PREPARATION OF CELL-WALL-FREE
PROTOPLASTS FROM THE CHESTNUT BLIGHT FUNGUS

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Abstract.--Certain isolates of Cryphonectria parasitica (Murr.) Barr (=Endothia parasitica (Murr.) P. J. & H. W. Anderson) contain cytoplasnically transmitted dsRNA which is believed to render mycelium hypovirulent. Practica. utilization of hypovirulence in fungi is limited because of the general)resence of mating incompatibility factors. A method of forcing vegetative fusion, and hence passible transfection of the dsRNA, involves use of electromanipulation. This research reports the protocol necessary to produce cell-wall-free protoplasts. Two strains were used. E7 is white in culture and is a hypovirulent strain which produces large titers of dsRNA. Strain 591 is virulent, is dsRNA free, and produces a yellowish-brown culture in Liquid Complete Medium (LCM) (Phytopathology 67:1393- 396). Two mm dia agar plugs of hyphal tips were removed from a 4-day-old culture on solid LCM and placed on a special cellophane membrane (6 x 6 cm) appressed to solid LCM (in 1% agar) and incubated in the dark for 2 dais at 27°C. The cellophane pieces, each with visible growth, were then floated on LCM and grown at room temp for 48 hr under a 16 hr photoperiod. Membranes with colonies were then placed on one-half strength LCM for 24 hr, then washed with 3 changes of 0.5 M MgSO4 in distilled water.

In order to remove the cell walls, mycelia were carefully removed intact from the cellophane membrane and resuspended in 15 ml of 0.5 M MgSO4 with 1 mg/ml of commercial wall-lytic enzyme and 1mM CaCl2 and incubated for 16 hr at 24°C. Protoplasts were collected by serial centrifugation and resuspended in MCT medium (0.5 M mannitol, 1mM CaCl2, and 50 μ wall-lytic enzyme). Three washes were done using centrifugal sedimentation, changing the MCT medium each time. The final protoplast pellet was suspended in MCT medium and adjusted to 1 x 10 protoplasts/ml for electromanipulation. The advantage of this procedure over liquid culture was the avoidance of tedious and time consuming filtration and washing.

Results showed that the cell walls of the E7 isolate were easy to remove enzymatically. E7 yielded approximately 10 times as many protoplasts as did 591 under identical growth conditions. Protoplasts from 591, however, were larger in overall size and had larger vacuoles than did E7. The cytoplasm of E7 appeared much denser.