A Simple Device for Sampling Spores to Monitor Fungicide Resistance in the Field

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ABSTRACT

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A simple device consisting of a standard-sized lipbalm tube filled with a solidified water agar core has been developed for use in field sampling of fungal spores from lesions. Spores are removed by pressing the agar surface of the core against a lesion. The top of the agar cores are sliced off, forming agar disks that are incubated in petri plates and then observed microscopically for spore germination. Incorporation of a fungicide into the agar allows for determination of resistance. The device has been used successfully for field sampling in epidemiological research as well as for monitoring fungal populations in commercial settings. Other potential uses for the device involve determination of spore production of individual lesions sampled in the field and monitoring fungicide residues on plant material.

A variety of methods have been used to monitor populations of fungicide-resistant foliar pathogens. In a bioassay for *Sphaerotheca pannosa* developed by Schroeder and Provvidenti (5), spores were dusted on benomyl-treated and untreated detached leaves of cucumber maintained in Hoagland's solution. MacKenzie et al (4) collected and stored as dry tissue large numbers of lesions of *Cochliobolus carbonum* on corn. The tissue was surface sterilized, placed on agar amended with cadmium chloride, and later examined for fungal growth. Littrell (3) determined the resistance of *Cercospora arachidicola* to benomyl by collecting diseased peanut leaves, removing conidia from the lesions with a glass rod, and then placing them on amended agar for later evaluation. A common method used in several studies (1,2,6) involved excision of sporulating lesions from leaf tissue, often by using a cork borer, followed by the sequential streaking of the lesions across amended and unamended agar to dislodge spores.

In epidemiological studies of the dynamics of fungal populations, it is crucial that the early stages of the epidemic be monitored. At such low disease levels, a relatively large sample would be necessary to detect a low frequency of the resistant or sensitive pathogen population. Furthermore, the sampling procedure should be nondestructive, since removal of a portion of the population at this time could significantly alter the course of the epidemic as well as the composition of the fungal population. These alterations could be particularly important for experiments involving small microplots. Thus, there is a need to develop a technique that is quick, nondestructive, and amenable to large sample sizes.

This paper outlines the use of a simple device, originally conceived by the second author, for large-scale field sampling of fungal populations.

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MATERIALS AND METHODS

Description of the sampling device. The sampling device consists of a standard size 1.27-cm (0.5 in.) diameter lipbalm tube containing a core of solidified water agar. The tubes, available from Penn Bottle & Supply Co., Philadelphia, PA, and other vendors, are composed of polystyrene plastic and measure 62 mm long by 15 mm in diameter. Each tube or vial consists of a hollow body, a dispensing knob with attached threaded shaft, an internally threaded cup, and a cap (Fig. 1).

The samplers are prepared in the following manner for use in the field. After empty tubes are cooled in a freezer, ~5.5 ml of a nonsterile melted water agar preparation (15 g agar in 1 L of water) is dispensed into each of the tubes via an automatic pipette. The cooling process allows for a rapid solidification of the media, which prevents leakage through the unsealed bottoms. While the agar is solidifying, the caps are placed on the vials to produce a moist agar surface through condensation. Water agar amended with a fungicide can be added to empty tubes in a similar manner. Capped tubes containing agar have been stored in closed containers lined with moist cheesecloth for up to 5 mo at 15 C without noticeable agar dehydration. Longer storage periods, especially at lower temperatures, are considered possible.

Description of technique. To remove spores from an individual lesion in the field, the cap of a sampler is removed and the agar core is extruded ~1–2 mm by turning the dispensing knob at the vial base. The exposed moist agar surface is then gently pressed against the lesion to transfer spores from it to the agar (Fig. 2). Without drawing the agar core back into the tube, the vial is capped and placed in a cool location until it is returned to the laboratory. The next lesion is chosen and the process is repeated with another sampler. Using this procedure, the experimenter establishes a one-to-one correspondence between the number of lesions and the number of vials employed.

Since spore removal with this device is incomplete, repeated sampling from the same lesion is possible. When both fungicide-amended and unamended agar are used for a single lesion, the latter should be used first. The samplers must be labeled appropriately for later identification and study. The degree of efficiency of the second and any subsequent samplings from the same lesion will be dependent on the number of spores available for removal; repeated sampling from very small lesions or those exhibiting minimal sporulation may not be feasible.

Upon completion of field sampling, the vials are returned to the laboratory for processing. The extended portion of each agar core with its accompanying sample of spores is sliced off flush with the top of the vial. A clean scalpel with an uncorrugated edge should be employed so that the new surface is smooth, allowing for easier microscopic viewing of spores collected in subsequent samples. The agar disks are then placed in the bottom of a petri plate which is inverted over a petri plate cover containing moist filter paper (Fig. 3). After incubation for a specified time and temperature, the spores on each disk are observed for germination or germ tube growth via an incident light microscope. Since the disks are not sterile, observations can be made with the petri plate lid removed. Approximately 20 agar disks can be obtained from the agar core of each vial. The slicing of very thin agar disks increases the number of lesions that can be sampled with each vial and provides for better lighting conditions during microscopic examination.

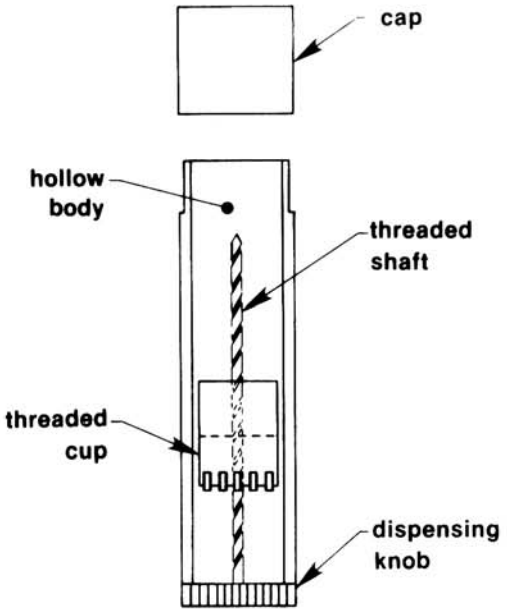

Fig. 1. Schematic drawing of lipbalm tube portion of the spore sampler. Rotation of dispensing knob moves the threaded cup vertically along the shaft and forces an added agar core out of the hollow body. The removable cap protects the agar surface and prevents short-term desiccation of medium.

RESULTS AND DISCUSSION

The sampling device has been used successfully for epidemiological studies in the field involving benomyl-resistant and -sensitive strains of *Venturia inaequalis*. Furthermore, the device has been extremely useful for monitoring populations of *V. inaequalis* in commercial orchards. The technique has also been employed successfully for removing spores of *Botrytis cinerea* from lesions on grape clusters.

Although initially intended for monitoring fungicide resistance in populations of pathogenic fungi, the device may prove useful for other purposes. For example, a survey of 40 lesions of *V. inaequalis* from a commercial Pennsylvania orchard indicated that 22 of the lesions had healthy germinating spores, in spite of previous applications of the fungicide captan. Since the moist agar surface readily removes fungicide residues along with spores, this result may have been an indication of poor fungicide distribution or that residue levels were beginning to drop below the threshold necessary for inhibition of spore germination. The device could also be used in field tests for measuring the effect of weather or fungicides on sporulation. However, because removal of spores is incomplete, only relative and not absolute spore counts could be compared. Other potential uses of the sampler could involve removal of pollen from anthers or sampling bacterial populations by employing specialized media.

The major benefits of the sampler are its utility in the field and the relatively large number of samples that can be obtained with minimal use of media and equipment. A simple experiment was performed with apple scab to compare this technique with another method involving the collection of diseased leaves, the excision of lesions with a scalpel and forceps, and the streaking of these lesions on agar to dislodge spores (7). Results from two observers, neither of which had previous experience with either technique, revealed

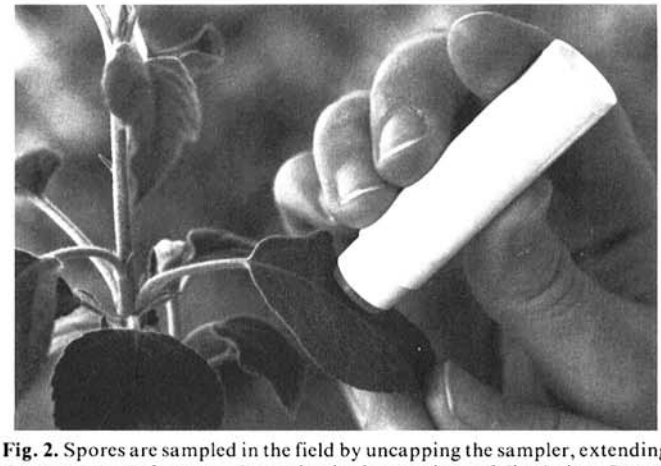

Fig. 2. Spores are sampled in the field by uncapping the sampler, extending the agar core 1–2 mm, and pressing its face against a foliar lesion. Spores adhere to the exposed, moist agar surface. The sampler is then capped for transport.

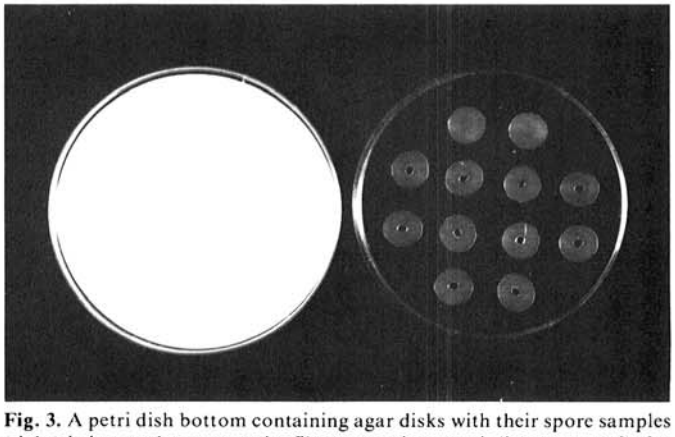

Fig. 3. A petri dish bottom containing agar disks with their spore samples (right) is inverted over a moist filter paper in a petri dish cover (left) for incubation. The first few disks cut from a sampler will be solid while the remainder will have a central hole formed by the threaded shaft.

that the agar disk method took twice as long to perform in the field but only one-third the time needed for processing in the lab; the time necessary for microscopic examination was nearly identical.

The limitations of the sampler and technique are related to the nonsterile nature of the substrate sampled in the field. Use of media capable of sustaining fungal growth such as potato-dextrose agar results in growth by contaminant fungi. Thus, this method cannot be used if the criteria for determination of fungicide resistance necessitates in vitro growth of the fungus on fungicide-amended agar. However, fungicide resistance in many fungal pathogens can often be determined by examination of spore germination or growth of germ tubes. In these cases, the use of water agar reduces contaminant growth, thus allowing for greater flexibility with respect to the length of incubation and the time of observation.

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