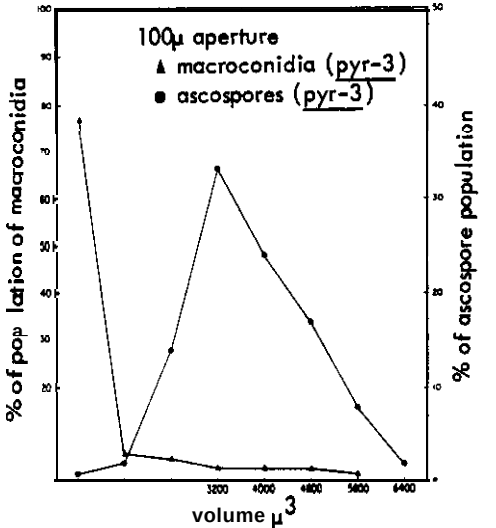
McDougall, K. J. The use of a Coulter counter for counting ascospores of Neurospom.

A Model F Coulter Counter equipped with a 100μ 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, 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 spares we 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 mode 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

Table 1. 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	323 1
1.48	3082
2.06	2929
2.24	2059
2.42	2658
3.00	2674
3.18	2517



each count. Generally, four counts ore made on each suspension. By stirring it is possible to get counts that ore 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 ore then pipetted into 80 ml of melted agar, heat shocked ond plated. Bacterial contamination has not been a problem using this procedure.

In order to determine the volume of on 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 con be seen that the curves overlap slightly. Using a 70 μ aperture tube, Gillie (1967 Neurospora Newsl. 11: 16) has shown that macroconidio 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, con be attributed to the coincident passage of macroconidia through the 100 μ aperture. These data point out the necessity of obtaining on ascospore suspension free of massive conidiol contamination when using a counting system such as the above. (This work was supported by NSF Grant No. GB-5998. ▪ ▪ ▪ Deportment of Biology, University of Dayton, Dayton, Ohio 45409.