Selectivity of *Fusarium* Culture Filtrates in Agar Media

R. Alconero and Alma G. Santiago[1]

INTRODUCTION

A number of agar media contain various antimicrobial agents for the isolation, enumeration and identification of *Fusarium* spp. from soil (*1,2,4,5*).[2] Papavizas (*3*) recently evaluated several of these media under standardized laboratory conditions.

In 1961 Parmeter and Hood (*4*) reported a selective medium containing culture filtrates of *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans. for its isolation from soil in dilution plates. They found that the medium restricted the numbers and growth of competing fungi, and simplified the identification of *F. solani* f. sp. *phaseoli* colonies. The potato-dextrose broth filtrate medium retained its inhibitory and selective properties when diluted to 50 and 70 percent. Autoclaving did not destroy the filtrate properties.

Media having selectivity for forms of *Fusarium* species would be very useful in studies of these forms and their responses to manipulation. We studied the selectivity of culture filtrates of *F. oxysporum* f. sp. *vanillae*, *F. oxysporum* and *F. solani* isolates on the linear growth in agar, spore germination, and growth in soil plates of these and other soil fungi.

MATERIALS AND METHODS

DETERMINATIONS OF LINEAR GROWTH IN AGAR

We incubated two isolates of *F. solani* and two isolates of *F. oxysporum*, including *F. oxysporum* f. sp. *vanillae*, at 28° C. for 14 days in potato dextrose (PDB) and nutrient broth (NB). Isolates (0.2 cm. dia. discs of a 1-week old Czapeck agar culture) were placed in 500 ml. of broth in 1000 ml. flasks. At the end of the incubation period the broth cultures were filtered, and agar was added to the filtrates to make 2-percent agar media. The media were autoclaved for 15 minutes, poured into sterile petri plates, and left for 1 day to harden. Potato dextrose agar (PDA) and nutrient broth agar (NBA) were prepared without the filtrates as controls.

A 4-mm. diameter water agar culture of one isolate was placed on the surface of the agar media. Diameter of each isolate was recorded daily in

[1] Research Plant Pathologist and Biological Laboratory Technician. Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Federal Experiment Station, Mayagüez, P.R.

[2] Italic numbers in parentheses refer to Literature Cited, p. 146.

the different media until the cultures covered the agar surface. Each isolate was replicated three times in each filtrate and control media. The experiment was repeated twice.

DETERMINATIONS OF SPORE GERMINATION

The isolates were incubated as previously described, except for use of 250 ml. of broth in 500 ml. flasks. Half of each filtrate was autoclaved for 15 minutes and the other half was filtered through a Seitz filter. The same procedure was followed for the control broths. Ten milliliters of a filtrate were poured in a small 2-inch diameter petri plate; and a sterile film of cellophane (first washed with acetone and distilled water) was placed on the filtrate surface.

Macro- and microspores of *F. solani* (B) were obtained from a 2-week-old Czapeck agar culture incubated at 28° C. To separate spores from the mycelium we used a spatula and sterile distilled water. The spores were washed three times by centrifugation and resuspended in water. About 10 spores were used per low-power field by diluting with sterile distilled water. After spreading the spore suspension (2 ml.) on the surface of the cellophane film, spore germination was observed hourly during 5 hours. One final reading was made 8 hours after the first observation. There were three replicates per trial in each of the three filtrates of each medium. The experiment was repeated twice.

DETERMINATION OF GROWTH IN SOIL PLATES

Unsterilized and sterilized soil infested with *F. oxysporum* f. sp. *vanillae* and *F. solani* (B) were employed to determine the selectivity of the filtrates on fungi developing from soil or humus particles. The artificially infested soil was prepared by autoclaving 40 cc. of rich garden soil for 1 hour in slanting, 100-ml. glass bottles. One day later a 2-cm. diameter disk of a 4-day old Czapeck agar culture of one isolate was placed on the soil surface and incubated for 1 week at room temperature (25 to 28° C.).

Soil samples (100 mg.) taken from the bottles after mixing the contents thoroughly, were distributed in 10 petri plates. Garden soil, soil infested with *F. oxysporum* f. sp. *vanillae*, soil infested with *F. solani* (B), and a mixture of the infested soils were represented in 10 petri plates for each filtrate and control medium.

Media consisted of potato-dextrose broth and nutrient broth (with and without a previous 2-week *Fusarium* culture). The broths were filtered and agar was added to make a 3-percent agar medium. The filtrates were autoclaved 15 minutes. Eighty milligrams of streptomycin sulfate and 60 mg. of rose bengal per 1,000 ml. were then added to the media. Approximately 15 ml. of medium were poured into each plate, and the soil particles were

distributed through the medium with a gentle motion of the plate. Readings of the developing colonies were taken 48 hours after the plates were incubated at 28° C. Twenty-five isolates were taken at random from each 10-plate group containing unsterilized garden soil. The experiment was repeated twice.

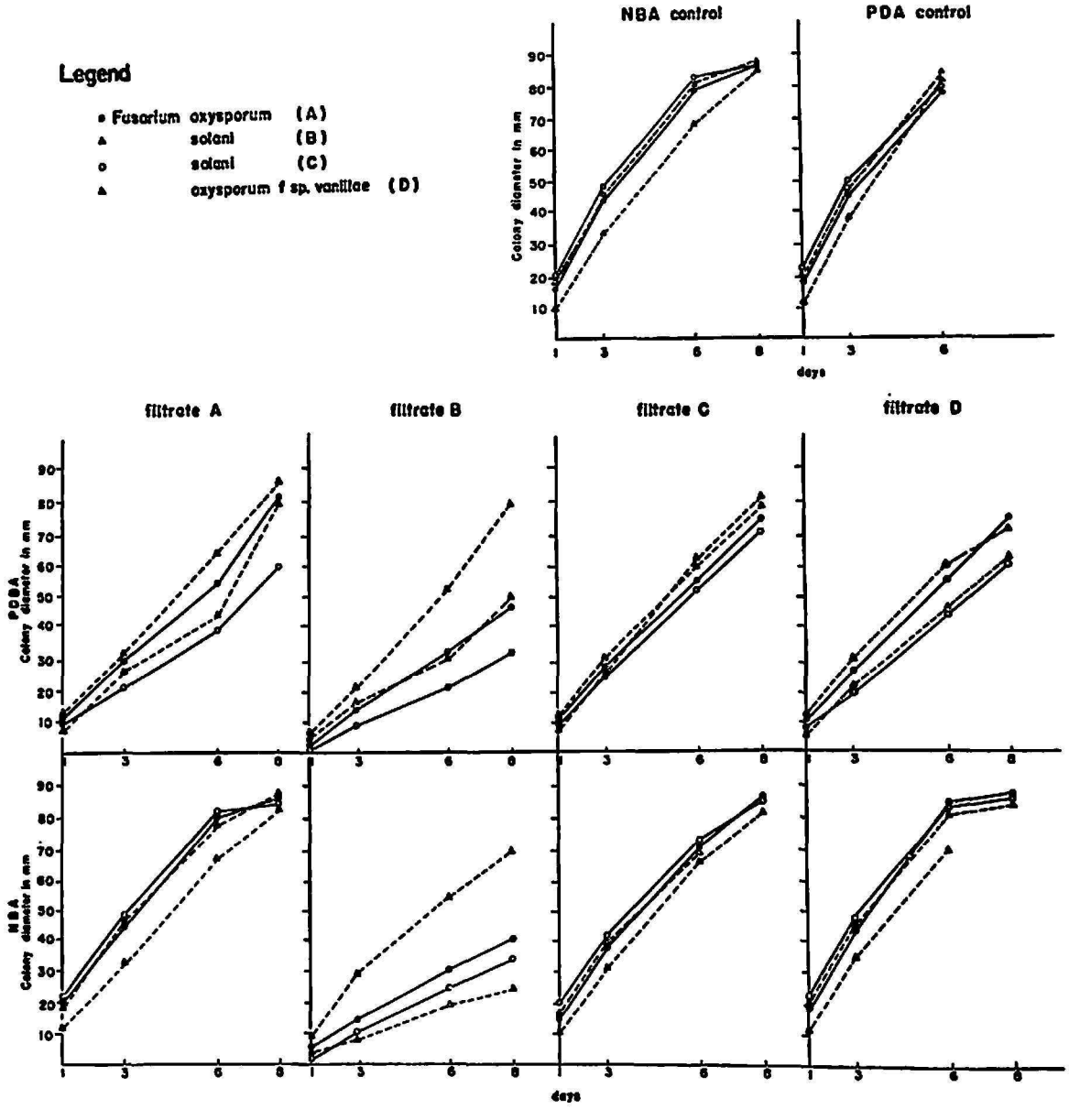

FIG. 1.—Linear growth in agar plus filtrates of *F. oxysporum* and *F. solani* isolates.

RESULTS

LINEAR GROWTH IN AGAR

Fusarium oxysporum in its own filtrate (A) media grew at approximately the same rate as in the controls. Inhibition was greatest in *F. solani* (C) in PDBA medium with filtrate A. Apparently other fungi were not inhibited (fig. 1).

The greatest inhibition of linear growth in agar, as compared with the controls, occurred in PDBA and NBA with filtrate B. The inhibition was selective in both media. *Fusarium solani* (B) grew best in its own filtrate media, whereas the other fungi grew more slowly than expected from observations in the controls. There was no appreciable inhibition in media with filtrates of *F. solani* (C) or *F. oxysporum* f. sp. *vanillae* (D).

SPORE GERMINATION

The germination of *F. solani* (B) spores was more rapid in both media without filtrates, whether sterilized with heat or with a Seitz filter. Nutrient broth apparently was the better medium, with 45-percent germination by the fourth hour of incubation as compared with 25 percent in potato dextrose broth (fig. 2). Germination was much slower in filtrate media, regardless of

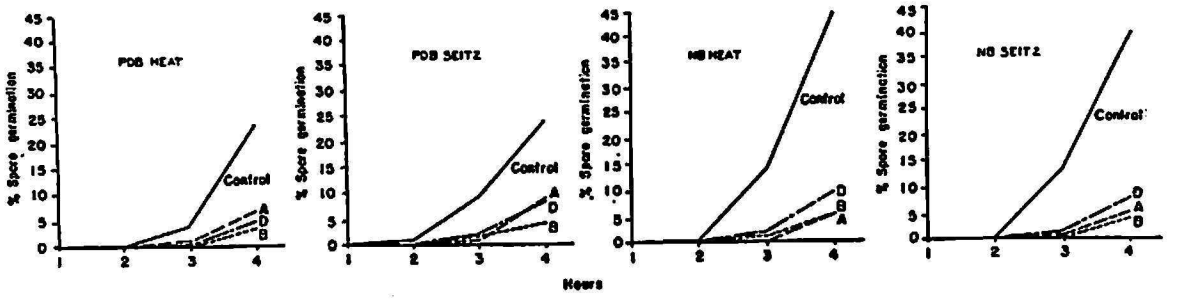

FIG. 2.—Spore germination rates of *F. solani* (B) over filtrates of *F. oxysporum* and *F. solani* isolates.

filtrate. Use of filtrate B from *F. solani* (B) did not accelerate germination of *F. solani* (B) nor did other filtrates inhibit germination rate.

GROWTH IN SOIL PLATES

Figure 3 illustrates the growth of fungi on filtrate media from *F. solani* and *F. oxysporum* f. sp. *vanillae* broth cultures and from control broths. Counts were lowest in soil plates with PDB filtrates, but much higher with the PDB control broth alone. The *F. solani* filtrate decreased the number of colonies appearing in all soil plates. The *F. oxysporum* f. sp. *vanillae* filtrate in PDB decreased the number of colonies of itself less than in NB. Colonies were more numerous than in the *F. solani* filtrate in the same media but the differences were small. In soil containing both fungi the use of filtrates did not, apparently, favor either fungus; and colonies were indistinguishable.

PDB agar without filtrates allowed a greater number of colonies to develop in nonsterile soil. At random selection of colonies from the soil plates resulted in a wide variety of heavily sporulating species, but there was no apparent selection of fusaria when filtrates had been added to the media (table 1).

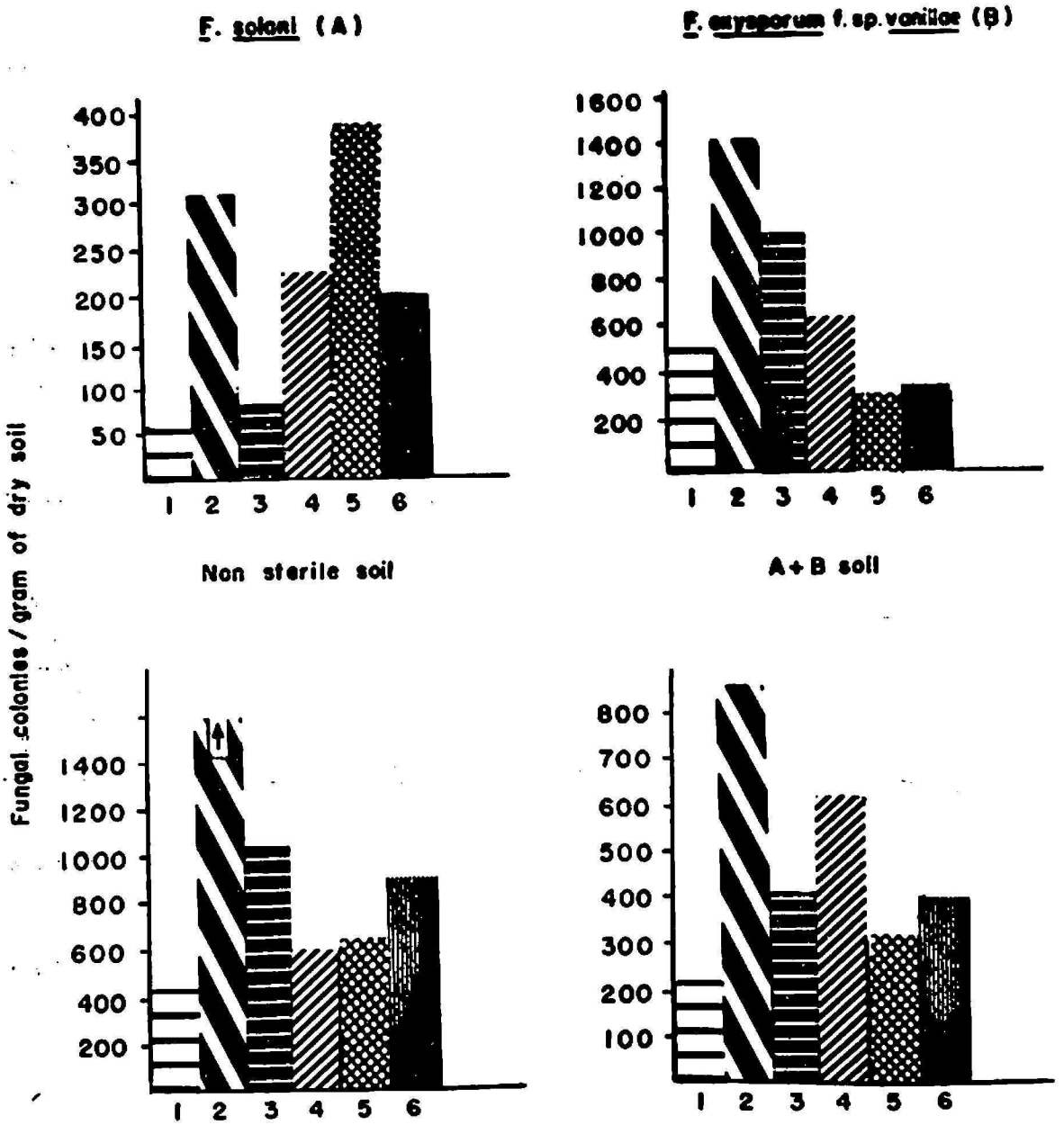

FIG. 3.—Fungal colonies in soil plates with filtrates of *F. solani* B in PDBA (1), no filtrates in PDBA (2), *F. oxysporum vanillae* in PDBA (3), *F. solani* B in NBA (4), no filtrate in NBA (5), and *F. oxysporum vanillae* in NBA (6).

ACIDITY OF MEDIA USED IN THE EXPERIMENTS

Table 2 illustrates the acidity of the media used. Generally, PDB was acidic (pH 3.6–5.0) and NB neutral to slightly basic (pH 6.6–8.8), with no marked differences between filtrates and controls.

DISCUSSION

There was little evidence of the selectivity of fusarial fungus filtrates in media. The only media with slight selective inhibitory properties were NBA and PDBA with filtrates of *F. solani* (B). Other fungi were inhibited in experiments of linear growth in agar only.

Spore germination of this fungus on the surface of washed cellophane did not exhibit the expected enhanced sporulation in its own filtrate. Spores germinated slowest over its own filtrate, whether the media had been

TABLE 1.—*Fungus isolations from unsterilized garden soil plates using filtrate media*[1]

Fungus	*F. oxysp. vanillae*		*F. solani*	(B)	Control	
	NBA[2]	PDBA[3]	NBA	PDBA	NBA	PDBA
Aspergillias spp.	3	10		16	2	9
Chatomium sp.	1				2	1
Circinella sp.	2		5		8	
Cunninghamella sp.	5	2	3	3	4	5
Curvularia sp.	1	1				
Fusarium spp.	4	2	5	3	1	
Mucor spp.		2		1	5	
Penicillium spp.	1	2		2		8
Rhizopus spp.	1	1	1		2	
Sclerotium sp.	1			1		
Stysanus spp.		1		1		
Trichoderma spp.		2				1
Unknown spp.	2		1	2	1	

[1] Results are averages of 2 trials.
[2] Nutrient-broth agar (NBA).
[3] Potato-Dextrose broth agar (PDBA).

TABLE 2—*pH's of filtrate and control media*[1]

Fungus	NB[2]		PDB[3]	
	Seitz	Heat	Seitz	Heat
F. oxysporum vanillae (D)	8.72	8.70	3.95	3.60
F. oxysporum (A)	8.15	8.30	4.25	4.40
F. solani (B)	8.10	8.78	3.60	3.72
F. solani (C)	8.12	7.85	4.20	3.65
Control	6.60	6.70	5.07	4.70

[1] Results are averages of 2 trials.
[2] NB: Nutrient Broth.
[3] PDB: Potato Dextrose Broth.

sterilized in the autoclave or through a Seitz filter. The control broths allowed a more rapid germination, but after 9 to 12 hours of incubation germination was similar to the controls (94 to 98 percent).

Selective inhibition of filtrates in soil plates was not apparent for any of the filtrate media, and species of *Fusarium* could not easily be distinguished from each other. There was no evidence that the numbers or types of fungi

developing in soil plates with filtrate media were different from each other or significantly different from the controls.

The large differences in spore germination rate observed between the controls and filtrates are possibly due to nutrient deficiency, although the presence of an inhibiting factor was not sought. The acidity of the media apparently had no effect. Our observations suggest that filtrates are not sufficiently selective to warrant their use in agar media.

SUMMARY

Filtrates of *Fusarium oxysporum* f. sp. *vanillae* (Tucker) Gordon, Schlecht. and two isolates of *F. solani* (Mart.) Appel & Wr., grown in nutrient and potato dextrose broth, effected spore germination, linear growth in agar, and colony development in soil plates of these and other fungi. There was not enough sign of selectivity in these filtrates to warrant their use for selective isolation of fungi from soil.

RESUMEN

Filtrados de *Fusarium oxysporium* f. sp. *vanillae* (Tucker) Gordon, Schlecht. y de *F. solani* (Mart.) Appel & Wr. cultivados en un caldo nutrimental y en agar-papa con dextrosa, produjeron esporas, lograron un crecimiento lineal en agar y el desarrollo de colonias en platillos con suelos que contenían éstos y otros hongos. No hubo suficientes señales de selectividad en estos filtrados que puedan justificar su uso para seleccionar y aislar hongos del suelo.

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