Method for Maintaining Three Selected Fungi

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ABSTRACT

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Glomerella cingulata from camellia, *Monilinia fructicola* from peach, and *Pestalotia guepini* survived freezing (−8 C) for 1 yr on filter paper, and *G. cingulata* and *P. guepini* survived for 4 yr. *M. fructicola* was not tested beyond 1 yr. *Phomopsis* sp. from azalea did not survive longer than 4 wk.

A strain of *Glomerella cingulata* (Stonem.) Spauld. & Schrenk causes camellia canker and graft failure (4). *G. cingulata* is easy to isolate from diseased camellia wood and grows readily on carrot juice agar (CJA). To produce an abundance of *G. cingulata* conidia, the fungus is seeded and grown on CJA in 90-mm culture dishes at 21 C for 4 days, then scraped and incubated for 3 more days in either constant or intermittent light (12 hr light/12 hr dark) at 21 C (2). *G. cingulata* mutates freely under these conditions. The sexual stage forms within 17–21 days (3) of seeding. Mutation and the sexual stage both provide a method for variability (L. W. Baxter, Jr., *unpublished*). Thus, frequent culture transfers are necessary to eliminate variability caused by hybridization and/or mutation. The work reported provides a method of long-term storage that greatly reduces the problem of variability. Three other fungi, *Monilinia fructicola, Pestalotia guepini,* and a *Phomopsis* sp. from azalea, were also tested.

MATERIALS AND METHODS

One hundred 1-cm disks of Whatman No. 1 filter paper were placed in each of 20 150-mm culture dishes on two layers of Whatman No. 1 filter paper and sterilized. Large quantities of *G. cingulata* conidia were produced on CJA, collected in sterile tap water, and the concentration standardized at 50 Klett units (Klett-Summersom colorimeter). One spore-laden drop was added to each of 2,000 sterilized disks. These were kept moist and incubated for 3 days at 21 C, then dried thoroughly (petri dish tops removed) in a sterilized oven at 21 C (usually 2–3 days). The 20 culture dishes were then covered and placed in the freezing compartment of a refrigerator at −8 C.

P. guepini from camellia leaves, *M. fructicola* from rotted peach fruit, and a *Phomopsis* sp. that causes stem dieback of azalea were similarly tested. At weekly intervals for 1 yr, 25 fungus-laden disks of each fungus were removed and plated on CJA, five per plate. A few of the remaining disks infested with *G. cingulata* and *P. guepini* were left in storage at −8 C for an an additional 3 yr, then 100 disks infested with each fungus were plated on CJA. All other disks were discarded after 1 yr. Five plants of *Camellia sasanqua* Thunb. were wound-inoculated after 6 mo, five after 12 mo, and five others after 4 yr from cultures grown from frozen disks (1). Controls consisted of *C. sasanqua* seedlings that were wounded but not inoculated.

RESULTS AND DISCUSSION

Identifiable colonies of *G. cingulata* and *P. guepini* grew out within 5 days from the spore-laden disks onto CJA even after storage at −8 C for 4 yr (Fig. 1). *M. fructicola* survived for 1 yr with no apparent loss of viability. Disks infested with *M. fructicola* were discarded and not tested after the first year. *Phomopsis* sp. survived this storage for less than 4 wk (Table 1).

All inoculations of camellia with *G. cingulata* fungal cultures resulting from colonies from previously frozen spore-laden disks (after 6 mo and 1 and 4 yr) caused lesions and cankers on camellia. The controls were not infected. The other fungi were not tested for pathogenicity.

The failure of the few spore-laden disks

Table 1. Growth of selected fungi from conidia incubated for 3 days on filter paper, dried, and frozen (−8 C) for various lengths of time[a]

Fungus	Year	
	1981	1985
Glomerella cingulata	1,273/1,300[b]	98/100
Monilinia fructicola	1,261/1,300	...[c]
Pestalotia guepini	1,283/1,300	100/100
Phomopsis sp.	73/300[d]	...[c]

[a] Twenty-five filter-paper disks were plated weekly.
[b] Numerator = successful growth and denominator = attempts.
[c] None available for testing.
[d] Not tested further after 12 wk. Only an occasional colony developed after 4 wk.

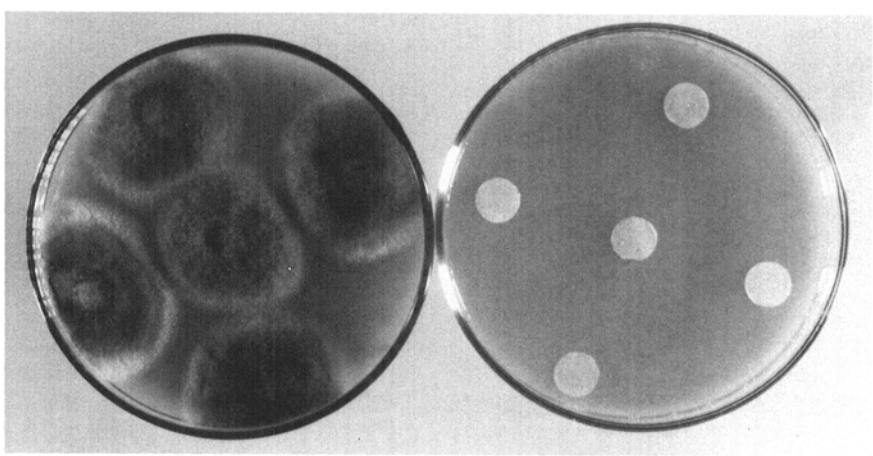

Fig. 1. Growth of *Glomerella cingulata* from filter-paper disks after 4 yr of storage at −8 C. (Left) Disk seeded with a conidial suspension and (right) disk not seeded.

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to produce colonies is thought to be due to human error, such as missing some disks during seeding. The spore-laden moisture wet some of the disks in advance, thus making it difficult to decide which ones had been wet with a spore-laden drop or with water that had diffused. Occasionally, a bacterial contaminant would grow and prevent fungal growth. This was checked a few times by placing contaminated disks (after washing gently in sterile water) onto acidified, streptomycin-amended CJA. The fungi then grew out.

Unless *G. cingulata* spores on disks were allowed to incubate and then dry before freezing, they did not survive well. If the fungal spores were not allowed to incubate on wet filter paper for 3 days but were frozen immediately at −8 C, they survived for less than 2 mo. It is possible that an appressorium-type structure was formed that allowed survival under frozen conditions for 4 yr (duration of test); however, no such structure was seen.

This is a method by which various fungi can be stored easily and in a small space for prolonged periods without having to transfer the cultures frequently. The failure of *Phomopsis* sp. to survive indicates that some fungi cannot be stored in this manner and that each fungus will have to be tested separately.

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