A Sorbose-Based Selective Medium for Enumerating Propagules of *Fusarium oxysporum* f. sp. *apii* Race 2 in Organic Soil

R. T. Awuah and J. W. Lorbeer

Graduate research assistant and professor, respectively, Department of Plant Pathology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca 14853.
Accepted for publication 13 March 1986.

ABSTRACT

Awuah, R. T., and Lorbeer, J. W. 1986. A sorbose-based selective medium for enumerating propagules of *Fusarium oxysporum* f. sp. *apii* race 2 in organic soil. Phytopathology 76:1202-1205.

A selective medium incorporating L-sorbose (20 g), Bacto agar (20 g), DL-asparagine (2 g), chloramphenicol sulfate (600 mg), pentachloronitrobenzene (PCNB as Terraclor 75WP, 120 mg a.i.), and sodium *p*-(dimethylamino) benzenediazo sulfonate (Dexon 5% granular, 120 mg a.i.) in 1 L of distilled water distinguished among four main types of colonies of *Fusarium* on soil dilution plates after 5–6 days. Colonies of *Fusarium oxysporum* f. sp. *apii* race 2 on the sorbose-based medium (SBM) exhibited a creamy to white color, smooth margins, compact mycelium, and slightly raised centers. The other three colony types of *Fusarium* were nonpathogenic to celery. Growth of colonies of *Fusarium* on SBM generally was slow, permitting colony counts up to 7 days after culturing. Background colonies of bacteria, mucoraceous fungi, and pythiaceous fungi were completely inhibited. Growth of *Penicillium* spp. was limited to whitish pinhead colonies. The efficiency of recovery of *F. o.* f. sp. *apii* race 2 with SBM was 78 and 85% when autoclaved organic soil was artificially infested with 10,000 and 5,000 conidia per gram of dry soil, respectively. Populations of the pathogen detected with SBM at five sites in a field with a history of severe Fusarium yellows ranged from 1,751 to 2,469 propagules per gram of air-dried soil. In another field with a history of a moderate incidence of the disease, the vertical distribution of the pathogen was 1,125 and 900 propagules per gram of air-dried soil in the top 0–20 and 20–40 cm, respectively. The pathogen was not detected at a soil depth of 40–80 cm. Celery plants grown in soil at a depth from 0–20 and 20–40 cm developed Fusarium yellows symptoms. Plants grown in soil 40–80 cm deep remained healthy.

In a previous study of Fusarium yellows of celery grown on organic (muckland) soil in Orange County, NY (3), it was determined that the pathogen, *Fusarium oxysporum* Schlect. emend. Snyd. & Hans. f. sp. *apii* race 2, had become established in a number of celery fields as a result of consistent use by several growers of infected celery transplants. Studies to determine vertical as well as other distribution patterns of the pathogen in commercial fields was not accomplished at that time because a medium for satisfactory quantitative determination of the pathogen in organic soil was lacking. The objective of the present study was to develop a selective medium that could facilitate colony recognition of *F. o.* f. sp. *apii* race 2 in combination with a soil dilution plate technique.

MATERIALS AND METHODS

Development of the selective medium. Puhalla (9) noted that *F. o.* f. sp. *apii* race 2 produced small buttonlike colonies on a sorbose complete medium, when the sugar L-sorbose was substituted for sucrose in the medium. Therefore, after a number of preliminary experiments, we chose L-sorbose as the sole source of carbohydrate in the selective medium. The basal medium developed consisted of 20 g of Bacto agar, 20 g of L-sorbose, and 2 g of DL-asparagine in 1 L of distilled water. This medium plus 600 mg of chloramphenicol sulfate was autoclaved for 20 min at 121 C. After cooling, two fungicides, sodium *p*-(dimethylamino) benzenediazo sulfonate (Dexon 5% granular) and pentachloronitrobenzene (PCNB, Terraclor 75WP) were added so that the concentration of each active ingredient was 120 mg per liter of medium. These fungicides had been effective previously in suppressing background fungi in organic (muckland) soil in New York on soil dilution plates (1). Aliquots (15 ml) of the sorbose-based medium (SBM) were dispensed into plastic petri dishes and stored at 5 C until needed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

Recovery from artificially infested soil. The effectiveness of SBM for recovery and growth of *F. o.* f. sp. *apii* race 2 and the accuracy of the enumeration procedure in organic (muckland) soil artificially infested with the fungus was determined as follows. Conidia (mostly microconidia) of isolate P-13 of the pathogen were suspended in sterile distilled water and quantified with a hemacytometer (Spencer Bright-Line, American Optical Co., Buffalo, NY). The quantified conidial suspension was used to infest 3 g (air-dried weight) of autoclaved organic soil samples in 5-cm-diameter glass petri dishes to give 5,000 and 10,000 conidia per gram of soil. Infested soil samples were thoroughly mixed and rapidly dried in an oven (28 C) for 180 min. From each petri dish, 2 g of the infested soil was shaken in 10 ml of sterile distilled water on a Vortex-Genie (Scientific Industries Inc., Bohemia, NY) for 2 min (rheostat setting 7). One milliliter of the final suspension was plated on 15 ml of solidified SBM in plastic petri dishes (four replications, five plates per relication). The plates were rotated to spread the suspension uniformly on the medium and incubated on laboratory benches for 5–6 days, after which colonies of *F. o.* f. sp. *apii* race 2 were recognized, counted, and expressed as the number of propagules per gram of air-dried soil.

Recovery from naturally infested soil. Several preliminary tests of SBM for its selective inhibition, enhancement, and differentiation of isolates of *Fusarium* from organic (muckland) soil naturally infested with *F. o.* f. sp. *apii* race 2 were conducted. Soil samples (500 g each, five from each of five sites) were obtained from the top 20 cm of a field in which celery production had been discontinued because of previous severe incidence of Fusarium yellows. (The field had been cropped to onion for 3 yr at the time the samples were taken.) Soil samples from each site were bulked in large plastic bags and mixed thoroughly by hand. Four 2-g representative samples then were taken from each of the five bulked samples and individually assayed for *F. o.* f. sp. *apii* race 2 on SBM (five plates for each representative sample). Single conidia from colonies of *Fusarium* growing on SBM were transferred to chloramphenicol-amended (600 mg per liter) potato-dextrose agar (CPDA) plates and incubated either under fluorescent white light (25–27 C) or under diffuse light on laboratory benches (average

temperature 25 C). Growth characteristics of distinct isolates of *Fusarium* from the soil dilution plates then were compared (based on macroscopic and microscopic characteristics) with known isolates of *F. o.* f. sp. *apii* race 2 grown on SBM and on CPDA. Pathogenicity of the distinct isolates of *Fusarium* also was tested. Also, any fungal colony appearing on SBM soil dilution plates not identified with certainty was transferred and grown on CPDA plates for positive identification.

The vertical distribution of *F. o.* f. sp. *apii* race 2 in the soil profile was determined as follows at two locations in another field with a moderate incidence of Fusarium yellows. Soil samples at different depths were obtained with a sampler (12) driven into the soil to a depth of about 85 cm. The intact soil core was divided into four depth units (0–20, 20–40, 40–60, and 60–80 cm). The 900 cm^3 of soil contained in each unit was thoroughly mixed. Two-gram samples from each unit then were assayed for *F. o.* f. sp. *apii* race 2 on soil dilution plates (four replications, five plates per replication) using SBM. Additionally, soil samples from each unit were placed into 7.5-cm-diameter plastic pots and bioassayed for their disease potential using the celery cultivar Florida 683. Three-week-old seedlings were transplanted into the pots (one seedling per pot) and rated for disease after 6 wk of growth at 25–28 C (16-hr photoperiod).

SBM also was compared with media incorporating different sources of carbohydrate. These were a medium incorporating dextrose (1), a potato-dextrose based medium incorporating potato-dextrose agar with the same fungicides and antibiotic as in SBM, and Komada's medium, which incorporates galactose as the carbohydrate source (7).

RESULTS

In general, four colony-growth-types of *Fusarium* were present and characterized on SBM after 5 days, when organic soils cropped to celery and naturally infested with *F. o.* f. sp. *apii* race 2 were plated. The colony characterizations were made by viewing the plates against a lighted background. In some instances only two or three colony types were present.

Colony type I exhibited morphological and microscopic characteristics atypical of *F. o.* f. sp. *apii* race 2. It was fast growing and circular in outline (Fig. 1A, a) and attained a diameter of about 10 mm in 5 days. On SBM, and especially when grown on CPDA, colony type I was characterized by abundant production of macroconidia with one to four septations. Microconidia usually were not produced. Inoculum from colony type I did not cause Fusarium yellows of celery.

Colony type II was intermediate in size, with raised centers and a circular outline (Fig. 1A, b) and ranged from 3–7 mm in diameter (usually 4–5 mm) after 5 days. The aerial mycelium of this colony type was compact and whitish in appearance. These colony characteristics generally were identical to those of known cultures of *F. o.* f. sp. *apii* race 2 (Fig. 1B). Production of abundant microconidia on both SBM and CPDA was a common feature of this colony type. Macroconidia were produced sparsely. This colony type consistently caused Fusarium yellows of celery. Based on these characteristics, colony type II was identified as that of *F. o.* f. sp. *apii* race 2.

Colony type III also was intermediate in size (usually 4–5 mm diameter after 5 days) with a circular shape and loose, floccose, whitish to creamy aerial mycelium. When viewed against light, some of these colonies appeared to possess two distinct margins as depicted in Figure 1A, c. Colony type III usually produced about equal quantities of macroconidia and microconidia on both SBM and CPDA. This colony type did not cause Fusarium yellows. Based on microscopic, macroscopic, and pathological observations, this colony type did not represent *F. o.* f. sp. *apii* race 2.

Colony type IV usually was small, although some colonies were intermediate in size. A major characteristic of this colony type on SBM was the spiderlike growth morphology (Fig. 1A, d). Most of these spiderlike colony types with wavy, irregular margins produced abundant macroconidia. Inoculum from this colony type did not cause Fusarium yellows.

Penicillium spp. were reduced to whitish pinhead colonies on SBM (Fig. 1A, e). Such colonies were so restricted in size that they did not interfere with growth of colonies of *Fusarium*. Colonies of *Aspergillus* (Fig. 1A, f), when present on SBM, also were restricted in size (generally 4–5 mm diameter after 5 days) and were characterized by circular outlines, smooth margins, and raised, grayish-green centers (indicating the presence of conidia).

Background bacteria were completely suppressed by chloramphenicol. Dexon and PCNB at the concentrations used satisfactorily suppressed background pythiaceous and mucoraceous fungi as well as other fast-growing fungi.

The efficiency of recovery of *F. o.* f. sp. *apii* race 2 from artificially infested autoclaved organic soil with SBM was 78 and 85% at 10,000 and 5,000 propagules per gram of soil (air-dried weight), respectively. Inoculum densities of *F. o.* f. sp. *apii* race 2 in organic soil from the field with a history of severe Fusarium yellows detected with SBM ranged from 1,751 to 2,469 propagules per gram of soil (air-dried weight) for five representative sites in the field. Assay of soil from four depths sampled at two locations in the celery field with a moderate incidence of Fusarium yellows indicated that the highest inoculum density of 1,125 propagules per gram of air-dried soil occurred in the top 0–20 cm soil layer (Table 1). A level of 900 propagules per gram of air-dried soil occurred in the 20–40 cm layer. The pathogen was not detected in soil samples from the 40–60 and 60–80 cm soil layers.

Celery plants grown in soil samples from 0–20 and 20–40 cm developed Fusarium yellows. None of the plants grown in soil from 40–60 and 60–80 cm developed Fusarium yellows (Table 1).

Media incorporating dextrose as the main source of carbohydrate permitted growth of colonies of *Fusarium* that were

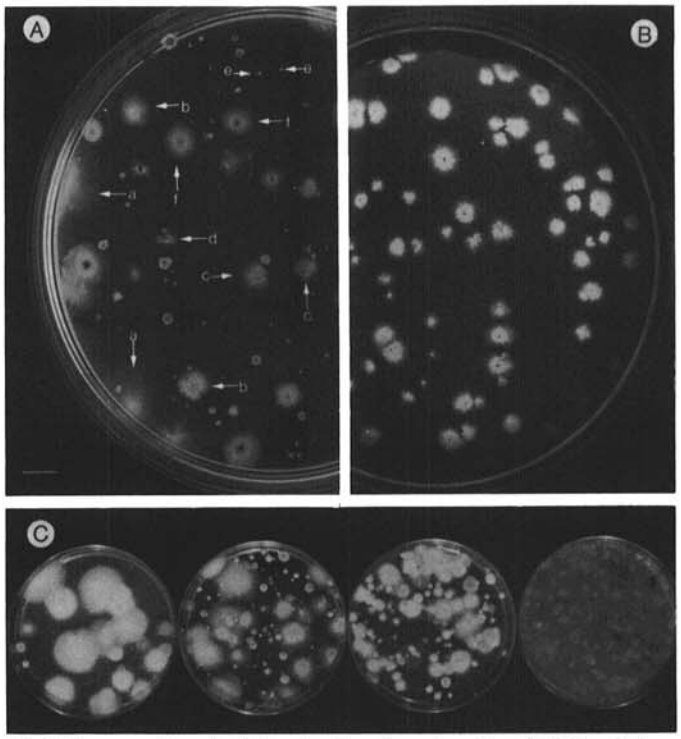

Fig. 1. Comparative selectivity of sorbose-based medium (SBM) and other media for *Fusarium* species in organic soil. The same organic soil suspension was used in both A and C. Dilution plates were 6 days old. **A,** SBM showing a large colony of *Fusarium* (a); intermediate-sized colony with circular outline and with compact mycelial morphology typical of *F. oxysporum* f. sp. *apii* race 2 (b); intermediate-sized colony of *Fusarium* with circular outline but with loose mycelial appearance (c); colony of *Fusarium* with spiderlike morphology (d); *Penicillium* species are reduced to whitish, pinhead colonies (e); and colonies of *Aspergillus* species are circular in outline with grayish-green centers (f); Bar = 5 mm. **B,** Colonies of a known culture of *F. o.* f. sp. *apii* race 2 on SBM. **C,** Growth of *Fusarium* species and other fungi on Komada's medium (left), potato-dextrose based medium (left center), Abawi and Lorbeer's medium (right center), and CPDA medium (right).

morphologically difficult to distinguish from each other on soil dilution plates (Fig. 1C). Such colonies of *Fusarium* generally were fast growing and were characterized by profuse, cottony aerial mycelium. Komada's medium, which employs galactose as the source of carbohydrate, also produced similar results. Colonies of *Penicillium* and *Aspergillus* spp. grew rapidly on dextrose-based media and sporulated profusely after 5–6 days of incubation.

DISCUSSION

Media employed in enumerating propagules of *Fusarium* spp. in soil may permit, at best, several species of the genus to grow. However, such media rely particularly on morphological characteristics of colonies of the desired species for positive identification on soil dilution plates. Attempts to quantify populations of *F. o.* f. sp. *apii* race 2 in organic soils with known Fusarium selective media (1,7) and the potato-dextrose based medium were unsuccessful because the morphology of *F. o.* f. sp. *apii* race 2 was similar to other colony types of *Fusarium*. This problem was overcome with SBM, which allowed differentiation among four main types of colonies of *Fusarium*. In the present study, the difficulty of obtaining satisfactory selective differentiation with media employing dextrose is supported by the findings of Komada (7). He reported that with one type of soil, identification of colonies of *F. oxysporum* on soil dilution plates was impossible when a modified Martin's medium (1) and a V-8 juice-dextrose-yeast-extract agar medium (8) were used. Both of these media incorporate dextrose as the source of carbohydrate. Because these media lack a satisfactory level of selective differentiation for *Fusarium* spp., use of these dextrose-based media to quantify populations of a specific forma specialis of *F. oxysporum* in natural soil could result in overestimation.

Another important feature of SBM is its complete selective inhibition of undesirable bacteria, pythiaceous fungi, and mucoraceous fungi. Colonies of *Penicillium* spp., which are fast growing and sporulate profusely on media incorporating dextrose, are restricted in radial growth to whitish pinhead colonies on SBM. Colonies of *Aspergillus* spp. also are restricted in radial growth. In general, colony growth of *Fusarium* spp. (in terms of radial growth and production of aerial mycelium) was adequately suppressed on SBM permitting some plates to be read for up to 7 days. This was not usually possible with media using dextrose.

The ability of SBM to restrict the spread of fungal colonies and thus prevent soil dilution plates from being overrun by fungi is attributed to the use of sorbose as the source of carbohydrate. Hopwood (6) indicated that sorbose, when used as a supplement in media, is able to limit radial growth of colonies of *Neurospora*, thus preventing the colonies from coalescing. Studies by Komada (7), in which the effect of 13 carbohydrate sources on fungal growth were compared on soil dilution plates, demonstrated that fewer colonies of *Penicillium*, *Aspergillus*, and *Fusarium* spp. were observed when sorbose was used as the carbohydrate source. It appears that sorbose is much less readily used by fungi than other carbohydrates, and this could explain the restriction in growth (radial growth and aerial mycelium growth) of fungal colonies on SBM. By similar reasoning, differential use of sorbose by different species of *Fusarium* or strains of *F. oxysporum* could explain the different colony morphologies of *Fusarium* observed on soil dilution plates employing SBM.

An additional advantage of SBM is that it is simple and relatively easy to prepare, as only seven ingredients are required. This contrasts with Komada's medium, which requires 12 ingredients in addition to pH adjustments with 10% phosphoric acid solution (7), or Abawi and Lorbeer's medium, which requires 11 ingredients (1).

The efficiency of recovery of SBM when used in combination with the soil dilution plate technique was 78 and 85% at pathogen population densities of 10,000 and 5,000 propagules per gram of soil, respectively. Failure to recover 100% of the population could be attributed at least in part to loss of microconidial viability when the infested soil samples were subjected to 180 min of oven drying at 28 C. Higher recovery efficiencies might have been obtained if the soil had not been oven dried and/or if chlamydospores rather than microconidia had been used to infest the soil samples.

In the celery field with the moderate incidence of Fusarium yellows, the vertical distribution of the pathogen decreased from 1,125 propagules per gram of air-dried soil in the top 0–20 cm to 0 in the deeper 40–80 cm. In the field with a history of severe Fusarium yellows, the pathogen population at five sites was evenly distributed, ranging from 1,751 at one site to 2,469 propagules per gram of air-dried soil at another site. A survey of disease incidence in this field made 3 yr earlier also suggested a uniform distribution of the pathogen (3). This field had been taken out of celery production and for 3 yr had been cropped to onion. The high pathogen population density in this field after 3 yr of continuous onion culture and the consistent disease conduciveness of the soil in several greenhouse pot experiments indicate the capacity of *F. o.* f. sp. *apii* race 2 to persist in the soil, most likely as chlamydospores in the absence of a susceptible host. Persistence of *Fusarium* spp. in soil in the presence of nonsusceptible crops is well known (2,5,10,11). A study by Schroth and Hendrix (10) attributed persistence of *F. solani* f. sp. *phaseoli* in soil to its ability to make limited vegetative growth and form chlamydospores in the rhizosphere of nonsusceptible plants. Other investigators (2,5,11) attribute persistence of *Fusarium* spp. in soil to actual penetration and parasitic growth of the fungus in roots of nonsuscepts. Elmer (4) has shown recently that roots of onion plants harbor *F. o.* f. sp. *apii* race 2. It thus appears that onion may be a symptomless host of *F. o.* f. sp. *apii* race 2 and, instead of decreasing pathogen populations, onion plants harbor the pathogen in their roots, thereby permitting long-term perennation of the pathogen in soil in the absence of celery.

TABLE 1. Population of *Fusarium oxysporum* f. sp. *apii* race 2 (FOA 2) at four depths in organic soil and the incidence of Fusarium yellows of celery in the soil samples

Sampling depth (cm)	Propagules per gram air-dried soil[a]		Fusarium yellows incidence[b] (no.)	Fusarium yellows index[c]
	FOA 2	Total *Fusarium*		
0–20	1,125	13,340	5/5	3.0
20–40	900	12,975	4/5	2.4
40–60	0	300	0/6	0.0
60–80	0	150	0/6	0.0

[a] Determined by the soil dilution plate method using sorbose-based medium. Each number is the mean of four replications (five plates per replication).
[b] Plants infected/number of plants used in the bioassay technique.
[c] On a scale of 0–5: 0 = healthy plant; 3 = leaves chlorotic or slightly blanched; 5 = dead plant.

LITERATURE CITED

1. Abawi, G. S., and Lorbeer, J. W. 1971. Populations of *Fusarium oxysporum* f. sp. *cepae* in organic soils in New York. Phytopathology 61:1042-1048.
2. Armstrong, G. M., and Armstrong, J. K. 1948. Nonsusceptible hosts as carriers of wilt Fusaria. Phytopathology 38:808-826.
3. Awuah, R. T. 1982. Occurrence of Fusarium yellows of celery in Orange County, New York and attempts to control the disease. M.S. thesis, Cornell University, Ithaca, NY. 71 pp.
4. Elmer, W. H. 1985. The ecology and control of Fusarium yellows of celery in Michigan. Ph.D. thesis, Michigan State University, East Lansing, MI. 146 pp.
5. Henrix, F. F., and Nielson, L. W. 1958. Invasion and infection of crops other than forma suscepts by *Fusarium oxysporum* f. *batatas* and other formae. Phytopathology 48:224-228.
6. Hopwood, D. A. 1970. The isolation of mutants. Pages 363-433 in: Methods in Microbiology 3A. J. R. Norris and D. W. Ribbons, eds. Academic Press, London and New York. 505 pp.
7. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. of Plant Prot. Res. 8:114-124.
8. Papavizas, G. C. 1967. Evaluation of various media and antimicrobial

agents for isolation of *Fusarium* from soil. Phytopathology 57:848-852.
9. Puhalla, J. E. 1984. A visual indicator of heterokaryosis in *Fusarium oxysporum* from celery. Can. J. Bot. 62:540-545.
10. Schroth, M. N., and Hendrix, F. F. 1962. Influence of nonsusceptible plants on the survival of *Fusarium solani* f. *phaseoli* in soil. Phytopathology 52:906-909.
11. Waite, B. H., and Dunlap, V. C. 1953. Preliminary host range studies with *Fusarium oxysporum* f. *cubense*. Plant Dis. Rep. 37:79-80.
12. Wymore, L. A. 1984. The epidemiology and control of *Sclerotinia minor* causing drop of lettuce grown on organic soils in New York. Ph.D. thesis, Cornell University, Ithaca, NY. 305 pp.