)] zinc and 16.1% ethylenebis (dithiocarbamic acid) bimolecular and trimolecular cyclic anhydrosulfidedes and disulfides); TBZ [2-(4-thiazolyl)-benzimidazole]; and thiram.

Three concentrations (1, 10, and 100 ppm active ingredients) of each fungicide were tested separately and in combinations with various concentrations of the other fungicides. The selected amount of each fungicide and 2 ppm chlorotetracycline HCl (Aureomycin) were added to a rose bengal basal medium (8). The basal medium was autoclaved 15-20 min at 120 C, and after cooling to about 50 C, the fungicides and antibiotics were added. Assay plates were prepared by pouring 12-15 ml of the selected medium into each disposable petri dish. Two controls were used in all experiments. One was a water check (basal medium + sterile distilled water) and the other was an antibiotic

check (basal medium + distilled water + antibiotic mixture). Effects of the fungicides were determined by (i) placing mycelial discs of 2- to 3-week-old cultures on the assay plates; (ii) mixing conidia with the medium; and (iii) adding conidia to the surface of the medium and determining the number and diameter of colonies of F. oxysporum f. sp. cepae, usually after 5 to 7 days.

The effectiveness of each medium for isolation of the fungus and suppression of background microflora was determined using naturally and artificially infested organic soils. Soil suspensions were prepared by adding 2 g of soil to 200 ml of sterile distilled water. The suspensions were shaken for 30 min, after which the desired dilutions were made. One ml of each dilution was added/petri dish. In all experiments, three replicates were made/dilution per treatment.

Isolation from soil.—Populations of F. oxysporum f. sp. cepae and F. oxysporum in naturally infested soils were determined by the soil dilution plate count method, using the selective medium described in the RESULTS section. In one experiment, the most probable number method (7) utilizing the same selective medium was compared with the soil dilution plate count method. Soil samples were collected in onion rows at a distance of several centimeters from the base of the plants. Assays were conducted using subsamples equivalent in wt to 2 g of oven-dry soil. These were suspended in 200 ml of 0.1% water agar solution and shaken on a Burrell Wrist-Action shaker for 30 min; then a series of dilutions was made and assayed. Water agar was used in order to obtain a good colony spread and the least interaction between the developing colonies. One ml of each dilution was added/petri plate. Three to five replicates were made/dilution per treatment. Assay plates were incubated in the dark for 7 days at 22-24 C. The number of colonies of F. oxysporum f. sp. cepae and F. oxysporum counted per plate were recorded as the number of propagules per g oven-dry soil. In addition, six samples/field were mixed together and a subsample equivalent in wt to 2 g of oven-dry soil was similarly assayed for each of the three fields/farm. Hyphal tip transfers of suspect colonies were made to PDA slants for positive identification.

Morphological types.—Single spore transfers were made to PDA slants from all isolates of F. oxysporum f. sp. cepae obtained from naturally infested field soils, artificially infested sterilized soils, and diseased onion tissues. The cultures were incubated for 3-4 weeks at 22-24 C under 2,000 ft-c fluorescent light (Life line Sylvania warm-white bulbs) for 12 hr/day. Each isolate was classified as one of four morphological types: sporodochial, ropy, pionnotal, or mycelial.

RESULTS.—Selective medium.—Benomyl and Daconil 2787 caused complete inhibition of conidial germination at all concentrations tested. Polyram, TBZ, thiram, Dithane M-22 Sp., and captan caused complete inhibition of conidial germination at 10 ppm but not at 1 ppm. Ferbam, Botran, Dexon, and PCNB did not inhibit conidial germination at the concentrations tested (1, 10, and 100 ppm), but caused restriction of colony

size. Thiram, Dexon, and PCNB at 1, 100, and 100 ppm, respectively, allowed good growth of F. oxysporum f. sp. cepae in agar plates, and greatly suppressed the microflora of organic soils of New York. Thiram, Dexon, and PCNB were tested separately and in combination at different levels. A combination of 1, 100, and 100 ppm thiram, Dexon, and PCNB, respectively, with 2 ppm Aureomycin added to the basal medium (8), was satisfactory for the growth of F. oxysporum f. sp. cepae from conidial suspension (Table 1) and for the isolation of the fungus from organic soils (Fig. 2-A, B). This medium had a pH of 6.00-6.05 (measured by Coleman Model 39 pH meter). This pH was within the optimum range of 4-8 (Fig. 1) for initiating the growth of F. oxysporum f. sp. cepae in culture as determined by the following method. Monospore transfers were made to PDA plates, and the pH

TABLE 1. Recovery of Fusarium oxysporum f. sp. cepae from conidial suspensions plated on both an antibiotic check and the selective medium

Spore suspension	Number of colonies/plate ^a				
	Expectedb	Antibiotic check ^c	Selective medium ^d		
1	30	34.3	38.6		
2	40	39.3	35.3		
3	43	24.0	23.3		
4	50		56.2		
5	60	71.3	76.6		

- a Each number is an average of three or five replicates.
- ^b These numbers were calculated from hemacytometer counts.
- ^c Consisted of the basal medium plus 2 ppm Aureomycin.
 ^d Consisted of the basal medium plus 2 ppm Aureomycin and 1, 100, and 100 ppm thiram, sodium-*p*-(dimethylamino) benzenediozosulfonate, and pentachloronitrobenzene, respectively.

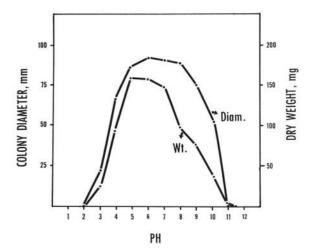


Fig. 1. Effect of pH on the growth of Fusarium oxysporum f. sp. cepae on potato-dextrose agar (PDA) at 24 C. The pH was adjusted using a dilute solution of NaOH or HCl. Monospore transfers were made to PDA plates and incubated for 162 hr, and colony diameter and dry wt were recorded in mm and mg, respectively. Each number is an average of four replicates.

adjusted by using a dilute solution of sodium hydroxide or hydrochloric acid. Twelve pH values (2-12) were tested. The cultures were incubated for 162 hr at 24 C, and colony diameter and dry wt determined. A medium with 1, 50, and 50 ppm of the same fungicides, respectively, added to the basal medium (8), plus 2 ppm Aureomycin, also was satisfactory for the selective isolation of the fungus, but the former was used in the studies which follow. Generally, 1 ppm thiram was the most effective of the three fungicides used in suppressing the background soil microflora.

The accuracy of the plating technique was tested by determining the frequency distribution of colonies of F. oxysporum f. sp. cepae from a particular soil dilution on the assay plates. The theoretical frequency of distribution of colonies on the plates was determined, using the Poisson distribution formula (10). Data obtained were analyzed statistically and found to be significant at the 5% level [Goodness of fit=12.598, Limits X^2 95/df (12) = 21.03], indicating the precision of the technique.

Effects of our selective medium on conidial germination and growth of *Fusarium* species was tested. Isolates of *F. oxysporum* forms tested grew well, while those of six other species of *Fusarium* failed to grow or were inhibited in colony size and number (Table 2).

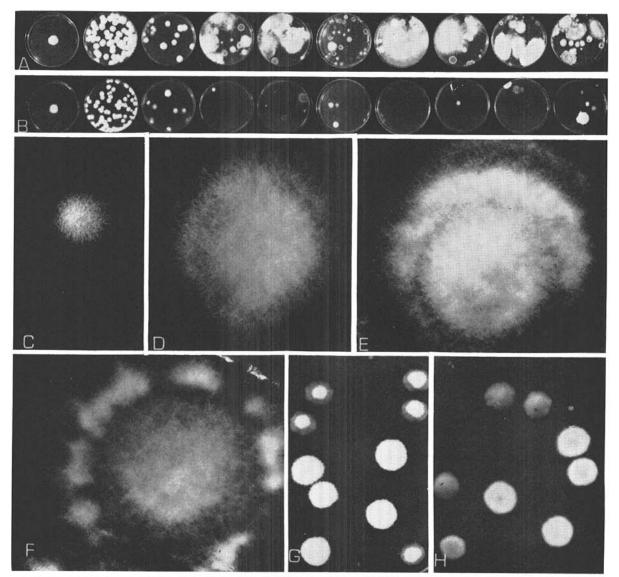


Fig. 2. Effect of the selective medium on the growth of Fusarium oxysporum f. sp. cepae and the natural soil microflora of organic soils. A, B) From left to right, mycelial disc, conidia on, and conidia in the agar. The remainder left to right are soil plates of seven soils prepared by mixing 1 ml of the organic soil suspension with the medium used. The media in A and B are the antibiotic check and the selective medium, respectively. Photographs were taken at 10 days of incubation. C, D, E, F) Colony development of the fungus on the selective medium. Photographs were taken at 3, 6, 9, and 14 days of incubation, respectively. G, H) Surface view and back view, respectively, of the fungus growing on the selective medium. Photographs were taken at 7 days of incubation.

TABLE 2. Effect on the selective medium on spore germination and growth of Fusarium species

	Per cent of controla			
Fusarium species ^b	No. colonies /plate	Colony diam		
F. oxysporum f. sp.				
lycopersici	95.1	84.9		
F. oxysporum f. sp.				
cepae	90.1	94.2		
F. oxysporum No. 5	90.0	57.9		
F. oxysporum No. 11	65.4	77.4		
F. solani	60.4	87.0		
F. moniliforme	32.3	57.7		
F. rigidiscula	32.0	54.3		
F. roseum	20.3	58.6		
F. tricinctum	16.6	68.4		
F. lateritium	0.0			
F. solani f. sp.				
phaseoli	0.0			

^a Each number is an average of three replicates. Control consisted of the basal medium plus 2 ppm aureomycin.

It was not always possible to visually differentiate F. oxysporum f. sp. cepae colonies from other members of the species F. oxysporum. In such cases, suspect colonies were transferred to PDA slants and maintained at 22-24 C under 2,000 ft-c fluorescent light (12 hr/day) for further identification. The pathogenicity of representative isolates was tested by the onion slice method (1) or by planting surface-sterilized onion seeds in artificially infested soil. Isolates of F. oxysporum f. sp. cepae obtained by selective plating from organic soils always were pathogenic. The morphological appearance of the fungus on the selective medium at different time intervals is seen in Fig. 2-C, D, E, F, G, H. Generally, the colonies were characterized by a very compact center with a loose uniform margin. The mycelial mat is composed of abundant, fine, cottonlike white hyphae.

Distribution in field soil.—The population of F. oxysporum f. sp. cepae in the organic soil of a farm at Linwood, N.Y., with a long history of Fusarium basal rot, ranged from 660 to 7,300 propagules/g oven-dry soil. This farm was divided into 15 fields, and a composite soil sample consisting of six subsamples was collected/field and analyzed. Data obtained (Fig. 3-A) clearly showed the uneven distribution of the fungus in these soils. Soils from another farm at East Pembroke, N.Y., with a history of severe outbreaks of the disease, was analyzed similarly and the results obtained also showed an uneven distribution with populations ranging from 600 to 4,500 propagules/g oven-dry soil. When the subsamples were analyzed separately, the same uneven distribution pattern occurred. In one sampling, the population in the Linwood soil ranged from 1,300 to 8,000 propagules/g oven-dry soil (Fig. 3-B).

Farms with a history of Fusarium basal rot consistently were found to have the highest populations of F. oxysporum f. sp. cepae (Fig. 3-C). Relative popula-

tions of the fungus and those of F. oxysporum in seven organic soils cropped to onion from western New York were determined. The highest population of F. oxysporum f. sp. cepae (an average of 4,600 propagules/g oven-dry soil) was found on farm A, which had a history of severe basal rot. Lowest population of the fungus (800 propagules/g oven-dry soil) was found on farm G which had no known history of the disease. Farm B, with a history of severe outbreaks of the disease (poorly managed, and covered with a variety of weeds), had an average population of 3,500 propagules/ g oven-dry soil. Farm B also had the highest population of F. oxysporum (an average of 14,000 propagules/g oven-dry soil) of the seven soils tested. The other farms had no histories of major outbreaks of the disease. There was no correlation between the populations of F. oxysporum and those of F. oxysporum f. sp. серае.

Seven organic soils from Orange County, N.Y., were also sampled. These soils did not have a known history of severe basal rot of onion. Populations of the fungus in these soils ranged from 300 to 6,500 propagules/g oven-dry soil (Fig. 3-D). Farm A was the only one with a high population of the pathogen, yet grower records indicate that *Fusarium* basal rot has not yet caused major economic loss on this farm, even when susceptible varieties have been grown.

Two organic soils at Elba and Linwood, N. Y., were selected for further study. The Elba soil had a history of freedom from outbreaks of Fusarium basal rot; the Linwood soil had a long history of severe outbreaks. Four fields were sampled for each soil at three depths (0-15.2, 15.2-30.5, and 30.5-45.7 cm). The population of F. oxysporum f. sp. cepae was determined by the dilution plate count method and the most probable number method using the selective medium. The Linwood soil had much higher levels of the fungus at all depths sampled than the Elba soil (Table 3). The highest population at Linwood occurred at 0-15.2 cm; the lowest occurred at 30.5-45.7 cm. These populations were 5,400 and 3,000 propagules/g oven-dry soil, respectively. Such differences were not found at Elba, which had populations of 1,500 propagules/g oven-dry soil for both the 0- to 15.2- and 30.5- to 45.7-cm samples. Close correlation was found between the population of F. oxysporum f. sp. cepae as calculated by the dilution plate count method and the most probable number method in both soils. Higher counts were obtained by the most probable number method (Table 3); however, the dilution plate count method was more consistent.

The amount of *Fusarium* basal rot that developed in four fields of both the Elba and Linwood soils was determined. Onion bulbs of the variety Downing Yellow Globe were collected from each field at harvest time, stored at 15 C for 1 month, and cut vertically and examined macroscopically. Onions from Linwood were found to have higher percentages of basal rot and stem plate discoloration (11.6 and 20.6%, respectively) than from Elba (2.5 and 6.4%, respectively). The natural infestation with *F. oxysporum* f. sp. *cepae* and the per-

b Fusarium species used either were obtained from C. W. Boothroyd, Department of Plant Pathology, Cornell University, Ithaca, N. Y., or were isolated personally during the study.

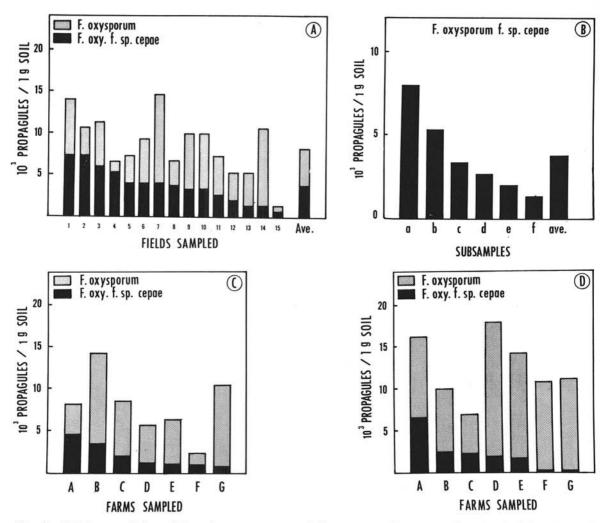


Fig. 3. Relative population of Fusarium oxysporum and F. oxysporum f. sp. cepae in naturally infested organic soils. A) Distribution of both fungi in a soil located at Linwood, N.Y., which had a history of Fusarium basal rot of onion. B) Distribution of Fusarium oxysporum f. sp. cepae within one field of the same soil. C) Population of both fungi in seven soils cropped to onion from western New York. Farms A and B had histories of Fusarium basal rot; farm G had no known history; the remainder had histories of no major outbreak of the disease, although low levels had occurred annually. Farm B had poor management and was covered with a variety of weeds when sampled. D) Population of both fungi in seven soils cropped to onion from Orange County, N.Y. None of these soils had major outbreaks of Fusarium basal rot of onion.

Table 3. Population of Fusarium oxysporum f. sp. cepae at three depths of sampling as measured by the dilution plate count (DPC) and the most probable number (MPN) methods in two organic soils from western New York

Depth, cm	Propagules/g oven dry soil $^{\rm a} imes 10^{\rm 3}$					
	Elba	farm ^b	Linwood farme			
	DPCa	MPN	DPCa	MPN		
0-15.2	1.5	2.5	5.4	7.8		
15.2-30.5	1.2	1.8	4.2	5.6		
30.5-45.7	1.5	1.9	3.1	4.1		

a Each number is an average of 20 replicates.

^b Farm located at Elba, N. Y., and had no known history of severe outbreaks of *Fusarium* basal rot.

^c Farm located at Linwood, N. Y., and had a long history of severe outbreaks of *Fusarium* basal rot.

centage of basal rot developed in the four fields tested at both farms are compared in Table 4.

Morphological types in soil.—Only the sporodochial type of F. oxysporum f. sp. cepae was isolated from naturally infested organic soils. However, sporodochial type isolates added to sterilized greenhouse soil (1:1 ratio of peat and sandy loam soil) columns and stored for 3-5 years mutated and gave rise to all variants found on PDA cultures. Sporodochial, ropy, pionnotal, and mycelial types were isolated from naturally infected stem and root tissues of onion grown in sterilized organic soils artificially infested with sporodochial isolates. All but the ropy type were isolated from similar tissues of onions grown in naturally infested organic soils under field or greenhouse conditions. Nevertheless, the sporodochial type was by far the most prevalent (Table 5).

TABLE 4. Population of Fusarium oxysporum f. sp. cepae measured by the dilution plate count (DPC) and the most probable number (MPN) methods and Fusarium basal rot of onion in four fields for each of two soils

Field	Elba farma			Linwood farm ^b		
	Propagules/g soil × 10 ³		% Onions with	$\begin{array}{c} \text{Propagules/g} \\ \text{soil} \times 10^3 \end{array}$		% Onions with
	DPCc	MPN	basal rot	DPCc	MPN	basal rot
1	2.0	4.2	4.3	5.7	4.9	11.1
2	1.5	3.1	2.0	5.8	9.7	9.8
2	1.3	1.9	1.8	4.0	6.2	14.0
4	1.3	0.8	2.0	6.2	10.4	
Avg	1.5	2.5	2.5d	5.4	7.8	11.6e

- a Farm located at Elba, N. Y., and had no known history of Fusarium basal rot.
- b Farm located at Linwood, N. Y., and had a long history of Fusarium basal rot.

e Each number is an average of five replicates.

d Average per cent of onions with stem plate discoloration was 6.4.
e Average per cent of onions with stem plate discoloration was 20.6.

DISCUSSION.—A new selective agar medium incorporating PCNB, thiram, Dexon, and Aureomycin with Martin's rose bengal medium (8) proved highly satisfactory for growth of F. oxysporum forms while suppressing background microorganisms. By using this medium, F. oxysporum f. sp. cepae was found to be concentrated in very high numbers (frequently as high as 8,000 and a single count of 13,000 propagules/g oven-dry soil) in the top 15.2 cm of several organic soils of New York. Vertical distribution studies to 45.7 cm indicated populations of 3,100 and 1,500 propagules/g soil 30.5-45.7 cm below the soil surface in fields with and without histories of the disease, respectively. These counts are higher than those reported previously for a number of species of the genus Fusarium (5, 10, 13, 14, 15) at these depths in mineral soils. The high populations of the fungus in organic soils are not entirely unexpected, however, since populations of most soil microorganisms generally tend to be higher in organic than in mineral soils. Comparative counts made on a volume rather than on a wt basis with mineral and organic soils could suggest a different relationship.

Fusarium oxysporum f. sp. cepae was found to be unevenly distributed in all organic soils cropped to onion in New York examined in this study. A similar pattern of distribution was reported earlier for F. oxysporum f. sp. cubense, f. sp. melonis, f. sp. lini, F. solani f. sp. cucurbitae, and f. sp. pisi (3, 4, 9, 13, 14). Nash & Snyder (10) reported F. solani f. sp. phaseoli

TABLE 5. Frequency of isolation of the morphological types of *Fusarium oxysporum* f. sp. cepae from naturally infested field organic soils, artificially infested sterilized greenhouse soils, and from onion plants grown in naturally infested and artificially infested sterilized organic soils

	% Isolation/morphological type				
Source	Sporodo- chial	Ropy	Pion- notal	Mycelial	
Field soils	100.0	0.0	0.0	0.0	
Sterilized soils	57.1	20.2	21.4	1.2	
Onion tissues Field soils	92.5	0.0	6.7	0.8	
Sterilized soils	72.6	15.5	2.4	9.5	

to be evenly distributed in the top 15.2-20.3 cm of the soil.

New York organic soils with histories of Fusarium basal rot consistently revealed high populations of F. oxysporum f. sp. cepae. However, it did not always follow that soils with high populations of the fungus had a history of a high incidence of basal rot even when susceptible varieties of onions were grown. This indicates that factors in addition to the inoculum density of the fungus influence the disease potential of naturally infested field soils. Similar observations have been reported for F. oxysporum f. sp. melonis, F. solani f. sp. phaseoli, and F. solani f. sp. pisi and the diseases they cause (3, 10, 14). It appears that factors in the soil (biotic, abiotic, or both) are involved which reduce infection in many cases, but in certain soils either their absence or nonfunction allows a greater incidence of infection. The effect is similar to the phenomenon reported in the studies conducted by Stotzky & Martin (12) on tropical soils in which an inverse correlation between the rapid spread of Fusarium wilt of banana (caused by F. oxysporum f. sp. cubense) and the absense of a swelling 3-layer silicate component was found in 67 soils in five tropical countries. Disease spread was slow in soils with the clay fraction, and rapid in those without. Recently, Burke et al. (3) suggested a correlation between soil hardness and incidence of pea root rot in Wisconsin. Sedimentary peat layers which are impenetrable to water and roots exist in certain New York organic soils at different depths, depending upon the origin of the soil. These layers could cause a similar phenomenon with the Fusarium basal rot disease in onions grown on such soils, and this possible association, particularly, is worthy of future study.

The isolation of only the sporodochial type of *F. oxysporum* f. sp. cepae from naturally infested field soil indicates that cultural variants either do not occur in field soil or exist in the soil at extremely low levels. Cultural variants of this fungus were isolated from naturally infected onion tissues and from greenhouse-prepared soils artificially infested with the fungus. It is possible that onion tissues may act as a selective substrate for the isolation and growth of the morphological variants isolated, but most probably the sporo-

dochial type mutates readily to these variants in infected tissues. In a sterile soil, it is expected that cultural variants would arise as on a medium such as PDA, and indeed all morphological types (sporodochial, ropy, pionnotal, and mycelial) were isolated from sterilized greenhouse soils artificially infested with sporodochial isolates.

In this study and those conducted previously (1, 2), morphological mutants always were less pathogenic than the wild type (sporodochial). If different biological forms of F. oxysporum f. sp. cepae occur in different organic soils cropped to onion, the results of the present study suggest that these forms are all of the sporodochial type. Differences in pathogenicity between sporodochial type isolates did occur, and suggest that these differences are regulated by a system other than that regulating the appearance of the morphological forms.

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