

McDougall, K. J. The use of a Coulter counter

for counting ascospores of *Neurospora*.

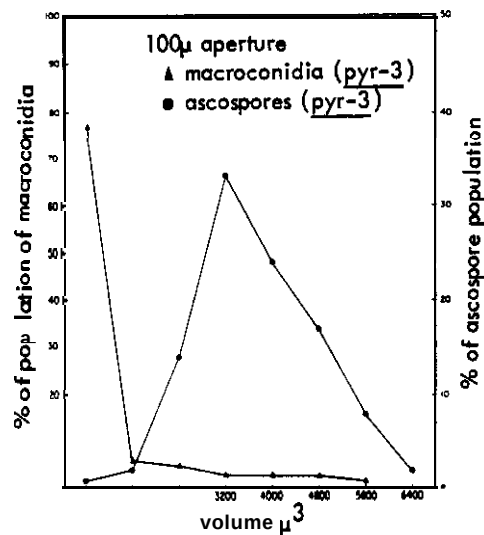
conidia of the perithecial parent are removed by vacuum (a Pasteur pipette attached to a vacuum pump) under a hood prior to crossing with the conidial parent. Prior to spore ejection, the petri dish lids are exchanged for new ones, thus providing a clean surface on which to collect the spores. The spores are then swabbed from the lid, using a small piece of sterile plastic sponge on the end of a microspatula, and suspended in Vogel's liquid medium N (0.1 g sucrose and 0.75 g sorbose per 100 ml) for counting. In the event that a particular cross exhibits considerable spontaneous germination, it is necessary to filter the suspension through five or six layers of gauze. This procedure increases the background count, but is necessary for an accurate count and to prevent plugging of the aperture by mycelia. Commercially prepared saline gives low background counts but drastically reduces germination.

The settings used for counting ascospores on this instrument are as follows: aperture current 64; attenuation 4; threshold 15. The counting chamber is a shell vial (70mm x 15mm OD). Counts are made on the actual suspension, thus avoiding dilution errors. Since ascospores settle relatively quickly (see Table I), it is necessary to stir the suspension with a glass rod prior to

A Model F Coulter Counter equipped with a 100 μ aperture tube has been used to count ascospores of *Neurospora*. The procedure employed is as follows. Crosses are made in petri dishes (100mm x 20mm) in the usual fashion. To aid in obtaining a clean preparation of ascospores,

Table I. Change in counts with time of a suspension of ascospores permitted to settle.

Time (min)	Count
0.00	3698
0.18	3588
0.36	3562
0.54	3491
1.12	3520
1.29	3231
1.48	3082
2.06	2929
2.24	2059
2.42	2658
3.00	2674
3.18	2517



each count. Generally, four counts are made on each suspension. By stirring it is possible to get counts that are in very close agreement (less than 1% deviation). The counter gives the actual count in 0.5 ml of suspension; therefore, it is a simple matter to calculate the total number of spores in the remaining suspension. The spores are then pipetted into 80 ml of melted agar, heat shocked and plated. Bacterial contamination has not been a problem using this procedure.

In order to determine the volume of an ascospore, the instrument was calibrated using paper mulberry pollen of known size obtained from the Coulter company. Figure 1 gives the size distribution of *Neurospora* ascospores obtained from a cross of two *pyr-3* mutants, and what appears to be the size distribution of a suspension of macroconidia of a *pyr-3* mutant. It can be seen that the curves overlap slightly. Using a 70 μ aperture tube, Gillie (1967 *Neurospora News* 1: 16) has shown that macroconidia of wild type (74-OR8-1a) do not exceed 400 μ^3 in volume. The data presented here were obtained with a 100 μ aperture tube and the discrepancy in the size of macroconidia, as measured by the two different aperture tubes, can be attributed to the coincident passage of macroconidia through the 100 μ aperture. These data point out the necessity of obtaining an ascospore suspension free of massive conidial contamination when using a counting system such as the above. (This work was supported by NSF Grant No. GB-5998. ■ ■ ■ Department of Biology, University of Dayton, Dayton, Ohio 45409.