Enumeration of Fusarium oxysporum f. pisi Race 5 Propagules from Soil

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Cooperative investigations of the Agricultural Research Service, U.S. Department of Agriculture, and the Washington Agricultural Experiment Station, Pullman 99163. Technical Paper No. 3927, Washington Agricultural Experiment Station.

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Accepted for publication 27 December 1972.

ABSTRACT

Five of 24 media tested distinguished races 1, 2, 4, and 5 of *Fusarium oxysporum* f. *pisi* when recovered from artificially infested soil. Of the five media, highest populations of *F. oxysporum* f. *pisi* race 5 in naturally infested soil were recorded with the PCNB medium of Nash and Snyder.

Phytopathology 63:765-768

Additional key words: soil-dilution plates, *Fusarium*-selective media, *Pisum sativum*.

Fusarium oxysporum Schl. f. *pisi* (Linf.) emend. Snyd. & Hans. race 5 Hag. & Kr. causes severe damage to peas (*Pisum sativum* L.) in western Washington (6). Other races of this organism have been reported in many pea-growing areas of the world (1, 4, 5, 8, 17). Whether race 5 will become important in areas other than western Washington is still unknown. The population of *F. oxysporum* f. *pisi* race 2 in artificially infested soil has been determined using selective media (8, 12), but thus far population levels of *F. oxysporum* f. *pisi* race 5 have not been measured in either naturally or artificially infested soil. Population estimates are important because inoculum density of *F. oxysporum* f. *pisi* is directly related to disease severity (5, 7, 10). A procedure for detecting, and determining levels of, race 5 in naturally and artificially infested soil is reported here.

MATERIALS AND METHODS.—The culture of *F. oxysporum* f. *pisi* race 5 used in this study (ATCC acc. No. 22554) was isolated from an infected plant of the pea cultivar 'New Wales' [resistant to races 1, 2, and 4 (1)]. Cultures of races 1 and 2 were isolated in our laboratory; race 4 was supplied by Bolton et al. (1). All cultures used in this study originated from single spores and were maintained in tubes of dried sterile soil (15).

Shake cultures of each *Fusarium* isolate were prepared by incubation for 7 days at 24 C in Kerr's medium (8). The growth medium was strained through a double layer of cheesecloth. This provided a conidial suspension which was used to infest separate soil samples with each organism (9).

The soil-dilution method (11) was used to determine which of the media listed in Table 1 were best suited to isolate races 1, 2, 4, and 5 from soil,

TABLE 1. Media and additives tested for differentiating the races of *Fusarium oxysporum* f. *pisi*

Media[a]	Modification	Antimicrobial additives[b] PCNB 100 ppm	Streptomycin sulfate 300 ppm	Chlorotetracycline HCl 100 ppm	Neomycin 100 ppm	Sodium azide 30 ppm
Potato dextrose agar (PDA)	A	+[c]	+	+	+	–
	B	+	+	+	+	+
	C	+	+	+	–	+
	D	+	+	–	–	+
	E	+	–	–	–	+
	F	+	–	–	–	–
	G	+	–	–	+	+
Cornmeal dextrose agar (CMDA)	A	+	+	+	+	+
	B	+	+	+	+	–
	C	+	+	+	–	–
	D	+	+	–	–	+
	E	+	–	–	–	+
	F	+	–	–	–	–
	G	–	–	–	+	+
Fusarium solani filtrate agar (FSF) (13)	A	–	–	–	–	–
	B	–	+	+	+	–
Fusarium oxysporum filtrate agar (FOF) (13)	A	–[c]	–	–	–	–
	B	–	+	+	+	–
Peptone-pentachloronitro-benzene agar (PCNB) (11)		+	+	+	+	–
V-8 juice-dextrose yeast extract-penta-chloronitrobenzene agar (VDYA-PCNB) (12)		+	–	–	–	–
Azide-rose bengal agar (ARB) (3)		–	–	–	–	+
	A	+	–	–	–	–
	B	+	+	+	+	–
	C[d]	+	+	+	+	–

[a] Media were prepared on a liter basis, with references where applicable.
[b] Antibiotics were added after autoclaving, when media were cooled to 60 C.
[c] + = added; – = absent.
[d] Rose bengal was deleted from ARB modification C.

and to distinguish one race from another. All media were autoclaved 15 min at 121 C and cooled to 60 C before the addition of antibiotics. Fifteen ml of each test medium were added to each petri dish and stored in the dark at 4 C.

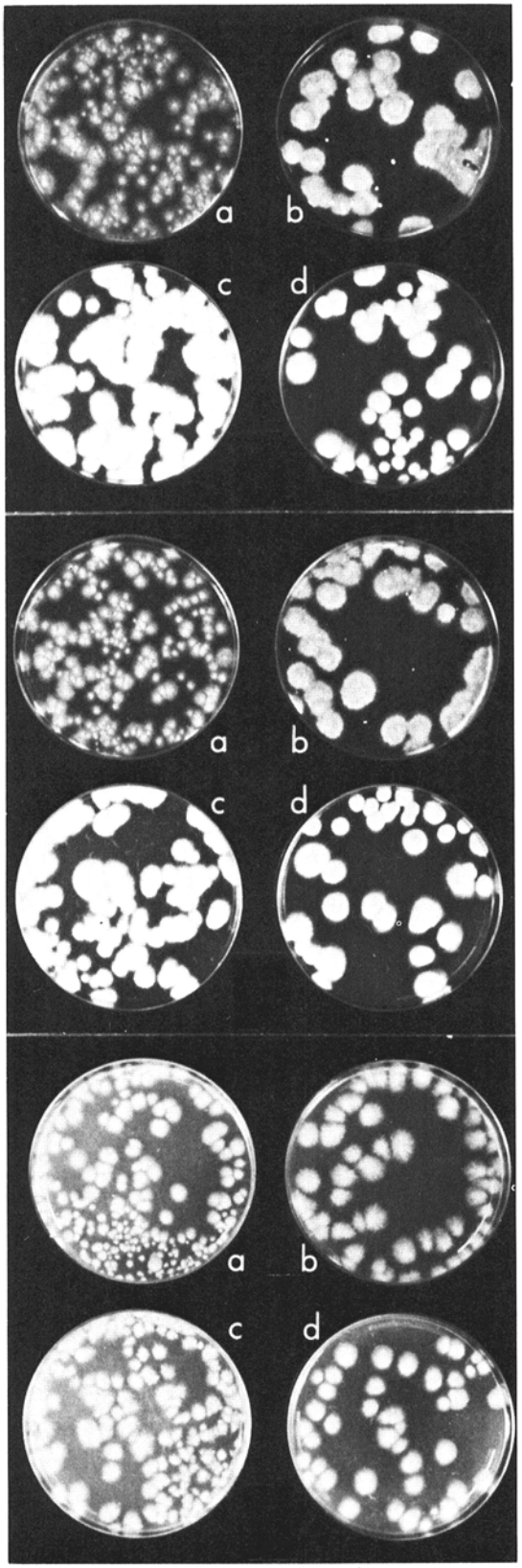
In preliminary tests, soils artificially infested were dispersed on each medium at 10^{-3} dilution, and incubated in the dark at 24 C. After 7 days, characteristics such as raised or appressed growth, color, size, and colony margins were used to determine which medium best distinguished colonies of the race 5 organism from colonies of races 1, 2, and 4.

Media selected from preliminary tests were then evaluated for use in estimating populations of race 5 in naturally infested field soil. All field soils were dispersed at a dilution of 10^{-3} (w/v). All tests included three petri dish replications per medium and were repeated at least twice. When colonies resembling an isolate of race 5 appeared on dilution plates of field soil, hyphal transfers were tested for

TABLE 2. Counts of *Fusarium oxysporum* f. *pisi* race 5 and other fungi in artificially and naturally infested soils on five media

Media	Colonies/Dish[w] Soil A[x] Race 5	Soil A[x] All other fungi	Soil B Race 5	Soil B All other fungi
PDA-A[y]	0.5 a[z]	7.8	1.8 b	6.8
PCNB	1.8 cd	10.0	2.7 c	10.2
ARB-A	0.8 ab	7.5	1.3 a	8.3
ARB-B	2.5 d	9.4	1.5 ab	8.0
ARB-C	1.4 bc	9.0	1.3 a	5.8

[w] An average of two experiments with three petri dishes of each medium.
[x] Soil: A = artificially infested with *F. oxysporum* f. *pisi* race 5. B = field soil naturally infested with *F. oxysporum* f. *pisi* race 5.
[y] The ingredients of each medium are listed in Table 1.
[z] Data followed by the same letter are not significantly different at the 5% level.



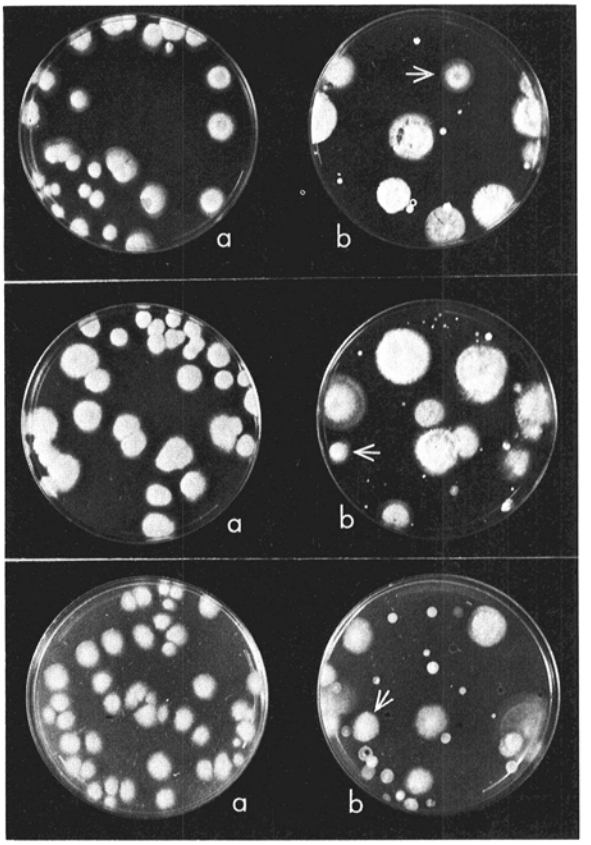


Fig. 2. Characteristic dilution plates of soil (a) artificially and (b) naturally infested with *Fusarium oxysporum* f. *pisi* race 5. Top two plates contain azide rose bengal agar, modification B (ARB-B). Center set of two contains azide rose bengal agar, modification C (ARB-C). Bottom set contains peptone pentachloronitrobenzene (PCNB) agar. A colony of the race 5 organism which originated from a dilution of naturally infested soil on each medium is indicated by an arrow.

pathogenicity on the cultivar 'New Wales' in the laboratory (14).

Analyses of variance and the Duncan Multiple-Range Test were made on colony counts race 5 in both artificially and naturally infested soil. All analyses were at the 95% confidence level.

RESULTS.—Differences in colony morphology between races of *F. oxysporum* f. *pisi* were more easily detected on the three modifications of Azide-rose bengal agar (ARB-A, ARB-B, ARB-C), modified potato-dextrose agar (PDA-A) and peptone

Fig. 1. Dilution plates of artificially infested soil, showing colony morphology of (a) *Fusarium oxysporum* f. *pisi* race 2; (b) race 1; (c) race 4; and (d) race 5. Top set (four plates) contains azide rose bengal agar, modification A (ARB-A). Center set contains azide rose bengal agar, modification C (ARB-C). Bottom set contains peptone pentachloronitrobenzene (PCNB) agar.

pentachloronitrobenzene agar (PCNB) than on any of the others tested (Table 1). Colonies of race 5 were more compact than those of races 1 or 4, whereas colonies of race 1 were more appressed than colonies of race 5 on all media (Fig. 1). When a dilution of soil artificially infested with race 5 was dispersed on PCNB medium, colonies readily identifiable as race 5 appeared in 3 to 4 days. In contrast, colonies of races 1, 2, and 4 were recognizable only after 5 days.

For isolation and determination of populations of race 5 in field soil, PCNB medium yielded higher counts than the other media tested (Table 2). Counts of race 5 on ARB-B and ARB-C media were not as high as were those on PCNB medium, but colonies were as easily recognized and these media were similar to PCNB in suppression of bacteria and other fungi (Fig. 2). In a group of 20 possible race 5 colonies, selected at random in two dilution platings of field soil on PCNB medium, only one was not pathogenic to the cultivar New Wales.

DISCUSSION.—Various researchers have used selective media to isolate and determine populations of various *Fusarium* species in soil (2, 8, 11, 12, 16). In this regard, the concentration, number, and types of antibiotics and antimicrobial chemicals used in this study were based on the findings in previous reports (3, 8, 11, 12, 13, 16). *Fusarium oxysporum* f. *pisi* race 5 can be isolated from and populations counted in field soil if one is familiar with the cultural characteristics of this fungus on ARB-B, ARB-C, or PCNB media. This organism is sufficiently distinct in colony morphology to permit detection on a dilution plate of infested field soil.

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