Nylon Mesh Discs Useful in the Transfer of Fungi and the Evaluation of Soil Fungitoxicants

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Evaluation of soil fungitoxicants often involves determining the survival of test fungi on agar plugs after exposure to toxicants in nutrient agar or in soil (8). Certain limitations of this technique have been noted (1). Toxicants can accumulate in the agar plugs and render the method ineffective for differentiating fungistatic and fungicidal properties. The toxicants may diffuse so poorly in the agar that fungal cells within the media are not killed. In soil, the plugs often become contaminated with other microorganisms, which may then obscure growth of the test fungi. Introduction of a fungus growing on a nutrient medium into the soil may also upset the microbiological equilibrium (2). A higher concentration of toxicant is required to kill a test fungus on a larger than on a smaller plug (8). Furthermore, a fungus growing on a medium that supports luxuriant growth may be more resistant to toxicants when transferred than one cultured on a medium less favorable for growth.

Various kinds of glass and nylon fibers have been employed as matrices in attempts to avoid the use of nutrient agar plugs. Legge (4) allowed *Phytophthora* spp. to grow from oatmeal agar onto glass fiber tape, which was then buried in garden soil to determine the fate of the reproductive structures. Waid & Woodman (7) used nylon screen to study hyphal growth and activity of various fungi in field soil. Old & Nicolson (5) grew *Rhizoctonia solani* Kühn on squares of monofilament nylon screen on potato-dextrose agar (PDA) and transferred the squares to soil to study lysis by *Streptomyces* spp. Schreiber (6) cultured *Fusarium* spp. on "limp, fine-mesh" nylon placed on a cellophane disc which was in contact with an agar medium in a petri dish. The mycelium-covered nylon was then applied to leaf surfaces to determine pathogenicity of the isolates.

The purpose of the present investigation was to determine whether certain plant-pathogenic soil fungi could be transferred on nylon discs from stock cultures or from soil to fresh media with a minimum concurrent transfer of nutrients, toxicants, or microbial contaminants.

Discs 6 mm diam were cut with a Gem paper punch from stiff, monofilament nylon screen cloth (No. 263 or 275 Nitex from Tobler, Ernst & Traber, 71 Murray St., New York, N. Y. 10007). Other similar-appearing grades of monofilament nylon screen from this source, such as ASTM 60-250, are not satisfactory because of excessive curling during autoclaving. After being autoclaved for 15 min at 15 psi, several discs were placed in a circle around a potato-dextrose agar (PDA-Difco) plug bearing the test fungus in the center of a petri dish containing PDA. Transfer of the discs with sterile forceps was made as soon as the fungus had grown 15 to 20 mm beyond the farthest edge of the discs. PDA-control plugs with mycelia were removed with a No. 2 cork borer along the arc of the circle made by the nylon discs and placed on the fresh media with the mycelium side down.

The following fungi grew over the nylon screen on PDA and continued to grow when the discs were transferred to fresh media: *Diplodia zeae* (Schw.) Lev., *Fusarium oxysporum* Schlecht. f. sp. *pisi* (Linford) Snyd. & Hans., *Gibberella zeae* (Schw.) Petch, *Helminthosporium sorokinianum* Sacc. ex Sorok., *Pythium debaryanum* Hesse, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* (Lib.) dBy.

Transfer of mycelia of most of the test fungi on nylon discs did not result in carry-over of enough nutrients from the original culture media to support growth equivalent to that from PDA. Washing of the discs did not appear necessary to remove nutrients. Nylon discs bearing mycelia of *P. debaryanum* were washed in sterile, deionized water with three consecutive changes of 2 hr each and placed on water agar in petri dishes with unwashed discs as controls. Growth from washed and unwashed discs were equally sparse over a 5-day period. Nylon discs and PDA plugs with mycelia of *P. debaryanum*, *R. solani*, *G. zeae*, *F. oxysporum*, *S. sclerotiorum*, and *D. zeae* were similarly washed and placed on fresh PDA in petri dishes with unwashed, mycelia-bearing discs and plugs as controls. Radial growth of all fungi from the nylon discs, with the exception of *G. zeae* and *R. solani*, was from 15 to 35% less than that from the PDA plugs (Fig. 1). Growth of *G. zeae* from the nylon discs was approximately 35% greater than from the PDA plugs, while that of *R. solani* was essentially the same from each matrix. There was no consistent difference between washed and unwashed discs or plugs.

There was no appreciable concomitant transfer of toxicants when nylon mycelia discs were removed from fungitoxicant-impregnated PDA and transferred to fresh media. Nylon discs and PDA plugs with mycelia of *D. zeae* were placed on PDA in petri dishes in which 25 ppm of Arasan 75—75% thiram—[bis(dimethylthiocarbamoyl) disulfide] had been incorporated. After 72 hr, *D. zeae* had not made appreciable growth on either discs or plugs. Upon being transferred to fresh PDA without washing, the fungus began to grow almost immediately from the nylon discs, but initiation of growth from the agar plugs was delayed for 48 hr (Fig. 1).

Toxicants are able to contact certain fungi and inhibit their growth on nylon discs more readily than on PDA plugs. *F. oxysporum* was exposed as in the preceding experiment to 100 ppm of captan 80—80% captan—[*N*-(trichloromethylthio)-4-cyclohexene-2,2-dicarboximide]. At the end of 60 hr on the captan-impregnated PDA, slight growth was noted only from the agar plugs. The

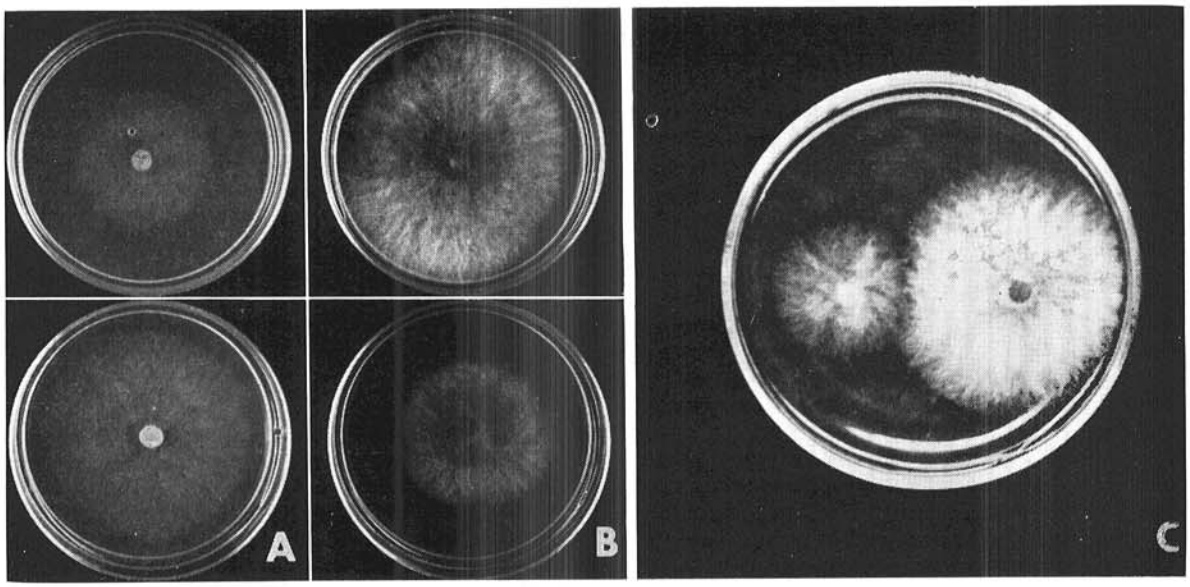

Fig. 1. A) Growth of *Sclerotinia sclerotiorum* and **B)** *Gibberella zeae* on potato-dextrose agar (PDA) after transfer on nylon discs (upper) and potato-dextrose plugs (lower). **C)** Growth of *Diplodia zeae* from PDA plug (left) and nylon disc (right) after transfer from Arasan-impregnated PDA to fresh media.

discs and plugs were transferred at this time to fresh PDA. Mycelia continued to grow from the plugs, but no growth was observed from the nylon discs.

Nylon discs were a satisfactory matrix for placing test fungi in a soil column and for recovering them after exposure to a toxicant without excessive contamination with other organisms. Nylon discs and PDA plugs bearing mycelia of *P. debaryanum* and *R. solani* were placed in screened, silt-loam soil in segmented Lucite tubes at depths of 7.5 and 12.5 cm, using techniques previously described (3). Five discs and five plugs with *P. debaryanum* were placed at each of the depths in one tube, and the same number of discs and plugs with *R. solani* were set at the two positions in a second tube. Terraclor (25% pentachloronitrobenzene emulsion) at 500 ppm based on soil wt was poured on top of the soil, which was at 27% moisture content or field capacity. The columns were covered and left intact for 96 hr, after which the discs and plugs were removed, washed in three changes of sterile deionized water, as in the earlier experiments, and placed on PDA in petri dishes. After 72 hr, both fungi had grown from discs and plugs extracted at each depth. Radial growth from the discs was approximately three times that from the plugs. Terraclor was apparently nontoxic to these fungi at the concentration employed. Eighteen of the 20 plugs were contaminated with unidentified species of bacteria and fungi, whereas only one of the 20 nylon discs was thus contaminated.

The results indicate that nylon discs are superior to nutrient agar plugs as matrices for fungi in the evaluation of soil toxicants, since they are less likely to become contaminated with soil microflora and to absorb chemicals differentially. Nylon discs should also provide a convenient base for the quantitative assay of a wide variety of fungi in culture and in soil. However, since there is evidence that nylon may stimulate growth of certain fungi, as with *Gibberella zeae,* the cloth should be tested for this effect before it is used as a transfer matrix.

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