EFFECT OF FREEZING ON VIABILITY OF THE LIMA BEAN
DOWNY MILDEW FUNGUS (PHYTOPHTHORA PHASEOLI THAXT.)

R. E. Wester, Charles Drechsler, and Hans Jorgensen

The lima bean downy mildew fungus (Phytophthora phaseoli Thaxt.) has been kept at Beltsville, Maryland, in a viable condition at -10° F temperature for over 100 days without any reduction in pathogenicity.

In the past it has been difficult to maintain this fungus for any considerable period in the laboratory. In the greenhouse the organism has been kept viable during fall, winter, and spring by inoculating a new lot of lima bean seedlings every 10 days with spores from previously infected seedlings. This procedure requires much time and some facilities for maintaining proper humidity and temperature. During the summer the fungus dies because of excessively high temperatures. The freezing method described here removes these hazards and makes the storing of the downy mildew fungus until required for use a simple matter.

FIGURE 1. Inoculating lima bean seedlings with a spore-suspension of downy mildew fungus.

FIGURE 2. Downy-mildew-inoculated lima bean seedlings being placed in the humidity chamber.

1Horticulturist, Mycologist, and Agricultural Aid, respectively, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland.
The method of preparing, freezing and storing the sporulating downy mildew fungus is as follows: Lima bean seedlings emerging from soil in a flat are sprayed with a water suspension of downy mildew conidia (Fig. 1) and placed in a saturated humidity chamber (Fig. 2), in which the temperature ranges from 65° to 75° F. After they have been kept 4 days in this chamber, the seedlings usually become overgrown rather extensively with young conidiophores, which form a downy turf visible to the naked eye. The flat is then transferred to a neighboring 50 percent humidity chamber where within 24 hours further growth of conidiophores usually takes place and conidia are produced in enormous quantity. The seedlings with sporulating conidiophores are cut into short pieces, which are placed in a 2-1/2-ounce

FIGURE 3. Short pieces of downy-mildew-infected lima bean stems in 2-1/2-ounce screw-top jar for storage in refrigerator at -10° F.

FIGURE 4. A flat of lima bean seedlings, 5 days after inoculation with the downy mildew fungus from infected seedling pieces that had been stored in a refrigerator for over 100 days at -10° F.
bottle with screw top (Fig. 3) and stored in a -10° F refrigerator.

When required for use, the seedling pieces are placed in an Erlenmeyer flask with water immediately upon removal from the refrigerator and shaken for about 30 seconds. By this treatment the conidia are dislodged from the tangle of conidiophores and set free in the water. The water with the conidia or sporangia in suspension is then atomized on a new stand of lima bean seedlings (Fig. 1). Figure 4 shows a close-up of lima bean seedlings infected with the downy mildew fungus that had been stored for over 100 days at -10° F.

CROPS RESEARCH DIVISION, UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, BELTSVILLE, MARYLAND