# A Technique for Cryogenic Storage of the Septoria Leaf Blotch Pathogen of Barley

W. H. Anderson and B. Skovmand

Research Assistant and Laboratory Technician, respectively, Department of Plant Pathology, University of Minnesota, St. Paul 55101.

Scientific Journal Series Paper No. 7500, Agricultural Experiment Station, University of Minnesota, St. Paul.

ABSTRACT

Isolates of *Septoria passerinii* retained viability through cryogenic storage when applied to wood segments that were sealed in glass ampules. Phytopathology 61:1027.

*Septoria passerinii* Sacc. is one of the pathogens used in our program for the development of disease-resistant, malting-quality barley (*Hordeum vulgare* L.). Cultures of this pathogen must be available that produce abundant conidia and retain the virulence representative of the disease response evoked by the pathogen in the field. Although isolates of *S. passerinii* grow well on a 2% cornmeal agar supplemented with 2% glucose and 2% peptone, these cultures must be transferred to fresh medium every 4 or 5 days to maintain optimum growth and to prevent loss of viability. To eliminate the time-consuming task of transferring cultures, a method of long-term storage was sought that would allow cultures to be increased quickly for use as inoculum.

Storage of *S. passerinii*, the incitant of leaf blotch of barley, has not been successful using liquid nitrogen techniques. When grown on an artificial medium, *S. passerinii* has a sparse mycelial development, forms no pycnidia, but produces copious amounts of conidia. This yeastlike growth habit makes it impossible to remove conidia from the substrate to be stored dry as rust conidia are stored. In our tests, conidia scraped from sporulating cultures and placed directly into ampules or suspended in water or 10% peptone solution have not survived nitrogen storage. We have devised a modification in the liquid nitrogen storage technique which has resulted in good survival of the pathogen.

Wellman & Walden (2) made quantitative and qualitative estimates of the viability of 50 species of fungi after periods of storage in liquid nitrogen. They observed no significant differences in the viability between cultures tested 24 hr after immersion in liquid nitrogen and those tested after longer periods of storage. These workers included several yeasts, but no species of *Septoria* in their tests. Our concern is not so much with the quantitative aspects of survival, but rather with the qualitative viability and virulence of *S. passerinii* after long-term cryogenic storage.

Cultures of *S. passerinii* were grown on a glucose: peptone-supplemented cornmeal agar medium. A small amount of the yeastlike fungus material was transferred aseptically from a 4-day-old culture to a sterile microscope slide. Dry-sterilized 2-cm segments of the wooden shaft of a cotton-tipped applicator were rolled through the fungus material on the slide with a sterile forceps. These wood segments were placed individually into sterile 2.5 mm × 3.5 cm glass ampules. The ampules were sealed with an oxygen natural-gas flame, placed on semitubular aluminum canes (1), and stored in liquid nitrogen.

To determine the viability and pathogenicity of the cultures after storage, ampules were removed from liquid nitrogen after intervals of 1 week, 3 weeks, 6 months, and 1 year. These were immediately thawed in a 45-C water bath for 5 min; ampules were broken, and the fungus-coated wood segments were rolled over the surface of a sterile, 2% glucose-peptone supplemented cornmeal agar with a flamed forceps.

All cultures of *S. passerinii* that had been applied to wood segments and stored in liquid nitrogen for up to 1 year produced abundantly sporulating cultures when the wood segments were rolled over a fresh agar medium. Seedlings of the cultivars C.I. 4780 and Larker, which are resistant and susceptible, respectively, to *Septoria* leaf blotch, were sprayed with a conidial suspension from these cultures and incubated in a moist chamber 4 days. The response of these cultivars to the liquid nitrogen-stored *S. passerinii* was the same as to those cultures of the pathogen that had not been stored in liquid nitrogen.

The advantages of this method of storage of *S. passerinii* are: (i) It eliminates the time-consuming task of frequent transfers of the cultures to a fresh medium; (ii) when applied to wood segments, *S. passerinii* survives cryogenic storage, and can easily and quickly be increased for use as inoculum; and (iii) the danger of loss of virulence that may result from continuous propagation on artificial medium is minimized.

LITERATURE CITED

1. Leath, K. T., R. W. Romig, and J. B. Rowell. 1966. A system for storing rust spores in liquid nitrogen. Phytopathology 56: 570.
2. Wellman, A. M., & D. B. Walden. 1964. Quantitative and qualitative estimates of viability of some fungi after periods of storage in liquid nitrogen. Can. J. Microbiol. 10:585-593.