A second experiment, using *pan* -2 cultures ranging in age up to 18 days, was done using Fries \#3 medium throughout. Conidia were incubated in liquid Fries using the method of Ryan (loc. cit.) and the percent of conidial germination was determined after 8 hours. The same suspensions were also plated as described in the previous experiment to determine the relative plating efficiencies. These two values are compared in Table 2.

Although we realize that the Plating efficiencies given in the two tables will vary from experiment to experiment and from culture to culture, we were impressed with the fact that although the plating efficiency is only about 50% for one-day-old cultures, it increases to a maximum on the second day and remains fairly constant for periods of up to ten days or longer. For our own purposes it seems clear that once a culture has enough conidia so that they can be easily collected, the viability is as good in a two-day-old culture as it is in a week-old culture. Furthermore, in growth tubes, where conidia in the proximal and distal ends are of different ages, the viability of the conidia from opposite ends is comparable.

In the second experiment using the medium of Fries, there was a good correlation between the colony counts on the plates and the percent of conidio which had germinated after 8 hours' incubation in liquid medium. This was surprising since we had found, although the data are not given here, that the percent of germination in liquid Vogel's medium after 8 hours was significantly lower than the percent of conidio which formed colonies.

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Wellman, A. M. Viability of *Neurospora macroconidia* after cryogenic storage by liquid nitrogen refrigeration.


Low temperature storage: The following procedure for the storage of *Neurospora* strains has been developed in this laboratory during the past 4 years. The fungi are grown on agar slants (on Fries minimal or supplemented medium) in plugged 2 ml cryogenic ampules (T. C. Wheaton Co., Millville, N. J.) for 7 days at 25°C. The ampules are then heat-waled, placed immediately on aluminum cans (Arnold Nasco Ltd., Guelph, Ont.), loaded into canisters and rapidly frozen (1-15°C/sec) by direct immersion into a liquid nitrogen refrigerator (Linde LR -35 -9). After various storage periods frozen cultures are warmed rapidly by transferring them to water both at 35-40°C for 2 min and then left at room temperature for 1/2-1 hour before testing for viability. On each occasion four ampules were sampled.

The results recorded here are part of an investigation of the effects of low temperature storage on several strains of fungi over a ten-year period. Viability of *Neurospora* was estimated as percentage germination. In order to distinguish between freeze/thaw injury and the effects of storage cultures frozen and immediately warmed were compared with unfrozen control cultures; thereafter frozen cultures stored for up to 30 months were compared with 7-day-old control cultures which had been maintained routinely on agar slants by successive transfer.

Germination tests: 0.5 ml of spore suspension (3 x 10⁴ spores/ml) was spread on the surface of each agar plate (4 plates per treatment) and the plates were incubated at 30°C. Discs were removed from the plates from 1-8 hours after incubation; a drop of 10% formalin was added to each disc and two randomly-chosen fields/disc were examined under a 40X high dry objective and scored for germination. Each field (16 fields/treatment/time interval) was recorded on 36 mm film using a Leitz Ortholux camera, so that on analysis of germ tube lengths under different treatments could be made from the projected negatives.

Conidia were also germinated on squares of sterilized dialyzing membrane on the surface of agar plates. The spores on the membrane can be fixed (in Helly's) and stained for more extensive morphological investigations. Some microorganisms are metabolically injured during freezing and thawing such that their nutritional requirements are altered. The nutritional requirements of one strain of *N. sitophila* in which 27% non-germinating spores are present after 6 hours' incubation on minimal medium following freeze/thawing are being investigated by transfer of spores on dialyzing membrane to supplemented media to determine whether non-germinating spores are non-viable or whether they have more demanding nutritional requirements.

Viability: As shown in Table 1, there is a slight decline in viability of frozen and thawed spores (significant at 1% level F = 18.56) but no significant decrease occurs with increased storage time (F = 2.09). A fairly good recovery has been obtained with wild type conidio of *N. crassa* strains 79A (FGSC#533) and 74-OR23-1A (FGSC#987) after 18 months storage and with 16 wild type and mutant strains after 3 months storage (in press).

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Table 1. % germination of *Neurospora* strain UWO 913 conidia after 6 hours incubation at 30°C (average of 4 sub-samples).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1 hr</th>
<th>1 month</th>
<th>6 months</th>
<th>12 months</th>
<th>18 months</th>
<th>24 months</th>
<th>30 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stored at -196°C</td>
<td>93.43</td>
<td>87.07</td>
<td>91.31</td>
<td>90.31</td>
<td>92.65</td>
<td>84.85</td>
<td>96.73</td>
</tr>
<tr>
<td>7-day-old control grown at 25°C</td>
<td>95.78</td>
<td>96.45</td>
<td>95.34</td>
<td>97.81</td>
<td>93.58</td>
<td>95.73</td>
<td>97.20</td>
</tr>
</tbody>
</table>

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