Cryogenic Storage of Conidia of Sclerospora sorghi

Anne W. Gale, C. G. Schmitt, and K. R. Bromfield

Biological Laboratory Technician and Research Plant Pathologists, respectively; Plant Disease Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 1209, Frederick, MD 21701.

ABSTRACT

Conidia of *Sclerospora sorghi* were suspended in 10 or 15% dimethyl sulfoxide (DMSO) and cooled to −30 C at 1 C per minute in a Linde BF-4-1 Biological Freezer. After storage for 7 days in the cold gas phase of liquid nitrogen, suspensions were quickly thawed, and the DMSO was washed out. Conidia sprayed on seedlings of *Sorghum bicolor* in dew consistently produced infections in a high percentage of plants.

Phytopathology 65:828-829

Additional key words: sorghum downy mildew of maize.

Preservation of the conidia of *Sclerospora sorghi* Weston & Uppal, the fungus inducing sorghum downy mildew of maize, has often been attempted, but without published success. We report a way to store conidial suspensions in the cold gas phase of liquid nitrogen and to recover conidia in a viable conditon.

Conidia were washed with cold distilled water from leaves of *Sorghum bicolor* (L.) Moench 'TX 412', as described by Schmitt and Freytag (7). They were resuspended in 10 or 15% (v/v) dimethyl sulfoxide (DMSO) after concentration on membrane filters by the method of Bromfield and Schmitt (1). Conidial concentrations ranged from 1.69×10^5 to 5.88×10^5/ml.

One milliliter of suspension was pipetted into each of six to eight polypropylene cryogenic storage vials (A/S Nunc serum and biological test tubes with a 2-ml capacity). Sealed vials were arranged in the freezing chamber of a Linde BF-4-1 Biological Freezing System (Linde Company, Tonawanda, New York). An unsealed vial of conidial suspension held one junction of a differential thermocouple. The BF-4 froze biological specimens at precisely controlled rates by regulating the differential temperature between a specimen sample and the cooling medium (liquid nitrogen). A similar Linde system has been more fully described by Cowley and Rinfret (2). A Honeywell ElectroniK 111 single pen strip

chart recorder (Honeywell, Inc., Fort Washington, Pennsylvania) monitored chamber temperatures.

We froze conidia by reducing temperature at 1 C per minute. To predetermine the required settings on the BF-4 controller, we made calibration runs with the thermocouple needle inserted in a polypropylene vial of either freshly harvested conidia or thawed conidia that had been cryogenically stored by other cooling regimes. A range setting of 0.2 milliamperes and a rate setting of 5 (on a scale of 1-60) usually cooled conidia in either 10 or 15% DMSO at 1 C per minute, though the most dense suspension required a rate setting of 6.

The onset of freezing was indicated on the strip chart by a short upward spike. After we determined the freezing temperature in the calibration run, we spanned that temperature in the runs for storage by using the BF-4's override feature to add a steady flow of liquid nitrogen. Increasing the cooling rate in the critical period when freezing began reduced the spread of temperatures among the vials and minimized supercooling and random freezing. Overrides of 20-25 seconds, starting 2-3 C above the freezing temperature and ending 2-3 C below it, were effective and permitted a return to the freezing temperature.

After 30-40 minutes in a freezing "plateau" with slow downward drift, the 1 C per minute cooling resumed. The rate setting was reduced to 4 and then to 3 during the last few minutes of both calibration and storage runs, to counteract the expected increase in cooling rate after the samples became completely frozen. When the vials reached −30 C, we quickly transferred them to the cold gas phase (−160 to −180 C) of a liquid nitrogen refrigerator.

When we removed vials of conidia after 7 days of storage, we plunged them immediately into a 40 C water bath and agitated them, until all ice was melted (90-100 seconds). We quickly washed out the DMSO and resuspended the conidia in cold water (2-3 C).

The conidial suspension (7 ml) was sprayed from an atomizer onto TX 412 sorghum plants in the two- to three-leaf stage, and the plants were exposed to dew overnight. In two replicate experiments, each involving 28 plants, 24 and 28 plants, respectively, inoculated with conidia that had been frozen in 10% DMSO developed local lesions within 3-6 days. Later, 6 and 24 of those plants, respectively, became systemically infected. Conidia frozen in 15% DMSO produced local lesions in all plants and systemic infections in 23 of 28 plants. Variation in inoculum concentration, from 1.80 to 2.67×10^5 conidia/ml, probably affected the relative numbers of plants which became systemically infected.

The two-step freeze described by Bromfield and Schmitt (1) was ineffective with conidia of *Sclerospora sorghi* in a variety of cryo-protective media. Conidia also did not survive a 1 C per minute freeze in water alone, nor a process involving slow-freeze slow-thaw in 10% DMSO. However, conidia in 15% DMSO from a calibration run, without the accelerated cooling possible with "override," did survive freezing and thawing. They produced systemic infections in 14 of 28 plants, from an inoculum (7 ml) of 1.69×10^5 conidia per ml. Conidia frozen in 10% glycerol, stored 7 days, and resuspended in water after a quick thaw survived poorly; only 5 of the 35 inoculated sorghum plants developed local lesions, and none was systemically infected. The greater protection evidently afforded by DMSO correlates with the findings of Hwang (4) and Hwang and Howells (5) who froze agar-culture plugs of 104 strains of mycelial fungi (mostly Basidiomycetes) in 10% glycerol at 1 C per minute to −35 C before storage at −150 to −196 C. They reported that 10% DMSO permitted the survival (though with decreased radiating growth) of the eight strains that had not survived freezing and thawing in the glycerol medium.

Cryogenic storage of plant pathogenic fungi has long been helpful, and is especially important for those pathogens whose conidia normally lose viability within a few hours at normal room temperatures and normal atmospheric conditions. Though our storage times to date have been fairly short, survival after freezing, equilibration to cryogenic temperatures, and thawing indicates that viability of *S. sorghi* conidia can be expected to be maintained for prolonged periods. This expectation is consistent with the findings of others with a variety of microorganisms (1, 3, 4, 5, 6).

LITERATURE CITED

1. BROMFIELD, K. R., and C. G. SCHMITT. 1967. Cryogenic storage of conidia of Peronospora tabacina. Phytopathology 57:1133.
2. COWLEY, C. W., and A. P. RINFRET. 1963. Technological solutions to problems in preservation at cryogenic temperatures. Pages 180-183 *in* S. M. Martin, ed. Culture collections: perspectives and problems. University of Toronto Press, Toronto, Canada. 221 p.
3. HWANG, S. 1966. Long term preservation of fungus cultures with liquid nitrogen refrigeration. Appl. Microbiol. 14:784-788.
4. HWANG, S. 1968. Investigation of ultra-low temperature for fungal cultures. 1. An evaluation of liquid-nitrogen storage for preservation of selected fungal cultures. Mycologia 60:613-621.
5. HWANG, S., and A. HOWELLS. 1968. Investigation of ultra-low temperature for fungal cultures. 2. Cryoprotection afforded by glycerol and dimethyl sulfoxide to 8 selected fungal cultures. Mycologia 60:622-626.
6. RINFRET, A. P. 1963. Cryobiology. Advan. Cryogenic Eng. 8:11-16.
7. SCHMITT, C. G., and R. E. FREYTAG. 1974. A quantitative technique for inoculating corn and sorghum with conidia of Sclerospora sorghi. Plant Dis. Rep. 58:825-829.