Techniques

Five-Year Preservation of *Fusarium* Species on Silica Gel and Soil

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ABSTRACT

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Cultures of 17 *Fusarium* spp. (461 isolates) that were prepared by the single-spore and hyphal-tip method were stored on both silica gel and sterile soil for 5 yr at 4.5 ± 0.5 C. Species tested included: *F. acuminatum*, *F. avenaceum*, *F. culmorum*, *F. dimerum*, *F. equiseti*, *F. graminearum*, *F. lateritium*, *F. merismoides*, *F. moniliforme*, *F. oxysporum*, *F. poae*, *F. proliferatum*, *F. sambucinum*, *F. semitectum*, *F. solani*, *F. sporotrichioides*, and *F. subglutinans*. Survival on silica gel after 3, 4, and 5 yr averaged 94, 90, and 89%, respectively, compared with survival on sterile soil, which averaged 95, 93, and 94%, respectively. Storage on silica gel is quick and easy; fungi do not grow in storage; and repeated isolations can be made from a single tube. Dense (turbid) suspensions of conidia are needed initially to ensure long-term preservation on silica gel.

Several methods have been described for long-term preservation of fungi (9). For *Fusarium* species, lyophilization (3) or preservation on soil (1,12) is commonly used. However, storage on soil is not always reliable and mutations can occur as the fungus colonizes the sterile soil before and during storage (1). Maintenance of "wild-type" isolates (with cultural and morphological characters of the culture originally isolated) is important because mutations can result in a loss of pathogenicity (12) and other characteristics. Lyophilization of *Fusarium* spp. growing on carnation leaf pieces in sterile skim milk ensures long-term viability with minimal cultural variation (3). However, the equipment is expensive and unavailable to some researchers; also, once a lyophilized culture is opened, the seal is broken and the vial cannot be returned to storage for reuse.

In 1962, Perkins (10) found that *Neurospora* conidia could be stored successfully on anhydrous silica gel. The technique was quick, easy, and maintained viability in an unchanged state. Subsequent studies showed that several genera of fungi (13), bacteria (13), plant-pathogenic bacteria (7,11), and blue-green algae (4) also could be preserved on silica gel. The technique has been described for use by plant pathologists (14), but its suitability for storing *Fusarium* spp. is unknown, except for a brief report (15).

The purpose of this study was to evaluate the effectiveness of silica gel compared with sterile soil for storing *Fusarium* spp. over a 5-yr period.

MATERIALS AND METHODS

Preparation of cultures. Cultures that were purified by the single-spore or hyphal-tip method (8) were grown on Difco potato-dextrose agar (PDA) or on carnation leaf agar (CLA) under fluorescent light (four General Electric or Sylvania 40W tubes) supplemented with black light (one Sylvania 40W tube, BLB series) for a 12-hr photoperiod (5,300 lx). Of 461 isolates prepared for storage on both silica gel and soil, 17 species were represented following the system of Nelson et al (8): *F. acuminatum* Ell. & Ev. sensu Gordon, *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Smith) Sacc., *F. dimerum* Penzig, *F. equiseti* (Corda) Sacc. sensu Gordon, *F. graminearum* Schwabe, *F. lateritium* Nees, *F. merismoides* Corda, *F. moniliforme* Sheldon, *F. oxysporum* Schlecht. emend. Snyd. & Hans., *F. poae* (Peck) Wollenw., *F. proliferatum* (Matsushima) Nirenberg, *F. sambucinum* Fuckel, *F. semitectum* Berk. & Rav., *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., *F. sporotrichioides* Sherb., and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas.

For storage on silica gel, suspensions of conidia were made from actively sporulating wild-type cultures on PDA by scraping the colony surface and placing conidia into screw cap culture tubes (100 × 13 mm) containing 2 ml of sterile Difco skim milk (autoclaved for 15 min at 121 C). When cultures were grown on CLA, two or three leaves on which *Fusarium* was sporulating were transferred to tubes of skim milk. For storage in soil, suspensions of conidia were made in sterile water following the same procedure (water blanks became turbid when spores were added). Tubes were placed on a vortex mixer to produce a uniform suspension of conidia and then placed in an ice bath to inhibit germination of conidia.

Because of low recovery of *F. graminearum* from silica gel after 3 yr of storage, preservation of this species was repeated on 30 November 1984 with 30 hyphal-tipped cultures. All cultures were checked microscopically for abundance of conidial production on CLA, and only carnation leaves with abundant sporulation were

transferred to skim milk and sterile water (three to six leaf pieces per tube) for preparation of suspensions of conidia.

Silica gel storage. Storage of *Fusarium* species on silica gel followed general procedures outlined by Tuite (14) and E. S. Luttrell (University of Georgia, *personal communication*), with some modifications. Screw cap culture tubes (100 × 13 mm) were filled with 3 cm^3 silica gel (specification Mil-D-3716 or Davison Chemical Corporation, commercial grade H and 05, mesh 6-16, Code 05-08-08-237). Silica gel (nonindicating, no dye) was dry-heat sterilized at 180 C for 1.5 hr and stored at 5 C until used. Tubes were placed in an ice bath at least 30 min before use.

For transfer of a suspension of conidia to silica gel, a precooled tube was held horizontally to distribute particles along the side of the tube. About 0.3 ml of a suspension of conidia was evenly distributed over the silica gel with a sterile Pasteur pipet. Enough suspension was added to moisten the particles (if too much moisture is added, the silica gel fuses). The culture tube was placed immediately on a vortex mixer to enhance distribution of conidia on silica gel and then placed in an ice bath until the tube cooled (some heat is released when moisture contacts silica gel). Before storage, each isolate was checked for viability by shaking a few particles onto PDA. Finally, a single layer of Parafilm was stretched around the screw cap and the culture was stored at 4.5 ± 0.5 C in custom-made stainless steel test tube holders, covered with stainless steel covers.

Soil storage. About 3 cm^3 of a soil, peat moss, and sand mixture (2:1:1, v/v/v) was added per culture tube (100 × 13 mm), moistened, and autoclaved at 121 C for 1 hr on each of two consecutive days. About 0.3 ml of a suspension of conidia was added to the soil surface. Tubes were held at room temperature for 3 or 4 days, tested for viability by sprinkling a few particles onto PDA, and then stored as described previously.

Viability tests. Approximately 3, 4, and 5 yr after storage, each isolate stored on both silica gel and soil was tested for viability by sprinkling a few particles onto PDA. If the first attempt after 5 yr of storage was unsuccessful, additional particles were tested until a viable colony appeared or until the stored material was depleted. Viability of *F. graminearum* was checked at 5–8-mo intervals for isolates stored on 30 November 1984 through January 1987.

RESULTS

Viability tests made 1–5 days after adding conidia to silica gel and soil (inoculation) and before storage at 4.5 C resulted in nearly 100% of the silica gel and soil particles yielding colonies. For the first 1 or 2 days after silica gel particles were on PDA, growth of *Fusarium* sometimes was delayed compared with growth from soil. But colony appearance and spore morphology of all species stored on silica gel were normal, regardless of the duration of storage. As storage time increased, the percentage of silica gel or soil particles producing colonies declined.

Survival of the 461 isolates of *Fusarium* spp. on silica gel after 3, 4, and 5 yr averaged 94, 90, and 89%, respectively, compared with survival on soil, which averaged 95, 93, and 94%, respectively. Because results changed very little from year to year for each *Fusarium* sp. and substrate, the results of storing the 17 *Fusarium* spp. on silica gel and soil after 5 yr are given in Table 1.

Although the overall recoveries were similar for silica gel and soil, 100% viability of all isolates of a given species occurred more frequently when stored on soil than on silica gel. Survival of *F. oxysporum*, *F. solani*, and *F. sporotrichioides* was greater on silica gel than soil. Survival of *F. acuminatum*, *F. avenaceum*, *F. culmorum*, *F. dimerum*, *F. equiseti*, *F. graminearum*, *F. lateritium*, *F. sambucinum*, and *F. semitectum* was greater on soil than on silica gel; however, the number of cultures stored of four of these species was low (range: 2–8). *F. avenaceum* and *F. lateritium* did not survive as well as other species on either silica gel or soil.

Viability of cultures of *F. graminearum* declined considerably after storage for 3 yr on silica gel (6% recovery), so the species was retested on silica gel and soil. Precautions were made to ensure that *F. graminearum* was sporulating abundantly before suspensions of conidia were prepared. Although not shown in Table 1, viability of 30 isolates of *F. graminearum* tested at 8, 13, 19, and 25 mo after storage were 100, 100, 97, and 97%, respectively, for silica gel and 100% for soil for all storage durations.

DISCUSSION

Most isolates of *Fusarium* spp. tested in this study were satisfactorily preserved on silica gel for 5 yr. The amount of inoculum of the fungus on silica gel may affect the ability to detect viable propagules of the fungus. Initial tests with *F. graminearum* resulted in low survival after 3 yr of storage on silica gel, but when the test was repeated using cultures that were microscopically examined for abundant sporulation before preparing a suspension of conidia, viability of the 30 *F. graminearum* cultures stored for 25 mo was 97%. *F. graminearum* does not sporulate prolifically, so for this species and others that produce a limited number of conidia on agar (e.g., *F. acuminatum* and *F. avenaceum*), carnation leaves or another natural substrate (3) should be used (to increase concentrations of conidia) and examined microscopically to ensure that the fungus is sporulating abundantly before suspensions of conidia are prepared for long-term storage. A high conidial density of *Neurospora sitophila* and *N. crassa* also enhanced survival on silica gel (2).

Long-term storage of *Fusarium* spp. on soil was more successful than reported (1), and may be because the present study was done at 4.5 C, rather than at room temperature (about 24 C). Similarly, survival of bacteria and fungi on silica gel was enhanced two- to threefold when cultures were stored at 4 C compared with room temperature (13). Loss of viability of *Fusarium* spp. stored on soil or silica gel does not appear to be related to the ability to form chlamydospores. *F. moniliforme* does not form chlamydospores, yet all isolates survived on both substrates.

Reduction of metabolic activity (hypobiosis) is induced by low temperature or water loss (11). In principle, a portion of the lyophilization process (quick drying of colonized carnation leaves in skim milk) in preparing *Fusarium* spp. for storage is similar to the storage process on silica gel. Rapid water loss occurs when conidia suspended in milk are transferred to a small quantity of silica gel particles with a large surface area, which rapidly absorbs the water of the suspension to leave conidia and milk solids adsorbed to the particle surfaces. While skim milk is a protective substance, the conidia are relatively exposed compared with lyophilized carnation leaves (which contain conidia and mycelium); this may account for some loss in viability on silica gel as the length of storage time increases. The atmosphere in test tubes

TABLE 1. Percent survival of 461 cultures of 17 *Fusarium* spp. stored on soil or silica gel at 4.5 ± 0.5 C after 5 yr

Fusarium spp.	Cultures (no.)	Survival (%)[a]	
		Soil	Silica gel
F. acuminatum	58	100	97
F. avenaceum	8	75	63
F. culmorum	11	100	91
F. dimerum	4	100	75
F. equiseti	27	100	96
F. graminearum	18	100	28
F. lateritium	16	94	69
F. merismoides	10	100	100
F. moniliforme	11	100	100
F. oxysporum	154	91	99
F. poae	6	100	100
F. proliferatum	15	100	100
F. sambucinum	16	100	88
F. semitectum	2	100	50
F. solani	63	83	100
F. sporotrichioides	29	97	100
F. subglutinans	13	100	100

[a] Viability was determined on potato-dextrose agar for one tube of each isolate stored on silica gel and soil.

sealed with plastic wrap is higher in CO_2 and lower in O_2 compared with the atmosphere in tubes not sealed with plastic wrap (5), and this factor also may account for decreasing survival. For isolates where viability is decreasing (as evidenced by the lower percentage of particles producing a colony compared with previous determinations), preparation of fresh wild-type cultures for reintroduction onto silica gel would help assure continued survival. Lower storage temperatures may also be beneficial in prolonging viability (10).

Characteristics of microorganisms stored on silica gel are reported to be retained. Sleesman and Leben (11) found that 13 species of phytopathogenic bacteria survived and were pathogenic after storage on silica gel at −20 C for 20 mo and at 5 C for 5 mo. *F. proliferatum* and *F. moniliforme* stored on silica gel for 5–6 mo caused stalk rot on corn (6). C. J. Mirocha (University of Minnesota, *personal communication*) stores some mycotoxin-producing strains of *F. moniliforme* on silica gel without loss of toxin-producing capabilities. On the other hand, storage of *Fusarium* spp. on soil results in vegetative growth until the soil dries and the fungus becomes dormant. This interval may allow mutation for saprophytic strains that can overgrow a pathogenic strain or for mutant vegetative strains to overgrow the wild type (1). Mutations also can occur before conidia are added to the storage substrate, but selection of wild-type cultures reduces this possibility.

For storage on silica gel, the silica gel particles replace the soil storage method and the suspension of conidia is prepared as for lyophilization. In contrast to storage on soil, *Fusarium* cultures added to silica gel do not colonize the substrate, and thus, reduce the chance of mutation. The procedure is simple, and repeated isolations can be made from a single tube. It is suggested that heavy suspensions of conidia be prepared for inoculation onto silica gel, and that culture viability be checked at regular intervals to determine if fresh cultures should be prepared for storage. This method is well-suited for situations where materials and equipment are limited.

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